Vascular Response to Angiotensin II in Upper Body Obesity

Søren Nielsen, John R. Halliwill, Michael J. Joyner, Michael D. Jensen

Abstract—Upper body obesity is associated with insulin resistance, hypertension, and endothelial dysfunction. We examined forearm vascular function in response to vasodilator (endothelium-dependent and endothelium-independent) and vasoconstrictor stimuli in 8 normotensive, upper body/viscerally obese men with a positive family history of hypertension and 8 age-matched nonobese men. We also measured body composition and insulin regulation of free fatty acid (FFA) and glucose metabolism. Forearm blood flow was measured before and during brachial artery infusions of acetylcholine (Ach), sodium nitroprusside (NTP), and angiotensin II (± nitric oxide synthase [NO]) synthase blockade with Nω-monomethyl L-arginine [L-NMMA]). On a separate day, baseline and insulin-regulated glucose ([3-H]glucose) and FFA ([9,10-3H]palmitate) turnover were measured. The vasoconstrictor response to angiotensin II was greater (P<0.05) in obese men than in nonobese men, whereas endothelium-dependent vasodilation was similar. The slope of the angiotensin II dose-response curve correlated significantly with the basal plasma palmitate concentration. Basal and insulin-mediated glucose disposal was significantly reduced and FFA turnover significantly increased in viscerally obese men. No differences in endothelium-independent vasodilation or relationships between vascular responsivity and palmitate and glucose kinetics or body composition were found. Angiotensin II–stimulated forearm vasoconstriction is increased in viscerally obese normotensive men. (Hypertension. 2004;44:435-441.)

Key Words: fatty acids ■ blood flow

Human obesity and essential hypertension frequently coexist. However, the mechanisms linking these conditions are not well-characterized. Several factors, including insulin resistance/hyperinsulinemia, increased sympathetic nervous system activity, and increased renin-angiotensin system activity have been proposed.1,2 Normally, insulin is a potent skeletal muscle vasodilator and the endothelium-dependent relaxing factor, nitric oxide (NO), plays a pivotal role in mediating this effect.3 A number of conditions have been reported to be associated with reduced NO-mediated vasodilation, among them obesity4 and hypertension.5 Ultimately, however, the balance between vasodilator and constrictor stimuli determines vascular tone.

Defective counter-regulatory vasorelaxation secondary to impaired NO synthesis or action could facilitate the development of hypertension. Reduced NO availability is associated with increased vasoconstriction in response to endothelin-1 and cyclooxygenase-dependent contracting factor in patients with essential hypertension.6 Moreover, patients with type 1 diabetes mellitus7,8 and essential hypertensio9 have an increased blood pressure response to systemic angiotensin II infusion. Obesity is associated with impaired endothelium-dependent vasorelaxation in response to arterial infusion of methacholine and with resistance to insulin’s ability to enhance endothelium-dependent blood flow.4 Although the impaired endothelium-dependent vasorelaxation response to insulin is correlated with insulin resistance with respect to glucose uptake,10 Egan et al found that hypertension was more closely associated with abnormal free fatty acid (FFA) metabolism in abdominally obese, hypertensive adults.11 The link between vascular function and FFA was more directly tested by acutely increasing plasma FFA concentrations using lipid emulsion infusions in lean healthy humans; increases in systemic blood pressure12 and inhibition of endothelium-dependent vasorelaxation develop.13,14

Because insulin resistance with respect to glucose and FFA metabolism (greater FFA concentrations) are more pronounced in upper body obesity than lower body obesity and because circulating FFA may affect NO-mediated vasorelaxation, we hypothesized that regulation of vascular tone is abnormal in normotensive, upper body obese, insulin-resistant subjects compared with nonobese subjects. Therefore, we examined forearm vascular tone and response to endothelium-dependent (acetylcholine [Ach]) and independent (sodium nitroprusside [NTP]) dilatory stimulation and angiotensin II, and NO synthase blockade evoked constriction in normotensive, upper body obese, insulin-resistant men with a family history of hypertension and age-matched, nonobese men. Moreover, we examined whether forearm vascular response abnormalities are worse in subjects with abnormalities of effective adipose tissue lipolysis (FFA turnover/FFA concentrations) and decreased insulin-mediated glucose disposal.
Methods

The study was approved by the institutional review board at Mayo Clinic, and informed written consent was obtained from all participants.

