Insulin-Mediated Venodilation Is Impaired in Patients With High Cholesterol

Bong Hee Sung, Marilou Ching, Joseph Izzo, Jr, Paresh Dandona, Michael F. Wilson

Abstract—Recently we have reported that insulin attenuates norepinephrine (NE)-induced vasoconstriction via a cyclic GMP–NO synthase pathway. Because hypercholesterolemia has been associated with abnormal endothelial function, we investigated whether insulin-mediated vasodilation is impaired in hypercholesterolemia. To assess vasoreactivity, NE (12.5, 25, 50, and 100 ng/min), NE (100 ng/min) combined with insulin (8, 16, 24, and 32 μU/min), and NE (100 ng/min) combined with sodium nitroprusside (0.01, 0.1, 1, 10, and 100 ng/min) were infused into dorsal hand veins. Changes in venous diameter were measured by ultrasonography, using a 7.5-MHz transducer. Twenty-two healthy, normotensive hypercholesterolemic subjects (HC; mean total cholesterol 6.93 mmol/L, HDL 1.45 mmol/L, LDL 4.81 mmol/L) and 18 age-matched normal control subjects (NC; mean total cholesterol 4.81 mmol/L, HDL 1.16 mmol/L, LDL 3.18 mmol/L) were studied. All HC had normal glucose tolerance test results. Baseline vein diameters were similar between groups, and the vasoconstrictor response to NE was not significantly different between HC and NC. Insulin significantly attenuated NE-induced vasoconstriction in NC but not in HC (P<0.01). Both groups were able to venodilate with sodium nitroprusside. To investigate the effects of cholesterol reduction on vascular reactivity, venoreactivity studies were repeated in 12 HC after treatment with 20 to 40 mg/d lovastatin for 6 weeks. There were no significant venoreactivity changes with the treatment. Plasma LDL cholesterol concentration was inversely correlated to venodilator effect of insulin (r = −0.42, P < 0.02). In conclusion, insulin-mediated vasodilation is impaired in patients with high cholesterol. Absence of normal insulin-mediated but not sodium nitroprusside–induced venodilation in hypercholesterolemia suggests that insulin-mediated vasodilation is endothelium dependent. (Hypertension. 1998;31:1266-1271.)

Key Words: hypercholesterolemia • vasoreactivity • insulin • norepinephrine • endothelium

The importance of endothelial dysfunction in the regulation of coronary and systemic arterial vasomotion has been well established.1-6 Hypercholesterolemia, one of the principal risk factors for coronary artery disease, has been linked to impaired arterial endothelial function via impairment in the NO–cyclic GMP pathway.7,8 Recently it has been shown in our laboratory that hypercholesterolemia causes an exaggerated BP response to mental arithmetic test and that this enhanced BP response can be normalized by hepatic HMG CoA reductase inhibitors in proportion to the degree of lowering of the serum cholesterol.9 The role of endothelial dysfunction in this phenomenon is of considerable interest.

Assessment of endothelial function has been generally carried out by measuring flow-dependent dilation to acetylcholine in the human coronary or brachial arteries10,11; however, veins are easily accessible and share many structural and functional similarities with arteries. Recently we have developed a technique that provides direct visualization of veins using cross-sectional and M-mode ultrasonography. It has the advantage of examining direct local effects of vasoactive substances in amounts not causing systemic effects. Using this technique, we have reported that insulin induced a dose-dependent inhibition of the vasoconstrictor effect of NE and that methylene blue, a known inhibitor of NO synthase and guanylate cyclase, inhibited the vasodilator effect of insulin.12

If insulin exerts its effect by activating either NO synthase or guanylate cyclase, it would increase levels of cGMP. Thus, the vasodilator effects of insulin may be cGMP dependent. However, our data do not demonstrate whether the effect of insulin on guanylate cyclase is a direct one or whether it is mediated by the generation of NO. Two other groups of investigators13,14 have recently demonstrated that the vasodilator effect of insulin on the arterial side is NO mediated, which further strengthens our hypothesis.

