Insulin impairs endothelium-dependent vasodilation independent of insulin sensitivity or lipid profile

Umberto Campia,¹ Gail Sullivan,¹ Melissa B. Bryant,¹ Myron A. Waclawiw,² Michael J. Quon,¹ and Julio A. Panza¹

¹Cardiology Branch and ²Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Submitted 11 June 2003; accepted in final form 20 August 2003

Campia, Umberto, Gail Sullivan, Melissa B. Bryant, Myron A. Waclawiw, Michael J Quon, and Julio A. Panza. Insulin impairs endothelium-dependent vasodilation independent of insulin sensitivity or lipid profile. Am J Physiol Heart Circ Physiol 286: H76–H82, 2004. First published August 28, 2003; 10.1152/ajpheart.00539.2003.—Insulin resistance is a risk factor for atherosclerosis and is associated with hyperinsulinemia, abnormal lipid profile, and hypertension. Whether hyperinsulinemia affects vascular function independent of insulin resistance or other metabolic risk factors is unknown. This investigation aimed to assess the effects of hyperinsulinemia on endothelial function in subjects with a spectrum of insulin sensitivity and lipid profile. Endothelium-dependent (flow-mediated dilation, FMD) and -independent (nitroglycerin) responses of the brachial artery were studied by high-resolution ultrasound before and during hyperinsulinemia (euglycemic clamp) in 25 normoglycemic, normotensive subjects. Participants were divided into an insulin-sensitive and an insulin-resistant subgroup based on their sensitivity index values, with a cutoff of 5.2 mmol/l (200 mg/dl). In the whole population, FMD was lower during hyperinsulinemia compared with baseline (2.3 ± 0.6% vs. 6 ± 0.6%; \( P < 0.001 \)). Resting FMD was lower in the insulin-resistant subgroup compared with the insulin-sensitive subgroup (4.2 ± 0.9% vs. 7.4 ± 0.8%; \( P = 0.014 \)) and in the high-cholesterol subjects compared with the normal-cholesterol subjects (4.4 ± 0.7% vs. 8 ± 0.7%; \( P = 0.002 \)). Hyperinsulinemia decreased FMD in both the insulin-sensitive (from 7.4 ± 0.8% to 3.6 ± 0.4%; \( P < 0.001 \)) and insulin-resistant (from 4.2% to 1.22%; \( P = 0.012 \)) subgroups and in both the normal-cholesterol (from 8 ± 0.7% to 3.9 ± 0.4%; \( P < 0.001 \)) and high-cholesterol (from 4.4 ± 0.7% to 1.1 ± 0.8%; \( P = 0.01 \)) participants. Acute hyperinsulinemia impairs conduit vessel endothelial function independent of insulin sensitivity and lipid profile. Insulin may trigger endothelial dysfunction and promote atherosclerosis.

hyperinsulinemia; endothelial function; cholesterol; metabolic syndrome

INSULIN RESISTANCE, defined as impaired insulin-mediated glucose disposal, has emerged in recent years as a major link between metabolic risk factors and atherosclerotic vascular disease. Several epidemiological studies have demonstrated that insulin resistance is associated with the development of coronary artery disease and is usually part of a cluster of metabolic abnormalities that has been termed metabolic syndrome X, which includes abnormal blood lipoprotein profile and hypertension (13). Hyperinsulinemia, itself a marker of insulin resistance, has been shown to be an independent risk factor for ischemic heart disease (9), to exert synergistic effects when in association with elevated apolipoprotein B concentrations (9), and to predict the development of Type 2 diabetes mellitus, altered lipoprotein profile, and hypertension (14). This epidemiological evidence has sparked both interest and controversy regarding the role of insulin in vascular homeostasis and in the development of endothelial dysfunction and atherosclerosis in patients with metabolic syndrome X.