Subjects

Eight overweight/obese white men with an upper body fat distribution, age younger than 45 years, and 8 age-matched, nonobese men were recruited. Total body fat by dual-energy x-ray absorptiometry was >27% in overweight/obese men and <23% in nonobese men. Overweight/obese subjects were only considered if normotensive (blood pressure <140/90 mm Hg), waist-to-hip ratio >0.95, and if they had a positive family history for hypertension (n=8). Two of these volunteers also had a family history of type 2 diabetes. Nonobese participants could not have a family history of hypertension or type 2 diabetes. Additional criteria were normal fasting plasma cholesterol, nonsmoking, and using no medications, including antioxidants.15 All volunteers were weight-stable for the previous 3 months and all participants had a normal blood cell count and biochemistry panel.

Protocol

Total body fat and fat free mass (FFM) was measured using dual-energy x-ray absorptiometry (Lunar Radiation Corp, Madison, Wis); intraabdominal and abdominal subcutaneous fat was assessed using a single-slice abdomen computed tomography CT at the L2 to 3 interspace.16 All participants were provided weight-maintaining meals (40% fat, 40% carbohydrate, 20% protein) including a constant sodium intake of 140 mmol/d at the Mayo Clinic General Clinic Research Center (GCRC) for 3 days before the study. All subjects maintained their usual level of physical activity and were asked not to participate in heavy exercise the last 3 days before the study. Each participant was admitted to the GCRC the evening before each study, which were performed over 2 consecutive days, each after a 12-hour fast.

On day 1, a 2-step hyperinsulinemic, euglycemic clamp was performed using palmitate and glucose tracers to measure basal and insulin regulated FFA and glucose kinetics. Intravenous catheters were inserted in an antecubital vein and a dorsal hand vein (in a retrograde fashion) for infusion and sampling, respectively. The hand was placed in a heated box for collection of arterialized blood. Infusions of [3-3H] glucose and [9,10-3H] palmitate were started at 6:30 AM and 8:00 AM, respectively. Between blood samplings, the catheters were kept patent by infusion of 0.9% saline. After completion of all measurements, all catheters were removed and the participants were asked to return to the GCRC in the evening.

On day 2, participants were transferred to the GCRC Integrative Physiology Core (autonomic/neurophysiology laboratory). The brachial artery of the nondominant forearm was catheterized to measure forearm blood flow and intraarterial insulin kinetics. Use of these drugs and with and without concomitant NO synthase blockade (L-NMMA; CalBiochem) were measured as described.

Glucose Turnover

A 2-step euglycemic, hyperinsulinemic clamp in combination with indirect calorimetry was used to assess basal and insulin-regulated FFA flux and glucose disposal. A priming dose of 12 μCi [3-3H]glucose (New England Nuclear, Boston, Mass) was given at 6:30 AM, followed by a continuous infusion (0.12 μCi/min) throughout the basal (90 to 60 minutes) and the hyperinsulinemic periods (step 1: 60 to 180 minutes; step 2: 180 to 300 minutes) to measure glucose turnover rates. Insulin (Actrapid, Novo-Nordisk; Gentofte, Denmark) was infused at a rate of 0.25 and 1.0 mU·kg FFM⁻¹·min⁻¹ and plasma glucose was “clamped” at ~5 mmol/L by infusion of 50% dextrose labeled with 3-3H-glucose (73.7 μCi/100g dextrose). Plasma glucose was measured in duplicate every 5 to 10 minutes (Beckman Instruments, Palo Alto, Calif) to allow adjustment of the glucose infusion rate to maintain euglycemia. Blood samples for determination of glucose specific activity, insulin, growth hormone, and catecholamines were drawn every 10 minutes during the final 30 minutes of each interval.

Indirect Calorimetry

Resting energy expenditure and respiratory exchange ratios were assessed by indirect calorimetry with a ventilated hood (Deltatrac Metabolic Cart, Yorba Linda, Calif) during the last 30 minutes of the basal period and the low-dose and high-dose hyperinsulinemic periods. Protein oxidation rate was estimated from urinary urea excretion collected during the investigation. Net nonoxidative glucose disposal (ie, glucose storage) was calculated by subtracting the glucose oxidation rate from the isotopically determined glucose disposal.

