Insulin-Induced Decrease in Large Artery Stiffness Is Impaired in Uncomplicated Type 1 Diabetes Mellitus

Jukka Westerbacka, Antti Uosukainen, Sari Mäkimattiila, Anna Schlenzka, Hannele Yki-Järvinen

Abstract—Normal insulin action in vivo involves a decrease in stiffness of large arteries (a decrease in aortic pressure augmentation). We determined whether the ability of insulin to decrease arterial stiffness is altered in uncomplicated type 1 diabetes. Nine type 1 diabetic men (age 28±2 years, body mass index 24±1 kg/m²) and 9 matched normal men were studied under normoglycemic hyperinsulinemic (sequential 2-hour insulin infusions of 1 [step 1] and 2 [step 2] mU · kg⁻¹ · min⁻¹) conditions. Central aortic pressure waveforms were synthesized from those recorded in periphery with applanation tonometry on the radial artery and a validated reverse transfer function to construct the central aortic pressure wave every 30 minutes. This allowed the determination of aortic augmentation (the pressure difference between the first and the second systolic peaks) and the augmentation index (augmentation divided by pulse pressure), as the measure of stiffness of large arteries. Whole-body glucose uptake was 44% (step 1) and 37% (step 2) lower ($P<0.001$) in the diabetic patients than in the normal subjects. At baseline, before the insulin infusion, augmentation averaged 0±1 and 2±1 mm Hg (NS) and the augmentation index was −1.5±4.5% and 4.0±3.7% (NS) in the normal and diabetic subjects, respectively. After 1 hour of hyperinsulinemia, the augmentation index had decreased significantly ($P<0.01$) to −9.5±4.8% in the normal subjects but remained at 4.2±4.2% in the diabetic patients. A significant decrease was not observed in the diabetic patients until 150 minutes (−1.2±4.1%, $P<0.05$ versus baseline). Whole-body glucose uptake was significantly inversely correlated with the change in the augmentation index during step 1 ($r=−0.61$, $P<0.01$). Insulin resistance in type 1 diabetes involves a defect in the ability of insulin to decrease central aortic pressure. This defect could predispose these patients to premature stiffening of large arteries. (Hypertension. 2000;35:1043-1048.)

Key Words: arteries ■ aorta ■ blood flow ■ blood pressure ■ hemodynamics

Patients with type 1 diabetes are at a high risk of cardiovascular disease, which is inadequately explained by classic risk factors.1 Regarding hypertension, recent evidence from, for example, the Systolic Hypertension in the Elderly Program (SHEP) and Framingham Heart Study has suggested that the pulsatile component of the hemodynamic load of the heart is a better predictor of cardiovascular events than either systolic or diastolic blood pressure.2-3 In these trials, the pulsatile component was assessed from measurements of pulse pressure, a surrogate for arterial stiffening. With increasing stiffness, pulse wave velocity increases, causing an early return of pressure waves from reflectance sites. The early return of the pressure wave will increase or augment central systolic pressure and the afterload of the left ventricle and decrease diastolic pressure.4 The degree of augmentation of the central systolic pressure wave can be recorded noninvasively with applanation tonometry, a validated transfer function and pulse wave analysis.5

Type 1 diabetic patients have had stiffer large arteries in many6-11 although not all12,13 studies. Intensive insulin therapy has been shown to slow arterial stiffening in these patients.14 Insulin therapy is also known to enhance insulin sensitivity via effects on chronic hyperglycemia,15 the major cause of insulin resistance in these patients.16 We recently demonstrated that insulin decreases central pressure augmentation independent of any effects on blood flow or peripheral vascular resistance.17 These data raise the possibility that insulin resistance in type 1 diabetes involves a defect in insulin regulation of arterial stiffness, possibly as a consequence of hyperglycemia.1 In the present study, we determined whether the normal action of insulin to diminish central pressure augmentation independent of peripheral vascular resistance and blood flow is defective in type 1 diabetes.