Several lines of research have shown that insulin may exert beneficial vascular effects, increasing endothelial nitric oxide (NO) synthase gene expression (17) and activity (28, 29) and stimulating NO release and forearm vasodilation (5, 5a). Insulin resistance, by decreasing NO activity and reducing the positive effects of insulin on the vasculature, has therefore been considered a major cause of endothelial dysfunction in patients with metabolic syndrome X (12, 25), and hyperinsulinemia has been regarded as a compensatory mechanism (12). However, recent experimental evidence suggests that, on the background of metabolic insulin resistance, hyperinsulinemia may exert proatherosclerotic actions, including an amplified mitogenic activity of growth factors, an increased expression of endothelial adhesion molecules, and an enhanced monocyte adhesion to the endothelium (22). Furthermore, in healthy subjects, acute hyperinsulinemia increases the activity of endothelin-1 (ET-1), a vasoconstrictor and mitogenic peptide (5a) that may promote atherosclerosis (10).

Hence, the vascular actions of insulin appear to be in a complex balance, involving poorly defined systemic mechanisms (5), the activation of vasoactive systems such as the NO and the endothelin pathways (5a, 28), and the generation of oxidative stress (2). This balance may, in turn, determine the pro- or antiatherogenic effects of the hormone. A recent study showed that modest levels of hyperinsulinemia (i.e., similar to those observed in the fasting state in insulin-resistant subjects) reduce endothelium-dependent vasodilation of conduit arteries in healthy subjects (2). These findings suggest that endothelial dysfunction may be a mechanism by which insulin resistance predisposes to atherosclerosis. However, it is possible that the vascular effects of insulin may be related not only to its intrinsic actions on the biology of the vessel wall but also to the pathophysiological milieu in which the hormone exerts its effects. In fact, previous clinical studies have not clarified whether the endothelial dysfunction observed during hyperinsulinemia is related to the presence of insulin resistance or other commonly encountered metabolic risk factors (12, 25).
Furthermore, these investigations have not explored whether different metabolic risk factors may exert additive deleterious effects on vascular function. The present study was therefore designed to determine the effects of acute hyperinsulinemia on the endothelium-dependent vasodilator function of nondo- betic, normotensive subjects with a spectrum of insulin sensi-
tivity and lipid profile.

MATERIALS AND METHODS

Study Subjects

The study population consisted of 25 subjects (14 men and 11 women), whose clinical and biochemical characteristics are reported in Table 1. Before admission into the study, subjects were screened by clinical history, physical examination, electrocardiography, chest X-
ray, and routine chemical analyses. Exclusion criteria were history or evidence of hypertension (blood pressure ≥140/90 mmHg), diabetes mellitus, cardiac disease, peripheral vascular disease, coagulopathy, or any other disease predisposing them to vasculitis or Raynaud phenomenon. Subjects had not taken any medications or antioxidant vitamin supplements in the preceding month. No effort was made to change the subjects’ diet before the performance of the studies. None of the participants was an active smoker. To evaluate the effects of lipid profile on insulin sensitivity and on brachial artery reactivity at baseline and during hyperinsulinemia, participants were prospectively divided into two groups based on their total cholesterol levels, with a cutoff of 5.2 mmol/l (200 mg/dl) (Tables 1 and 2). This cutoff point was based on the definition of desirable cholesterol level of the National Cholesterol Education Panel (1). Similarly, to evaluate the effects of insulin sensitivity on brachial artery reactivity at baseline and during hyperinsulinemia, participants were divided into two groups according to their insulin sensitivity, with a cutoff value of the clamp sensitivity index ($S_{clamp}$) of 8. This value was arbitrarily chosen on the basis of values collected in our institution with the same technique (16). The study protocol was approved by the National Heart, Lung, and Blood Institute (NHLBI) Institutional Review Board (National Institutes of Health protocol no. 00-H-0184), and all participants gave written informed consent for all procedures. All procedures followed were in accordance with the NHLBI Institutional Guidelines.

Protocol

All studies were performed in the morning in a quiet room with a temperature of ~22°C. Participants were asked to fast for at least 10 h and to refrain from drinking alcohol or beverages containing caffeine for at least 24 h before the study.