Palmitate Kinetics

Basal and insulin-suppressed systemic FFA (palmitate) flux was measured using constant infusions of [9,10-3H] palmitate (Amer sham Corp, Arlington Heights, Ill) (0.3 μCi/min) at times 0 to 60 minutes, 120 to 180 minutes, and 240 to 260 minutes. Blood samples for measurements of palmitate concentration and specific activity were drawn before the first tracer infusion and at 10-minute intervals over the last 30 minutes of each infusion period. Plasma palmitate concentration and specific activity were determined by high-performance liquid chromatography.17,18

Forearm Blood Flow and Intraarterial Blood Pressure

Arterial pressure was measured throughout the forearm infusion experiments with a 5-cm, 20-gauge Teflon catheter placed in the brachial artery under local anesthesia using aseptic technique. This catheter was continuously flushed with low-dose heparinized saline (2 U/mL, 3 mL/h). Mean arterial pressure was obtained electronically.

Forearm blood flow was measured in the nondominant forearm using venous occlusion plethysmography with mercury-in-silastic strain gauges.19 In brief, an arterial occlusion cuff around the wrist was continuously inflated to suprasystolic pressures (250 mm Hg) during measurements (5 to 10 minutes at a time), while a venous occlusion cuff around the upper arm is inflated to 40 mm Hg for 7.5 seconds out of every 15 seconds, providing 1 blood flow measurement every 15 seconds. Forearm blood flow is expressed as mL/(dL of tissue) per minute. Forearm volume was calculated as determined by Pawelczyk and Levine.20 Pharmacologic agents were infused via a brachial artery catheter using a syringe pump. Ach (IOLAB Pharmaceuticals) was infused at doses up to 32 μg/min. NTP (Ekins-Sinn) was administered at doses up to 10 μg/min, and angiotensin II at doses up to 100 pmol/min. The infusion of angiotensin II was performed alone and during coinfusion of NTP at 4 μg/min. L-NMMA was infused at doses 50 mg over 15 minutes followed by a continuous infusion of 1 mg/min for another 30 minutes, during which response curves to Ach and NTP were repeated. L-NMMA in this dose reduces resting forearm blood flow and blocks Ach-mediated NO. It also has no effect on resting forearm blood flow in the contralateral arm. Use of these drugs allowed us to compare endothelial dependent (Ach) and independent (NTP) vasodilator responses, and to evaluate the how vasoconstrictor responses to angiotensin II interact with NO.

Analysis of Samples

Insulin and growth hormone were measured using chemiluminescent sandwich assays (Sanofi Diagnostics Pasteur Inc, Chaska, Minn), and catecholamines were measured using high-performance liquid chromatography. Plasma cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by enzymatic methods.

Statistics

Values are means±SEM. Comparisons between groups were performed using Student t test or the Mann–Whitney 2-sample test. Slopes of individual dose-response curves were evaluated using linear regression analysis. For angiotensin II dose-response curves
and remained stable during the clamp. Resting energy expenditure was not significantly different between nonobese and obese subjects (1929 versus 1971 kcal/d). Systemic palmitate flux was significantly greater in upper body obese subjects during the basal state and during insulin suppression. Insulin-stimulated glucose disposal rates were significantly less in obese than in nonobese men.

**Basal Forearm Blood Flow and Response to L-NMMA**

Basal forearm blood flow was similar in nonobese and obese men (Figure 1). Inhibition of NO synthase reduced blood flow and increased forearm vascular resistance in both groups. There was no significant difference between groups in the response to L-NMMA.

**Endothelium-Dependent Vasodilation**

Infusion of Ach resulted in an increase ($P<0.001$) in forearm blood flow in both groups (Figure 2). There was no significant difference in the Ach dose-response curves, neither before nor after L-NMMA. Ach increased blood flow after L-NMMA. Forearm vascular resistance was not different between nonobese and obese men.

**Endothelium-Independent Vasodilation**

Infusion of NTP resulted in an increase ($P<0.001$) in forearm blood flow in both groups (Figure 3). There was no difference in the NTP dose-response curves between the 2 groups, neither before nor after L-NMMA, and forearm vascular resistance was not different between nonobese and obese subjects.