Because hypercholesterolemia is associated with endothelial dysfunction, we examined whether insulin-mediated venodilation is impaired in the hypercholesterolemic population. We chose dorsal hand veins, substituting infused NE as a surrogate for sympathetic nervous activity. We also studied vasoreactivity to sodium nitroprusside, an endothelium-independent vasodilator, to investigate the underlying mechanism of the defect in insulin-mediated venodilation. In addition, we examined whether a reduction in cholesterol with HMG CoA

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Selected Abbreviations and Acronyms

BP = blood pressure
HC = hypercholesterolemic subjects
HMG CoA = hydroxymethylglutaryl coenzyme A
NC = normal-cholesterol subjects
NE = norepinephrine
NO = nitric oxide

reductase inhibitor improves insulin-mediated venodilation in hypercholesterolemia.

Methods

Study Population

Healthy normotensive volunteers were recruited through advertisements in the local community. Respondents were initially interviewed by telephone, and those who met the inclusion criteria underwent screening and physical examination. Smokers, hypertensives, diabetics, subjects with known cardiovascular disease, and those taking medications were excluded. Subjects with homozygous familial hypercholesterolemia were not included in the study. Lipid profile and glucose were measured after a 12-hour fast. Subjects with levels of LDL cholesterol >4.14 mmol/L and triglycerides <3.35 mmol/L were classified as HC, and with normal total cholesterol (<5.17 mmol/L) and triglycerides (<1.70 mmol/L) were classified as NC.

The final study population consisted of 22 HC and 18 NC. Glucose tolerance tests were performed in HC to determine possible insulin resistance. Glucose and insulin levels were measured at 30 minutes, 1 hour, and 2 hours after ingestion of 75 g glucose. Table 1 compares demographic and baseline hemodynamic variables between HC and NC groups.

Study Protocol

The study protocol was approved by the Human Ethics Committee of the Millard Fillmore Hospital, and informed consent was obtained from each volunteer after the procedures were explained. All study subjects were requested to refrain from alcohol and caffeine for at least 12 hours before the experiment. The experiments were conducted in a quiet room maintained at 26°C to 28°C. The experiments were conducted in a quiet room maintained at 26°C to 28°C.

With the subject supine, one arm was placed on an inclined padded support at an angle of 30° from the horizontal to empty the superficial hand vein. A 23-gauge needle was inserted into the dorsal hand vein, and an intravenous infusion of normal saline was conducted in a quiet room maintained at 26°C to 28°C. There was a 10- to 15-minute washout period with normal saline between drugs. BP and heart rate were monitored in the contralateral arm by an automated BP monitor (Colin Press-Mate, Colin Medical Instruments Corp). Readings were obtained before, during, and after the experiment to assess systemic hemodynamic changes.

TABLE 1. Group Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal Cholesterol (n=18)</th>
<th>Hypercholesterolemia (n=22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53±13</td>
<td>55±10</td>
<td>NS</td>
</tr>
<tr>
<td>Gender distribution, M/F</td>
<td>8/10</td>
<td>10/12</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24±3</td>
<td>27±4</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.81±.46</td>
<td>6.93±.7</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.18±.52</td>
<td>4.81±.52</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.16±.32</td>
<td>1.45±.31</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>64±5</td>
<td>68±7</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>118±9</td>
<td>122±11</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>70±5</td>
<td>74±8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Two sets of baseline hand vein diameters, one with the cuff uninflated and another with the cuff inflated at 40 mm Hg, were measured by ultrasonography. NE (12.5, 25, 50, and 100 ng/min), NE (100 ng/min) coupled with insulin (8, 16, 24, and 32 μU/min), and NE (100 ng/min) coupled with sodium nitroprusside (0.01, 0.1, 1, 10, and 100 ng/min) were infused for 5 minutes at each dose. Vein diameter was measured during the last 2 minutes of infusion. There was a 10- to 15-minute washout period with normal saline between drugs. BP and heart rate were monitored in the contralateral arm by an automated BP monitor (Colin Press-Mate, Colin Medical Instruments Corp). Readings were obtained before, during, and after the experiment to assess systemic hemodynamic changes.