Brachial ultrasound reactivity study. Endothelium-dependent (flow-mediated dilation, FMD) and -independent (nitroglycerin-me-
diated dilation, NMD) vasodilator function of the brachial artery was assessed at baseline and during the last hour of insulin infusion of a euglycemic hyperinsulinemic clamp study, following the forearm cuff occlusion technique (7). Briefly, subjects lay supine on a bed and were allowed to rest for at least 10 min. During the resting period, subjects were connected to a continuous ECG monitor and a pressure cuff was applied around the upper forearm. The left brachial artery was then visualized on the anterior aspect of the arm, 2–15 cm proximal to the antecubital fossa, with a high-resolution ultrasound probe (ATL HDI 5000 with a 12-MHz linear array transducer; Philips Medical Systems, Best, The Netherlands). The position of the transducer was marked to retrieve the same portion of the artery in the second study.

After baseline images and flow measurements were obtained, the pressure cuff applied on the forearm was inflated at 200–250 mmHg for 5 min. Blood flow was measured during the first 15 s after cuff deflation, and arterial image acquisitions for diameter measurements were performed between 60 and 90 s after cuff deflation.

After at least 10 min of rest, new baseline images and flow measurements were obtained and 0.4 mg of nitroglycerin spray was given sublingually to assess endothelium-independent vasomotor responsiveness. Blood flow and images for arterial diameter were recorded after 3 min.

Arterial diameter was measured from the anterior to the posterior “m” line (the interface between media and adventitia) at end diastole, incident with the R wave on the ECG. Images were analyzed by an investigator, different from the sonographer, blinded to image sequence and subjects’ clinical data. To evaluate the reproducibility of echographic measurements, all studies were reexamined by the same investigator. The coefficients of variability and repeatability were determined according to published criteria (20). The coefficient of variability was 0.5 ± 0.77 for the baseline study and 0.81 ± 1.17 for the hyperemia study. The coefficient of repeatability was 0.005 ± 0.007 for the baseline study and 0.008 ± 0.011 for the hyperemia study. To rule out the possibility that differences in FMD within the same individual were related to the time elapsed between measurements, on a different day, nine subjects underwent two additional FMD studies, separated by 3–4 h, without any intervening pharmacological intervention.

Hyperinsulinemic euglycemic glucose clamp. After performance of the baseline brachial ultrasound reactivity study, participants underwent a euglycemic hyperinsulinemic clamp study. A 20-gauge intra-
venous cannula was placed in a deep vein of one arm for infusion of glucose, insulin, and potassium phosphate. Another catheter was placed in the other arm for blood sampling. The arm was warmed with a heating pad to arterialize the blood. An insulin solution (regular Humulin; Eli Lilly, Indianapolis, IN) was prepared with normal saline at a concentration ranging from 0.8 to 1.2 U/ml. The insulin solution was allowed to dwell in the intravenous lines for at least 15 min, and the lines were flushed before the beginning of insulin infusion. Insulin was infused at a rate of 60 mU·m⁻²·min⁻¹ for 4 h with a calibrated syringe pump (model A-99; Razel Industries, Stamford, CT). A solution of potassium phosphate was infused at the same time (0.23 meq·kg⁻¹·h⁻¹) to prevent hypokalemia. Blood glucose concentrations were measured at the bedside every 5–10 min with a glucose analyzer (YSI 2700 Select, YSI, Yellow Springs, OH), and an infu-