**Response to Angiotensin II**

Figure 4 shows dose-response curves to angiotensin II infusion before and during concomitant NTP infusion. Without NTP, vascular resistance increased significantly in both groups ($P<0.001$). Whereas there were no differences at baseline, the increase in vascular resistance in response to angiotensin II was significantly greater in subjects with upper body obesity compared with nonobese subjects (slope, 82.1±20.5 versus 35.7±8.6 mm Hg·mL⁻¹·min⁻¹·μmol⁻¹; $P<0.05$). This difference was not seen during concomitant NTP infusion. When vascular resistance was broken-down into its

### TABLE 2. Hormone and Substrate Values

<table>
<thead>
<tr>
<th></th>
<th>Lean Men (Basal)</th>
<th>Lean Men (Low Insulin)</th>
<th>Lean Men (High Insulin)</th>
<th>Upper Body Obese Men (Basal)</th>
<th>Upper Body Obese Men (Low Insulin)</th>
<th>Upper Body Obese Men (High Insulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate, μmol/L</td>
<td>70±13</td>
<td>20±3</td>
<td>8±1</td>
<td>115±8</td>
<td>46±5</td>
<td>20±3</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>5.3±0.1</td>
<td>5.1±0.1</td>
<td>5.4±0.1</td>
<td>5.4±0.1</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>8.6±1.2</td>
<td>17.8±2.7</td>
<td>47.0±3.3</td>
<td>3.2±0.2†</td>
<td>10.1±0.7‡</td>
<td>43.5±3.0</td>
</tr>
<tr>
<td>Norepinephrine, nmol/L</td>
<td>135±16</td>
<td>154±18</td>
<td>168±23</td>
<td>144±15</td>
<td>152±13</td>
<td>186±25</td>
</tr>
<tr>
<td>Epinephrine, pmol/L</td>
<td>38±6</td>
<td>48±6</td>
<td>49±7</td>
<td>41±3</td>
<td>35±4</td>
<td>59±7</td>
</tr>
<tr>
<td>Glucose turnover, mg·kg·FFM⁻¹·min⁻¹</td>
<td>2.5±0.1</td>
<td>3.6±0.2</td>
<td>9.5±1.4</td>
<td>2.2±0.1*</td>
<td>3.1±0.2*</td>
<td>6.4±0.9*</td>
</tr>
<tr>
<td>Palmitate turnover, μmol/min</td>
<td>99±20</td>
<td>34±6</td>
<td>18±1</td>
<td>256±27‡</td>
<td>114±17‡</td>
<td>55±7‡</td>
</tr>
</tbody>
</table>

Mean±SEM of plasma concentrations of insulin, catecholamines, palmitate, and glucose, as well as palmitate and glucose kinetics. The values are for 8 nonobese and 8 upper body obese men before and during a 2-step hyperinsulinemic, euglycemic insulin clamp. $P<0.05$, †$P<0.01$, ‡$P<0.001$ vs lean.
basic components (ie, blood pressure and blood flow), we noted that intra-arterial blood pressure was significantly higher in obese subjects but increased the same in nonobese and obese subjects in response to angiotensin II (Figure 4, insert). Conversely, blood flow was similar but tended toward a greater decline in subjects with upper body obesity compared with nonobese subjects (slope [log transformed dose]: -0.76±0.16 versus -0.65±0.16 mL·dL⁻¹·pmol⁻¹; NS).

Correlations
Within groups (nonobese and obese), basal blood flow, vascular resistance, and slopes of dose-response curves were not correlated with basal and insulin-regulated palmitate or glucose kinetics or with visceral or subcutaneous fat. For the combined groups, there was a statistically significant correlation between baseline (not insulin-suppressed) plasma palmitate concentrations and the slope of the angiotensin II dose-response curve (Figure 5). There was no relationship between the basal or insulin-regulated palmitate concentration and the slope of the Ach or NTP dose-response curves.