Methods

Subjects
Nine type 1 diabetic men and 9 young normal subjects participated in the study. The physical and biochemical characteristics of the subjects are shown in Table 1. The normal subjects were healthy as judged on history and physical examination, ECG, and routine laboratory tests. They were not taking any medications. Their fasting plasma glucose and glycosylated hemoglobin $A_1c$ (HbA$1c$) concen-
TABLE 1. Physical Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Subjects (n=9)</th>
<th>Type 1 Diabetic Subjects (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>26±1</td>
<td>28±2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178±2</td>
<td>181±2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.3±0.7</td>
<td>23.9±1.0</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>13±1</td>
<td>15±2</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.3±0.1</td>
<td>7.4±0.8*</td>
</tr>
<tr>
<td>Fasting serum insulin, mU/L</td>
<td>3±1</td>
<td>8±1†</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>5.1±0.2</td>
<td>7.6±0.3†</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>4.3±0.3</td>
<td>4.4±0.2</td>
</tr>
<tr>
<td>Serum HDL-cholesterol, mmol/L</td>
<td>1.5±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Serum LDL-cholesterol, mmol/L</td>
<td>2.5±0.2</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>0.7±0.1</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>...</td>
<td>18±3</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. *P<0.05, †P<0.001.

The technique of pulse wave analysis was used to determine central aortic pressure and the augmentation index as previously described in detail.\(^5\)\(^7\) All measurements were made from the radial artery with planation tonometry with a Millar tonometer (SPC-301; Millar Instruments) twice at baseline and every 30 minutes during the insulin infusions. Data were collected directly into a desk top computer and processed with a SphygmoCor Blood Pressure Analysis System (BPAS-1; PWV Medical), which allows continuous on-line recording of the radial artery pressure waveform. The integral system software was used to calculate an average radial artery waveform and to generate the corresponding ascending aortic pressure waveform with a previously validated transfer factor.\(^20\)\(^21\) The aortic waveform was then subjected to further analysis for calculation of aortic augmentation, the augmentation index, central blood pressure, and ejection duration (duration of systolic period in milliseconds). The augmentation index was calculated by dividing augmentation with pulse pressure.\(^5\)\(^22\)

**Forearm Blood Flow, Peripheral Vascular Resistance, Glucose Extraction, and Forearm Glucose Uptake**

Forearm blood flow was measured every 30 minutes with venous occlusion plethysmography (model EC-4; Hokanson), a rapid cuff inflator (Rapid Cuff Inflator model E20; Hokanson), and computerized analysis of flow curves (MacLab/4e; AD Instruments), as previously described.\(^1\)\(^3\) Peripheral vascular resistance was calculated by dividing mean arterial pressure in the brachial artery by forearm blood flow. Glucose extraction was calculated from the glucose concentration difference between arterialized and deep venous blood (glucose arteriovenous difference). Forearm glucose uptake was calculated by multiplying glucose extraction by forearm blood flow.\(^1\)\(^8\) The data for hemodynamic and glucose uptake measurements during the normoglycemic part of the clamp (i.e., from the last hour of step 1) were used for data analysis.

**Other Measurements**

Fat free mass and the percentage of body fat were determined with bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model BIA-101A; RJL Systems). Serum free insulin was measured before and at 30-minute intervals during the insulin infusions with double antibody radioimmunoassay (Pharmacia Insulin RIA kit; Pharmacia) after precipitation with polyethylene glycol. Plasma glucose concentrations were measured in duplicate with the Beckman Glucose Analyzer II (Beckman Instruments). HbA₁c was measured by HPLC with a fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad).

**Statistical Analysis**

Analyses of group, time, and group×time effects between normal subjects and type 1 diabetic patients were made with ANOVA for repeated measures followed by Bonferroni’s test. Correlation analyses were performed with Spearman’s nonparametric correlation coefficient. The best fit to characterize the relationship between hemodynamic parameters over time was determined by comparing the goodness of fit of linear and multiple nonlinear equations with the use of Prism v. 2.01 (GraphPad Software Inc). The results are expressed as mean±SEM, and P<0.05 was considered statistically significant.