Table 1. Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>IS</th>
<th>IR</th>
<th>NC</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (males/females)</td>
<td>25/14 (11)</td>
<td>12 (6/6)</td>
<td>13 (8/5)</td>
<td>11 (8/3)</td>
<td>14 (8/6)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>48 ± 2</td>
<td>45 ± 3</td>
<td>49 ± 2</td>
<td>47 ± 3</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76 ± 3</td>
<td>71 ± 4</td>
<td>78 ± 4</td>
<td>78 ± 4</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 2</td>
<td>172 ± 4</td>
<td>175 ± 3</td>
<td>178 ± 3</td>
<td>170 ± 3</td>
</tr>
<tr>
<td>BMI</td>
<td>25 ± 1</td>
<td>24 ± 0.8</td>
<td>25 ± 0.8</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.82 ± 0.28</td>
<td>5.2 ± 0.4</td>
<td>6.37 ± 1.33</td>
<td>4.37 ± 0.16</td>
<td>6.96 ± 0.13</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>4.09 ± 0.23</td>
<td>3.58 ± 0.12</td>
<td>4.56 ± 1.15</td>
<td>2.99 ± 0.13</td>
<td>4.94 ± 0.60</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.37 ± 0.16</td>
<td>1.43 ± 0.14</td>
<td>1.33 ± 0.1</td>
<td>1.11 ± 0.10</td>
<td>1.58 ± 0.08</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.52 ± 0.14</td>
<td>1.34 ± 0.20</td>
<td>1.71 ± 0.17</td>
<td>1.43 ± 0.23</td>
<td>1.60 ± 0.17</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. IS, insulin sensitive; IR, insulin resistant; NC, normal cholesterol; HC, high cholesterol; BMI, body mass index.
sion of 20% dextrose was adjusted to maintain the blood glucose concentration at the fasting level. Blood samples were also collected every 20–30 min for determination of plasma insulin concentrations (IMX assay, Abbott Laboratories, North Chicago, IL). The steady-state period of the clamp was defined as a 60-min or longer period (at least 1 h after the beginning of insulin infusion) during which the coefficient of variations for blood glucose, plasma insulin, and glucose infusion rate were <5%. The glucose clamp-derived index of insulin sensitivity (SI Clamp) was defined as $M/(G \times \Delta I)$, where $M$ is the steady-state blood glucose concentration (mg/dl), $G$ is the steady-state blood glucose concentration (mg/min), $I$ is the difference between basal and steady-state plasma insulin concentrations (μU/ml).

**Statistical Analysis**

All group data are reported as means ± SE. Group differences were analyzed by Student’s t-test. Within-group comparisons were performed with paired t-test. Correlations between variables were calculated with Pearson’s coefficient. Univariate and multivariate analyses of associations were assessed with standard regression techniques. All calculated $P$ values are two-tailed. The primary hypothesis of this study was that hyperinsulinemia induces a reduction in the magnitude of FMD, and its significance was tested at $\alpha = 0.05$. All other comparisons were regarded as exploratory, and, as such, no adjustments to $\alpha$ were made for these. Statistical analyses were performed with SigmaStat 2.03 (SPSS).

**RESULTS**

**Effects of Hyperinsulinemia on Brachial Artery Reactivity**

Steady-state hyperinsulinemic conditions were achieved ~2 h after the initiation of the euglycemic glucose clamp and were maintained for at least 60 min. Mean steady-state insulin levels were 118 μU/ml, ~12-fold higher than the mean basal fasting insulin value of 9 μU/ml. These levels are within the physiological range observed in a postprandial state and ensured complete inhibition of hepatic glucose production under clamp conditions.

Resting brachial artery diameter during hyperinsulinemia was similar to baseline (4.8 ± 0.02 and 4.5 ± 0.02 mm, respectively; $P = 0.22$). Peak hyperemic flow after cuff deflation did not change during hyperinsulinemia (994 ± 82 ml/min during clamp vs. 1,000 ± 77 ml/min at baseline; $P = 0.95$). FMD was significantly lower during hyperinsulinemia compared with baseline (2.3 ± 0.6% during insulin infusion vs. 6 ± 0.6% at baseline; $P < 0.001$). Similarly, NMD was decreased during clamp compared with baseline (10 ± 0.9% vs. 13 ± 1%; $P = 0.04$) (Fig. 1). To estimate a possible contribution of the increase in resting diameter between baseline and hyperinsulinemia to the decrease in FMD and NMD, a linear regression model using the difference between hyperinsulinemic and baseline diameter as independent variable was applied. Clamp FMD could not be predicted by the difference in diameter ($r = 0.13; P = 0.657$), whereas clamp NMD was significantly predicted by diameter increase ($r = 0.619; P < 0.001$). To rule out a potential contribution of the decrease in NMD during hyperinsulinemia to the decrease in FMD, a linear regression analysis was performed with the difference between hyperinsulinemic and baseline NMD as independent variable. In this model, clamp FMD could not be predicted by the difference in NMD ($r = 0.107; P = 0.611$). In addition, a
multiple linear regression model using the difference between hyperinsulinemic and baseline diameter and the difference between hyperinsulinemic and baseline NMD as independent variables was also applied. Similar to the previous analyses, FMD could not be predicted by differences in baseline diameter ($P = 0.848$) or in NMD ($P = 0.770$).