Discussion
An upper body, especially visceral, fat accumulation and insulin resistance are risk factors for hypertension. This pattern of obesity could potentially contribute to increased blood pressure via an abnormal regulation of endothelium-dependent vasodilation and/or by greater response to vasoconstrictor stimuli. In this study we found an increased vasoconstrictor response to angiotensin II in upper body obese men, whereas resting forearm blood flow, vascular resistance, and responses to Ach, NTP, and 1-NMMA were not different in upper body obese and nonobese men. There was no relationship between the vascular responses we examined and basal or insulin-regulated substrate turnover rates or with body composition. There was a significant, positive correlation between the vasoconstrictor response to angiotensin II and fasting palmitate (FFA) concentrations. The increased responsiveness to angiotensin II we found in upper body obesity is consistent with its effects in other insulin-resistant conditions in humans and animals. The mechanism is unknown but could result from a greater direct responses, reduced compensatory vasodilator responses, or modulation through the sympathetic nervous system; angiotensin II facilitates adrenergic vasoconstriction. Interestingly, hyperinsulinemia, a hallmark of upper body obesity, may increase sympathetic nervous system activity in obese humans. In rats, pharmacological impairment of NO synthase activity augments the pressor response to angiotensin II and norepinephrine. Insulin sensitivity has also been reported to correlate with the vasoconstrictor response to 1-NMMA, but not to norepinephrine. Regarding the vasoconstrictor response to 1-NMMA, we did not find a difference between lean and obese or a correlation with insulin action, but this may relate to our use of high doses of 1-NMMA designed to result in complete blockade of NO synthesis.

To minimize fluctuations in renin-angiotensin system activity, sodium intake was fixed and volunteers spent the night in the GCRC to avoid unnecessary physical activity on the day of the study. Only men were investigated. However,
preliminary data indicate that women with upper body obesity have normal endothelium-dependent and independent vasodilation when compared with lean women. We could not confirm the finding of impaired endothelium-dependent vasorelaxation in obese subjects. Possible explanations for the differences include issues of gender differences, obesity phenotype (upper versus lower body obesity), antioxidant intake, serum lipid concentrations, and the responsiveness of the examined vasculature (forearm versus leg). Of note, we found similar endothelium-dependent vasodilation in upper body obese and nonobese men, despite the fact that upper body obese men had a positive family history of essential hypertension and type 2 diabetes. Our upper body obese men represent a group susceptible to angiotensin II and to the development of hypertension, dyslipidemia, and abnormal glucose and FFA metabolism.

Plasma FFA concentrations largely reflect the release of FFA via adipose tissue lipolysis, and higher FFA in upper body obesity is attributable primarily to dysregulation of this process in upper body subcutaneous fat. To uncouple obesity from increased FFA plasma concentrations, intravenous lipid emulsion and heparin infusions have been used to increase circulating FFA in nonobese volunteers. Higher plasma FFA concentrations obtained using this approach have increased the pressor response to phenylephrine. Moreover, infusion of FFA in rats, especially into the portal vein, increased blood pressure and plasma catecholamines. These observations indicate that vasodilatory and constrictor mechanisms are affected by FFA concentrations, and suggest that increased hepatic delivery of FFA (a hallmark of visceral obesity) elicits neuroregulatory responses to cause increased sympathetic discharge and peripheral vasoconstriction. The positive correlation between plasma FFA concentrations and pressor response to angiotensin II we observed is consistent with the concept that FFA may influence vascular tone. The lack of a significant correlation between basal palmitate concentrations and measures of obesity/body composition and angiotensin II dose-response curves suggest that the relationship between basal palmitate concentration and angiotensin II–mediated vasoconstriction is not merely a reflection of obesity per se.
Perspectives
Upper body obese, insulin-resistant men with a family history of hypertension had an exaggerated vasoconstrictor response to angiotensin II, but comparable endothelium-dependent and independent vasodilation. This abnormality may represent an early pathophysiological change predisposing to hypertension. The association between the abnormal angiotensin II response and plasma FFA concentrations is consistent with the concept that increased FFA may be involved in the development of endothelial dysfunction, especially as it relates to angiotensin II. It is not clear whether increased plasma FFA concentrations are responsible for the abnormalities or are merely a reflection of an underlying common defect. It will be necessary to manipulate FFA concentrations and assess the vasoconstrictor responses to address this question.

Acknowledgments
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References


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