Measurement of Venous Reactivity by Ultrasonography

Dorsal hand vein diameter was measured by ultrasonography as described previously. An Acuson TM 128xp ultrasound machine with a 7.5-MHz linear array transducer was used. The transducer was held stationary with a stand specially designed for this purpose. An interface of gel was applied between the skin and transducer to improve coupling. Measurements were made 1 cm distal to the tip of the cannula, showing two-dimensional images of the vein’s cross-section. An M-mode image was generated from which vein diameter was measured. In our laboratory, this device has been shown to have a sensitivity of 0.1 mm and an interobserver and intrasubject coefficient of variation of <5%.

Treatment

To examine whether lowering cholesterol would modify venous reactivity to NE and insulin, we treated 12 HC with 20 to 40 mg/d lovastatin for 6 weeks. A fasting lipid profile was performed and a venous reactivity study was repeated after the treatment.

Statistical Analysis

All data are expressed as mean±SD. The baseline difference between NC and HC groups in resting hemodynamics and demographic variables was evaluated by unpaired t test. Effects of drug treatment with lovastatin on lipid profiles and vasoreactivity were analyzed by one-way ANOVA. The changes in venous diameter with NE, insulin, and sodium nitroprusside between groups were examined by a two-way ANOVA with repeated measures using Systat software. Multiple regression analysis was performed to examine the relationship between LDL levels and vasodilator effects of insulin. A value of P<0.05 was considered to be significant.

Results

As summarized in Table 1, both groups had similar age and gender distribution. The HC group was heavier and taller than the NC group, but body mass index was not significantly different. As expected, HC had significantly higher total cholesterol levels than NC (6.93±0.7 versus 4.81±0.46 mmol/L, P<0.001). Although LDL cholesterol was significantly higher in HC than NC (4.81±0.52 versus 3.18±0.46 mmol/L, P<0.001), HDL cholesterol was not significantly different between the groups. Baseline heart rate (68±7 versus 64±5 bpm, NS), systolic BP (122±11 versus 118±9 mm Hg, NS), and diastolic BP (74±8 versus 70±5 mm Hg, NS) were not significantly different between HC and NC groups.

All study subjects had normal fasting glucose (mean, 4.9±0.6 mmol/L) and insulin levels (mean, 8±3.8 μU/mL). To rule out insulin resistance in the HC group, glucose tolerance tests were performed in all HC. Their mean glucose levels for 30, 60, 90, and 120 minutes after glucose challenge were 6.7±1.4, 7.3±1.8, 5.7±1.2, and 5.2±1.7 mmol/L; mean insulin levels were 46±12, 65±14, 50±11, and 28±7
μU/mL. These results confirm that the HC group did not have glucose intolerance.

**Effects of NE and Insulin on Venous Diameter**

The mean venous diameter at baseline was similar between NC and HC (1.6±0.5 and 1.54±0.45 mm, respectively; $P=\text{NS}$). On cuff inflation, mean resting venous diameter increased to 2.9±0.8 mm for NC and 3.03±0.6 mm for HC. NE caused a dose-dependent decrease in venous diameter for both groups (significant drug effect, $P<0.001$). Although ranges of vеноconstriction to NE were greater in HC than NC (19% to 58% versus 12% to 42%, $P=0.09$), these results were not statistically significant. The highest dose of NE returned venous diameters similar to those before cuff inflation.

There was a significant difference in venodilatory response to insulin between HC and NC groups. Infusion of insulin in increasing doses of 8, 16, 24, and 32 μU/min increased venous diameter to 2.17, 2.28, 2.64, and 2.84 mm ($P<0.01$) in NC, respectively, whereas there was an insignificant increase in venous diameter during insulin infusion in HC (1.67, 1.74, 1.71, and 1.79 mm, respectively; $P=\text{NS}$). There was a significant drug-by-group interaction ($P<0.01$). Thus, NE-mediated vеноconstriction was significantly attenuated by insulin in NC, but this effect was blunted in hypercholesterolemia. The comparison of venous diameter changes to NE and insulin between NC and HC is illustrated in Figure 1.

**Comparison of Vasodilation Mediated by Insulin and Sodium Nitroprusside**

In contrast to the response to insulin, both groups were able to venodilate with sodium nitroprusside, and there was no significant group difference in venodilation to sodium nitroprusside between HC and NC. Vasodilator responses to sodium nitroprusside between HC and NC are compared in Figure 2. The HC group was able to venodilate to sodium nitroprusside, an endothelium-independent vasodilator, but not to insulin.