Effects of Insulin Sensitivity and Lipid Profile on Baseline Brachial Artery Reactivity

In the whole group, the mean value of SI Clamp was $9.2 \pm 1 \times 10^{-4} \text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \mu \text{U}^{-1} \cdot \text{ml}$. A significant direct correlation was found between baseline FMD and SI Clamp, whereas total cholesterol plasma levels showed a significant inverse correlation with FMD (Fig. 2). Similar to total cholesterol, LDL cholesterol was inversely correlated with FMD ($r = -0.365; P = 0.07$) or triglyceride ($r = 0.09; P = 0.66$) plasma values.

Baseline insulin levels were significantly higher in insulin-resistant compared with insulin-sensitive subjects and in the high-cholesterol compared with normal-cholesterol subgroups (Table 2). The mean values of SI Clamp were $13.1 \pm 1.2$ and $5.2 \pm 1.3$ in the insulin-sensitive and insulin-resistant subjects, respectively ($P = 0.0001$), and $12.3 \pm 1.7$ and $7 \pm 1$ in the normal-cholesterol and high-cholesterol participants, respectively ($P = 0.02$).

Interaction of Effects of Hyperinsulinemia and Insulin Sensitivity on Brachial Artery Reactivity

To determine whether insulin differentially affects FMD depending on insulin sensitivity, we compared the effects of hyperinsulinemia on endothelium-dependent dilation between insulin-sensitive and insulin-resistant subjects. During the resting study, baseline diameter was similar in the insulin-resistant compared with the insulin-sensitive subgroup ($4.7 \pm 0.03 \text{mm}$ vs. $4.2 \pm 0.02 \text{mm}; P = 0.20$). Peak hyperemic flow was comparable between the insulin-resistant and insulin-sensitive subgroups ($1,073 \pm 128$ and $984 \pm 116 \text{ml/min}$, respectively; $P = 0.60$). Resting FMD was significantly lower in the insulin-resistant compared with the insulin-sensitive subjects ($4.2 \pm 0.9\%$ vs. $7.4 \pm 0.8\%; P = 0.014$; Fig. 3A), whereas resting NMD was not different between the two groups ($13.9 \pm 1.7\%$ vs. $11.8 \pm 1.5\%; P = 0.37$; Fig. 3B). To determine a possible contribution of baseline diameter and NMD to FMD, a multiple linear regression model using baseline diameter, NMD, and insulin status as independent variables was applied. In this model, baseline diameter and NMD were not significant predictors of FMD ($P = 0.07$ and $P = 0.34$, respectively).

During hyperinsulinemia, a significant reduction of FMD from the resting values was observed in both the insulin-sensitive (from $7.4 \pm 0.8\%$ to $3.6 \pm 0.4\%; P < 0.001$) and the insulin-resistant (from $4.2 \pm 0.9\%$ to $1.22 \pm 1\%; P = 0.012$)
subgroup (Fig. 3A). NMD was similar to baseline in insulin-sensitive (13.9 ± 1.7% resting vs. 10.5 ± 0.8% during clamp; P = 0.08) and insulin-resistant (11.8 ± 1.5% resting vs. 10.2 ± 1.9% during clamp; P = 0.09) subjects (Fig. 3B). Hyperinsulinemic baseline diameter was similar in the insulin-resistant and insulin-sensitive participants (4.9 ± 0.03 vs. 4.5 ± 0.02 mm; P = 0.21) as well as peak hyperemic flow (1,071 ± 140 vs. 947 ± 101 ml/min; P = 0.48). Clamp FMD was significantly lower in the insulin-resistant subjects compared with the insulin-sensitive subjects (1.2 ± 1% vs. 3.6 ± 0.7%; P = 0.05), whereas NMD was similar between the two subgroups (insulin sensitive 10.5 ± 0.8% vs. insulin resistant 10.2 ± 1.9%; P = 0.89). To evaluate a potential contribution of baseline diameter and NMD to FMD, a multiple linear regression model using baseline diameter, NMD, and insulin status as independent variables was applied. In this model, FMD could not be significantly predicted by baseline diameter or NMD (P = 0.36, and P = 0.30, respectively). The reduction in FMD during hyperinsulinemia was not different between the insulin-sensitive and insulin-resistant subjects (−3.8 ± 0.7% vs. −3 ± 1%; P = 0.53).