**Results**

**Glucose and Insulin Concentrations and Glucose Uptake**

Fasting plasma glucose and serum free insulin concentrations are given in Table 1. During the insulin infusions, serum free insulin concentrations averaged 61±3 (step 1, 30 to 120 minutes) and 132±5 (step 2, 120 to 240 minutes) mU/L in the normal subjects and 63±5 (step 1) and 139±9 (step 2) mU/L in the patients with type 1 diabetes (NS for normal subjects versus patients with type 1 diabetes). Plasma glucose averaged 5.2±0.1 (step 1) and 5.2±0.1 (step 2) mmol/L in the normal subjects and 5.3±0.1 (step 1) and 5.1±0.1 (step 2) mmol/L in the patients with type 1 diabetes (NS). Whole-body glucose uptake was 44% lower during step 1 (22.2±2.2 versus 39.4±3.3 μmol·kg⁻¹·min⁻¹) and 37% lower during step 2 (44.2±2.2 versus 70.0±5.0 μmol·kg⁻¹·min⁻¹) for patients with type 1 diabetes versus normal subjects, P<0.001.
pressure), decreased significantly at 60 minutes (−9.5±4.8%, P<0.01 versus baseline) (Figure 2). The augmentation index averaged −1.5±4.5% at baseline: −8.7±4.5% during step 1 (P<0.01 versus baseline) and −10.2±4.0% during step 2 (P<0.01 versus baseline, NS versus step 1) (Figure 2).

At baseline, before the insulin infusion, augmentation and the augmentation index were comparable between type 1 diabetic patients and normal subjects. In contrast to the normal subjects, however, augmentation did not decrease by insulin in the diabetic patients during the first hour. Mean augmentation averaged 1.6±1.3 mm Hg at baseline: 1.3±1.2 mm Hg during step 1 (NS versus baseline in the patients with type 1 diabetes) and 0.1±1.5 mm Hg during step 2 (NS versus baseline and step 1). The augmentation index averaged 3.7±3.8% at baseline: 3.5±4.0% during step 1 (NS versus baseline) and −0.9±4.5% during step 2 (NS versus baseline, P<0.05 versus step 1). The first significant decrease in the augmentation index occurred at 150 minutes (0.9±4.0%, P<0.05 versus baseline) in the patients with type 1 diabetes (Figure 2). The changes in the augmentation index were significantly different between the patients with type 1 diabetes and normal subjects from 30 until 90 minutes (Figure 2). The rate of whole-body glucose uptake was significantly inversely correlated with the change in the augmentation index by insulin during step 1 (r=−0.61, P<0.01, Figure 3). The concentration of glycosylated HbA1c was correlated with the change in the augmentation index during step 1 (r=0.65, P<0.01, Figure 1).

### Hemodynamic Parameters

Data on heart rate, ejection duration, peripheral and central blood pressures, pulse pressure, mean arterial pressure, forearm blood flow, and peripheral vascular resistance at baseline and during steps 1 and 2 are shown in Table 2. Basal heart rates, ejection duration, and peripheral vascular resistance were comparable at baseline. Heart rate remained unchanged in both groups during step 1, as did forearm blood flow and peripheral vascular resistance (Table 2). Brachial and aortic systolic and pulse pressures were slightly higher in the type 1 diabetic patients at baseline than in the normal subjects. Diastolic blood pressures were not significantly different between the groups.