**Effects of Cholesterol Lowering on Vasoreactivity**

The last question we addressed in this study was whether cholesterol lowering reverses abnormal venoreactivity in HC. To answer this question, 12 hypercholesterolemic subjects...
were treated with 20 to 40 mg/d lovastatin for 6 weeks, and the venoreactivity experiment was repeated. There was significant reduction in total cholesterol (7.24 to 5.43 mmol/L, \(P<0.001\)) and LDL cholesterol (4.78 to 3.49 mmol/L, \(P<0.001\)). Changes in triglycerides (176 to 155 mg/dL) and HDL cholesterol (1.34 to 1.5 mmol/L) were not significant. Table 2 summarizes change in lipid profile by treatment.

Table 2. Lipid Profile Changes After Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Treatment</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.24±.59</td>
<td>5.43±.54</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>4.78±.51</td>
<td>3.49±.46</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>1.34±12</td>
<td>1.5±12</td>
<td>0.063</td>
</tr>
<tr>
<td>Triglycerides, mL/dL</td>
<td>176±24</td>
<td>155±22</td>
<td>0.061</td>
</tr>
<tr>
<td>Ratio LDL/HDL</td>
<td>3.6</td>
<td>2.3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Figure 3 illustrates the effects of cholesterol lowering on venous reactivity to NE and insulin among the three groups: NC, HC, and treated HC. Venous diameter change to NE and insulin of the treated group was between NC and untreated HC. Although there was a small increase in venous diameter to insulin with the treatment (average increase of 0.33±0.54 mm to 32 \(\mu\)U/min insulin), these changes were not statistically significant. Reduction in LDL cholesterol ranged from 0.75 to 1.55 mmol/L, with a mean of 1.29 mmol/L. With this narrow range of reduction, there was no significant relationship between the magnitude of reduction in LDL cholesterol and change in venous diameter to insulin with treatment \((r=0.25, \(P=NS\)).

Furthermore, we examined the relationship between plasma cholesterol levels and vasodilator effects of insulin; there was a significant inverse relationship between plasma LDL concentration and venous diameter changes with insulin \((r=-0.42, \(P<0.02\)). Figure 4 demonstrates this relationship.

Local infusion of NE alone or combined with insulin or sodium nitroprusside at the dose ranges used for this study did not significantly change heart rate or BP.

**Discussion**

The present study demonstrates that insulin-mediated venodilation is attenuated in HC compared with NC. The nitrate donor sodium nitroprusside caused equal degrees of venodilation in both groups. This finding suggests that insulin-mediated venodilation is dependent on endothelial NO generation. A vasodilatory effect of insulin has been reported by several investigators, although underlying mechanisms have not been agreed on. Recent work by Kahn et al\(^{15}\) has demonstrated that insulin attenuates vascular smooth muscle calcium influx by means of both voltage-operated channels and receptor-mediated mechanisms. Creager et al\(^{16}\) have reported that insulin induces \(\beta\)-adrenergic–mediated vasodilation, and Lembo and colleagues\(^{17}\) suggested that insulin blunts sympathetic vasoconstriction via an \(\alpha\_2\)-adrenergic pathway. We have previously reported that insulin attenuates NE-induced vasoconstriction via the cGMP pathway.\(^{12}\) The present study of blunted insulin-mediated vasodilation in hypercholesterolemia further strengthens the hypothesis that insulin-mediated vasodilation is endothelium dependent and extends the observations to veins, which are more easily studied than arteries.

Another noteworthy finding of the present study is that our hypercholesterolemic subjects had normal glucose and insulin responses to a glucose challenge but showed abnormal vascular responses to insulin. Our results clearly demonstrate that impaired vasodilatory effects of insulin are not always accompanied by glucose intolerance. This means that selective resistance to insulin action on vasculature may occur in pathophysiological conditions that cause abnormal endothelial function. Thus, altered vasoreactivity to insulin may occur in clinical populations with or without metabolic insulin resistance.