Interaction of Effects of Hyperinsulinemia and Cholesterol Levels on Brachial Artery Reactivity

To establish whether cholesterol levels modulate the action of insulin on brachial artery reactivity, we compared the effects of hyperinsulinemia on endothelium-dependent dilation between the normal-cholesterol and high-cholesterol subjects. During the resting study, baseline diameter was similar in the normal-cholesterol and high-cholesterol subjects (4.6 ± 0.02 vs. 4.4 ± 0.03 mm; P = 0.71). Peak hyperemic flow was comparable between the two groups (1,048 ± 120 vs. 963 ± 120 ml/min, normal cholesterol and high cholesterol, respectively; P = 0.59). As previously reported, high-cholesterol individuals showed lower values of FMD compared with normal-cholesterol subjects (4.4 ± 0.7% vs. 8 ± 0.7%; P = 0.002; Fig. 4A), whereas NMD was similar between the two subgroups (12 ± 1.3% vs. 13.7 ± 1.6%; P = 0.41; Fig. 4B). To evaluate a possible contribution of baseline diameter and NMD to FMD, a multiple linear regression model using baseline diameter, NMD, and insulin status as independent variables was applied. In this model, baseline diameter and NMD were not significant predictors of FMD (P = 0.10 and P = 0.45, respectively).

During hyperinsulinemia, a significant reduction of FMD was observed in both the normal-cholesterol (from 8 ± 0.7% to 3.9 ± 0.4%; P < 0.001) and high-cholesterol (from 4.4 ± 0.7% to 1.1 ± 0.8%; P = 0.01) subjects (Fig. 4A), whereas NMD was similar at baseline and during clamp [normal cholesterol 12.1 ± 1.3% vs. 9.7 ± 1% (P = 0.17), high cholesterol 13.7 ± 1.6% vs. 10.3 ± 1.5% (P = 0.12)]. Clamp baseline diameter was similar in normal-cholesterol and high-cholesterol subjects (4.8 ± 0.02 and 4.8 ± 0.03 mm, respectively; P = 0.84) as well as peak hyperemic flow (1.146 ± 154 and 875 ± 69 ml/min, respectively; P = 0.13). FMD was significantly lower in the high-cholesterol subgroup compared with the normal-cholesterol subgroup (1.1 ± 0.9% vs. 3.9 ± 0.4%; P = 0.006; Fig. 4A), whereas NMD was similar between the two groups (9.7 ± 1% vs. 10.3 ± 1.7%, normal cholesterol and high cholesterol, respectively; P = 0.87; Fig. 4B). To determine a potential contribution of baseline diameter and NMD to FMD, a multiple linear regression model using baseline diameter, NMD, and insulin status as independent variables was applied. In this model, FMD could not be predicted by baseline diameter or NMD (P = 0.77 and P = 0.12, respectively). The reduction in FMD during hyperinsulinemia was similar between the normal-cholesterol and high-cholesterol subgroups (−4.1 ± 0.8% vs. −3.3 ± 1; P = 0.55). To determine whether in the normal- and high-cholesterol subgroups the changes in FMD were related to differences in insulin sensitivity, we performed specific correlation analyses. In the whole population, the variation in FMD induced by hyperinsulinemia (∆FMD) did not correlate with SIClamp (r = 0.31; P = 0.162). Similarly, no significant correlations between ∆FMD and SIClamp were found in the normal-cholesterol (r = −0.03; P = 0.94) and high-cholesterol (r = 0.43; P = 0.14) subgroups and in the insulin-sensitive (r = 0.32; P = 0.33) and insulin-resistant (r = 0.57; P = 0.08) subgroups.