### Discussion

In the present study, we determined whether insulin resistance in type 1 diabetes involves a defect in the action of insulin on large artery stiffness and, if so, the clinical or biochemical parameters to which such a defect is related. We found the type 1 diabetic patients to be resistant not only to the action of insulin to stimulate glucose uptake but also to its ability to decrease central aortic pressure augmentation. Furthermore, the rate of insulin-stimulated glucose uptake was inversely correlated with the change in augmentation index by insulin, implying that the greater effect of insulin to stimulate glucose uptake, the more it diminished central aortic augmentation. The latter effect was, as described previously,\(^{17,23}\) independent of peripheral vascular resistance and blood flow.
Changes in the augmentation index provide a measure of changes in stiffness provided both heart rate and peripheral vascular resistance remain unchanged.\(^5,24\) This was true in the present study during the 1 mU \cdot kg\(^{-1}\) \cdot min\(^{-1}\) insulin infusion. During the higher-dose insulin infusion (2 mU \cdot kg\(^{-1}\) \cdot min\(^{-1}\)) in the normal subjects, heart rate increased and ejection duration shortened. This will result in a greater portion of wave reflection to occur in the diastole and decrease the augmentation index independent of any change in stiffness. This did not, however, influence interpretation of the present data because the augmentation index decreased maximally in the normal subjects during the first step, when ejection duration and heart rate remained unchanged. Similarly, the lack of change in the augmentation index in the type 1 diabetic subjects could not be attributed to heart rate or ejection duration because these remained unchanged.

The degree of insulin resistance in type 1 diabetic patients was similar to that previously reported.\(^16\) Because whole-body and forearm glucose uptakes were significantly correlated and forearm blood flows were similar between the 2 groups during the first 2-hour insulin infusion, the insulin resistance can be attributed to a cellular rather than a vascular defect in peripheral tissues.\(^25\) Also, as in previous studies that include larger numbers of type 1 diabetic patients, the defect in peripheral glucose uptake tended to relate to the degree of chronic hyperglycemia as measured with Hb\(\text{A}_1\)c (Figure 1).\(^16\) The new finding in the present study was that insulin resistance was also characterized by a defect in the ability of insulin to decrease large artery stiffness, as determined from the effect of insulin on the augmentation index. Under non—insulin-stimulated baseline conditions, the augmenta-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Subjects (n=9)</th>
<th>Type 1 Diabetic Subjects (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Step 1</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>55±2</td>
<td>58±3</td>
</tr>
<tr>
<td>Ejection duration, ms</td>
<td>349±4</td>
<td>341±4</td>
</tr>
<tr>
<td>Brachial systolic blood pressure, mm Hg</td>
<td>114±3†</td>
<td>117±2‡</td>
</tr>
<tr>
<td>Brachial diastolic blood pressure, mm Hg</td>
<td>70±2</td>
<td>71±2</td>
</tr>
<tr>
<td>Aortic systolic blood pressure, mm Hg</td>
<td>100±2*</td>
<td>100±2</td>
</tr>
<tr>
<td>Aortic diastolic blood pressure, mm Hg</td>
<td>70±2</td>
<td>72±2</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>44±2*</td>
<td>46±2</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>84±2*</td>
<td>86±2</td>
</tr>
<tr>
<td>Forearm blood flow, mL \cdot dL(^{-1}) \cdot min(^{-1})</td>
<td>2.6±0.2</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>Peripheral vascular resistance, mm Hg \cdot mL(^{-1}) \cdot dL \cdot min</td>
<td>34±3</td>
<td>31±2</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.02 for type 1 diabetic patients vs normal subjects at baseline or during step 1 or 2.
‡P<0.05, §P<0.02 for change in parameter step 1 or step 2 vs basal.

**Figure 2.** The augmentation index during insulin infusions of 1 (0 to 120 minutes) and 2 (120 to 240 minutes) mU \cdot kg\(^{-1}\) \cdot min\(^{-1}\). Euglycemia was maintained with the use of the insulin-clamp technique. *P<0.05, **P<0.01 for difference between the changes in the augmentation index in the normal subjects and type 1 diabetic patients. *P<0.05, †P<0.01 for change in the augmentation index at a given time point versus 0 minutes. DM indicates diabetes mellitus.