The present study used a preconstricted venous model to examine vasodilator effects of insulin. Previously, we used the venous model and found that insulin alone did not have a vasodilator effect on relaxed vein.\(^{12}\) Our experience supports earlier observations in the literature\(^{19}\) that in subjects who are supine and comfortably relaxed, the forearm veins are usually fully dilated, in part because of low sympathetic output. In

![Figure 3. Venous reactivity to NE and insulin after the treatment with HMG CoA reductase inhibitors for 6 weeks compared among NC, HC, and treated HC.](http://hyper.ahajournals.org/)}
these circumstances, no response is seen if a dilator substance is given. Thus, there must be some vasoconstriction to demonstrate any vasodilator effect. In our studies, the cuff inflation, which was necessary to minimize measurement variability, may cause “artificial vasodilation.” Consequently, it was necessary to preconstrict vessels with NE to demonstrate vasodilator effects of insulin.

Assessment of endothelial function has been generally carried out by measuring flow-dependent dilation to acetylcholine in the human coronary or brachial arteries. Our ultrasonographic method provides the cross-sectional image of the vein and permits direct measurement of absolute vein diameter while avoiding systemic effects of infused drugs. Several animal and human studies have reported that hypercholesterolemia is associated with impaired endothelial function and abnormal forearm vasoreactivity. Our finding in the dorsal hand vein indicates that the potential effect of insulin on vascular tone or vascular responses to physiological vasoconstrictors is not limited to the arterial system and may be important in the study of phenomena affecting venous return to the heart.

Although veins are capacitance vessels that have less smooth muscle than arteries, they are easily accessible and share many structural and functional similarities with arteries. The role of arterioles in the regulation of BP has long been the subject of intense investigation. In contrast, the role of veins has received less attention. Because veins have sympathetic innervation, there is physiological variation in venomotor tone. At least two thirds of the circulating blood volume is normally contained in the venous system, so changes in venous capacitance will directly affect cardiac filling (“preload”) and may have effects on regional blood flow patterns. Dorsal hand veins have been widely used for pharmacological studies by measuring changes in venous diameter as reflected by a linear variable differential transformer. Venoconstrictor responses to NE measured by ultrasonography in this study were comparable to those found in other studies. In this study, NE-induced constriction was greater in HC than in NC, although the data did not reach statistical significance.

Previously we have reported that patients with high cholesterol show exaggerated BP response to mental stress. The dorsal hand vein responses to NE and insulin in this population may explain the greater BP response to stress with hypercholesterolemia. These findings suggest that venous reactivity to vasoactive substances may reflect systemic BP regulation. Our venous model was able to identify abnormal insulin-mediated vasodilation in subjects with high cholesterol. Thus, an impaired endothelium-dependent vasodilation may be a mechanism by which hypercholesterolemia contributes to increased BP response during stress.

Further, treatment of hypercholesterolemia with HMG CoA reductase inhibitors reduced BP response to mental stress. Significant improvement of endothelium-dependent vasodilation has been reported with cholesterol reduction treatments for 6 months or longer. Our high-cholesterol group was treated with HMG CoA reductase inhibitor for 6 weeks, and it may take longer than 6 weeks of cholesterol lowering to achieve optimal reversal of endothelial dysfunction. Nevertheless, there was a significant inverse relationship between plasma LDL concentration and venodilator effects of insulin. Therefore, reversing or normalizing endothelial dysfunction at an early stage with nonpharmacological and pharmacological interventions may prevent subsequent endothelial damage and may normalize exaggerated cardiovascular reactivity and reduce the risk of cardiovascular disease.

In summary, the present study documents impaired insulin-mediated vasodilation in patients with high cholesterol. Furthermore, our study demonstrates a significant inverse relationship between venodilator effect of insulin and plasma LDL cholesterol concentration. Our observation that blunted insulin-mediated vasodilation is not specific for metabolic insulin resistance and can occur before overt glucose intolerance is a significant finding and has potential clinical impli-
cations. Metabolic abnormalities are associated with hypertension and often cluster together. The interaction of these risk factors on vascular reactivity may be an important component for overall regulation of BP. The demonstration of impaired vasodilator effect of insulin in patients with high cholesterol may provide insight into the possible detrimental interaction of these risk factors.

Acknowledgments
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References