Effects of Time on Brachial Artery Reactivity

In the nine subjects who participated in the additional brachial reactivity studies, resting brachial artery diameter was similar between the first and the second study (4.4 ± 0.02 and 4.4 ± 0.02 mm, respectively; P = 0.9). FMD was not significantly affected by the time elapsed between the two studies (6.8 ± 0.7% and 7.1 ± 0.7% in baseline and repeat studies, respectively; P = 0.79).

Fig. 4. Comparison of flow-mediated dilation (A) and nitroglycerin-mediated dilation (B) at baseline (filled bars) and during hyperinsulinemic clamp (hatched bars) between the normal-cholesterol and high-cholesterol subgroups. The P values represent the results of unpaired Student’s t-test. Values represent means ± SE.
DISCUSSION

The main new finding of the present investigation is that acute, systemic hyperinsulinemia impairs FMD in nondiabetic, normotensive subjects with a spectrum of insulin sensitivity and lipid profile. The reduction in NMD might suggest that hyperinsulinemia also impairs endothelium-independent dilation. However, the changes observed in NMD during hyperinsulinemia correlated with the concomitant increase in baseline artery diameter also induced by hyperinsulinemia, suggesting that these changes in NMD are likely not related to impaired endothelium-independent vasodilation. Conversely, the decreased FMD during clamp was not predicted by the changes observed in baseline artery diameter, indicating a predominant effect of hyperinsulinemia on endothelial function. Thus the discrepancy between FMD and NMD in their correlations with baseline brachial artery diameter during clamp demonstrates that hyperinsulinemia preferentially impaired endothelium-dependent vasodilation, a concept further supported by the significantly greater reduction in FMD than in NMD induced by hyperinsulinemia (65% vs. 18%; \( P = 0.003 \)).

A potential limitation of the brachial artery reactivity testing is the use of a fixed dose of nitroglycerin. Because no dose response to nitroglycerin is determined by the standard technique, we cannot rule out the possibility that the use of smaller doses of nitroglycerin might have detected the development of reduced vascular smooth muscle sensitivity to NO.

Although our results may appear to differ from those of previous studies indicating that insulin may exert favorable actions on the vascular endothelium (17, 29) and may induce NO-dependent dilation of the skeletal muscle microcirculation (5, 24), our findings confirm and expand the recent report by Arcaro et al. (2), who showed a marked decrease in FMD during mild hyperinsulinemia, mimicking the fasting hyperinsulinemia of insulin-resistant states, in young, healthy subjects. In particular, we demonstrate that a level of plasma insulin similar to the physiological hyperinsulinemia present in the postprandial state may induce endothelial dysfunction of conduit vessels independent of the metabolic background, suggesting additive effects of independent risk factors on vascular function. Multiple mechanisms may explain the observed actions of insulin on FMD. In our laboratory, we have shown that hyperinsulinemia may affect vascular function through the activation of systemic mechanisms (5) and by increasing endothelin activity (5a). Furthermore, acute insulin infusion may stimulate sympathetic nerve activity, with significant consequences on cardiovascular homeostasis (23). These different effectors may ultimately impair endothelium-dependent dilation by reducing vascular NO activity, possibly by increasing oxidative stress, as suggested by the results from Arcaro et al. (2), who reversed insulin-induced endothelial dysfunction by administering vitamin C. However, the results of our study do not allow clarification of the pathophysiological and biochemical pathways responsible for the effects observed during clamp, and further studies are needed to investigate the mechanisms of impairment of endothelial function by hyperinsulinemia.