**Figure 3.** Relationship between whole-body glucose uptake and the change in augmentation index during the 1 mU \cdot kg\(^{-1}\) \cdot min\(^{-1}\) insulin infusion. Spearman’s nonparametric correlation coefficient was −0.61 (P<0.01). DM indicates diabetes mellitus.
tion indexes were similar between the groups. If the ability of insulin to diminish large artery stiffness would also characterize type 1 diabetic patients under postprandial conditions, this would be expected to expose the left ventricle to repeated bouts of increased afterload.

Regarding the site at which insulin acts to diminish wave reflection, it is clear that this effect cannot be explained by changes in peripheral vascular resistance, which are dominated by the caliber of the arterioles. Even low concentrations of insulin, which have no other hemodynamic effects, increase sympathetic nerve activity in muscle. This action of insulin is thought to produce vasoconstriction at the level of arterioles and has been suggested to counteract insulin-induced peripheral vasodilatation at physiological insulin concentrations such as those induced with the 1 mU · kg⁻¹ · min⁻¹ insulin infusion in the present study. Small increases in systolic blood pressure, which were also observed in the present study (Table 2), may reflect these direct sympathetic effects of insulin. Regarding preresistance arteries (arteries larger than arterioles), where insulin acts and the site of abnormal action of insulin on central pressure augmentation in patients with type 1 diabetes are presently unclear. This could theoretically be sorted out with a single vessel rather than a global approach to study arterial stiffness. It is, however, uncertain whether insulin would change the diameter of a single artery of any size measurably under conditions in which very small, if any, changes are observed in systemic hemodynamic parameters. This is because even in a 20-kg dog, there are ≈40 arteries with a mean diameter of 4 mm (the size of a human brachial artery) and 500 arteries with a diameter of 1.3 mm. An anatomic localization of the effects of insulin would, however, be important to establish whether the defect in insulin action might be localized at sites later predisposed to arteriosclerosis. The latter include the usual type of arteriosclerosis characterized by intimal calcifications, especially in central large arteries, and medial artery calcification (Mönckeberg’s arteriosclerosis). Diabetic patients are particularly prone to develop the latter type, which is characterized by uniform arterial narrowing and is most commonly found in muscular arteries, especially those in the thigh and those affected by neuropathy.

The cellular mechanism that underlies resistance to the vascular effects of insulin is poorly understood. Recent data have, however, demonstrated that both the aorta and smaller arteries contain all of the signaling molecules necessary to directly respond to insulin and that these tissues can be resistant to insulin action. In obese Zucker (fa/fa) rats, insulin-induced tyrosine phosphorylation of insulin receptor substrates 1 and 2 and their protein levels were decreased in the aorta. In contrast, the mitogen-activated protein kinase pathway was intact. This study thus documented selective insulin resistance in vascular tissues in obesity. Whether similar alterations characterize humans with hyperglycemia-induced insulin resistance remains to be investigated.

To conclude, insulin resistance in patients with clinically uncomplicated type 1 diabetes extends to large artery function and is characterized by the failure to normally decrease central pressure augmentation. This defect could predispose these patients to hypertension. The Stockholm Diabetes Intervention Study showed that intensive insulin therapy can retard the stiffening of large arteries in type 1 diabetic patients. Insulin resistance in type 1 diabetic patients also is ameliorated via normalization of glycemia. These data provide a rationale to test whether the insulin action of stiffness might respond to improved glycemia.

Acknowledgments
This work was supported by grants from the Sigrid Juselius Foundation (Dr Yki-Järvinen), the Academy of Finland (Dr Yki-Järvinen), and the Finnish Diabetes Research Society (Dr Westerbacka). We wish to thank Kati Tuomola for excellent technical assistance and volunteers for their help.

References


Insulin-Induced Decrease in Large Artery Stiffness Is Impaired in Uncomplicated Type 1 Diabetes Mellitus
Jukka Westerbacka, Antti Uosukainen, Sari Mäkimattila, Anna Schlenzka and Hannele Yki-Järvinen

Hypertension. 2000;35:1043-1048
doi: 10.1161/01.HYP.35.5.1043

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/35/5/1043

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/