To our knowledge, our study is the first to report a correlation between FMD and insulin sensitivity measured by the euglycemic clamp technique. Previous data have shown a significant relationship between FMD and the insulin area under curve (AUC) after glucose load in nondiabetic subjects (4). However, the insulin AUC is only an indirect assessment of insulin sensitivity under dynamic conditions, whereas the euglycemic clamp, which measures the direct effects of insulin on glucose disposal under steady state, is considered the reference standard test (11). This observation indicates that, in conduit vessels, vascular homeostasis is significantly affected by the metabolic milieu and in particular by insulin sensitivity. In line with previous reports, in our whole population, FMD was inversely correlated with total cholesterol (6) and LDL cholesterol (19) plasma levels.

An interesting finding of our study is the evidence that subjects with high cholesterol levels were significantly less insulin sensitive than those with normal cholesterol values. Of note, despite the presence of insulin resistance, the high-cholesterol subjects showed a lipid profile characteristic of a hypercholesterolemic population at large (increased total cholesterol and LDL with normal HDL and triglyceride levels) and not the lipid abnormalities typical of the insulin resistance syndrome (normal or slightly elevated LDL, increased triglycerides, and decreased HDL). Thus we believe that our high-cholesterol individuals were representative of typical hypercholesterolemic subjects. Because the high-cholesterol and normal-cholesterol subgroups were well matched for the clinical characteristics known to affect insulin sensitivity (age, body mass index, triglyceride levels, blood pressure, smoking status), it seems reasonable to conclude that the high total cholesterol levels, or some other unrecognized factor(s), underlie the insulin resistance observed in these subjects.

Our findings may have relevant pathophysiological and clinical implications. In metabolic syndrome X, hyperinsulinemia secondary to insulin resistance is usually associated with major cardiovascular risk factors such as abnormal lipid profile and hypertension (12). Despite the epidemiological evidence indicating that hyperinsulinemia is a predictor of vascular disease in nondiabetic men (9), an independent role of insulin in the pathogenesis of endothelial dysfunction and atherosclerosis in patients with metabolic syndrome X has not been clearly established. The recent in vitro data from Montagnani et al. (22) suggest that, in endothelial cells, insulin may exert proatherosclerotic actions on a biochemical background of insulin resistance, which determines an imbalance between the phosphatidylinositol 3-kinase and the mitogen-activated protein kinase signaling pathways. Our results indicate that, in vivo, acute hyperinsulinemia impairs FMD independent of insulin sensitivity, suggesting that physiological levels of insulin per se may cause endothelial dysfunction in conduit vessels and independently contribute to the increased vascular risk of insulin-resistant states. Together, these observations shed new light on the potential involvement of insulin in atherogenesis and challenge the current paradigm that, in insulin-resistant states, hyperinsulinemia plays only an indirect role (12) by affecting lipoprotein metabolism (15), blood pressure (8, 18), and fibrinolysis (4). In addition, our data suggest a potential role of hyperinsulinemia in the negative effects of a meal on FMD (4). However, it is likely that during the postprandial phase of a mixed meal, multiple factors, including hyperglycemia and hypertriglyceridemia, may affect endothelial function, as shown by previous investigations (26). From a clinical viewpoint, our data indicate that, in patients with metabolic syndrome X and other insulin-resistant states,
including the early stages of Type 2 diabetes mellitus, primary treatment should not simply correct the secondary metabolic abnormalities but mainly improve insulin sensitivity and the accompanying hyperinsulinemia. In this respect, the thiazolidinediones, a relatively new class of insulin-sensitizing drugs, seem to hold great promise, because they have been shown to ameliorate endothelial function in patients with diabetes mellitus and may have similar beneficial effects in patients without diabetes (27).

In summary, in normotensive, normoglycemic subjects, acute hyperinsulinemia similar to the hyperinsulinemia present in the postprandial state impairs conduit vessel endothelial function independent of insulin sensitivity and lipid profile. These data indicate that physiological insulin levels may trigger endothelial dysfunction and exert negative additive effects in the presence of metabolic abnormalities and support the epidemiological evidence that hyperinsulinemia is an independent risk factor for cardiovascular disease.

REFERENCES


