The Effects of Plasma Insulin and Glucose on Myocardial Blood Flow in Patients With Type 1 Diabetes Mellitus

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OBJECTIVES The objective of this study was to determine the impact of insulin and glucose on myocardial vasodilator function in patients with type 1 diabetes mellitus (T1DM).

BACKGROUND The relative importance of plasma insulin and glucose levels on the abnormal vasodilator function observed in T1DM is unknown.

METHODS Twenty T1DM patients underwent positron emission tomography studies to measure myocardial blood flow (MBF) (in ml/g/min) at rest (MBFr) and during adenosine (MBFa), both under baseline metabolic conditions and then during either hyperinsulinemic-euglycemic clamp (HE) (n = 10; 40 ± 9 years, 8 female subjects, hemoglobin A1c [HbA1c] 7.8 ± 1.1%) or hyperinsulinemic-hyperglycemic clamp (HH) (n = 10; 44 ± 12 years, 8 female subjects, hemoglobin A1c 7.7 ± 0.6%).

RESULTS Both groups showed similar MBFr and MBFa under baseline metabolic conditions (p = NS). Compared with baseline conditions, MBFa increased in the HH group (p < 0.005), whereas it did not change in the HE group. Compared with baseline conditions, MBFa decreased in the HH group (p < 0.05) but did not change in the HE group. Myocardial perfusion reserve (MPR) (MBFa/MBFr) was similar between the HE and HH groups at baseline (p = NS). During clamp, MPR tended to decrease in the HH group (p < 0.1) but did not change in the HE group (p = NS) when compared with baseline conditions. However, during the clamp MPR was significantly lower in the HH group when compared with the HE group (p < 0.0001).

CONCLUSIONS In the short term, hyperglycemia has a deleterious effect on myocardial vasodilator function, which outweighs the beneficial effect of hyperinsulinemia. (J Am Coll Cardiol 2005;46:42–8) © 2005 by the American College of Cardiology Foundation

Diabetes mellitus afflicts more than 10 million patients in the U.S. Cardiovascular disease is the leading cause of excess mortality observed in this population (1). Accelerated atherosclerosis has been well documented (2), but microvascular and macrovascular dysfunction, autonomic neuropathy, and specific structural, functional, and biochemical alterations may contribute to the excess cardiac morbidity and mortality in diabetes (3). Animal models of diabetes mellitus have shown impairment of both endothelial-dependent and endothelial-independent vasodilation (4). Human studies have confirmed the impairment of vasodilator reserve in the coronary circulation of patients with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) (5).

Type 1 diabetes mellitus by definition requires exogenous insulin therapy. Insulin administered either subcutaneously or intravenously produces peripheral hyperinsulinemia at levels needed to keep the blood glucose normalized because of the lack of first-pass hepatic extraction of the hormone. Patients with T1DM also have hyperglycemia for prolonged periods of time in the setting of both hyperinsulinemia and hypoinsulinemia because of mismatched timing of insulin peaks with food intake or inadequate insulin regimens. Insulin has known vasodilatory properties that are dose dependent (6), whereas hyperglycemia impairs vasodilation (7). In animal models of T1DM, acute insulin administration normalizes coronary artery sensitivity to adenosine (8). In humans with diabetes, tight glycemic control results in a 41% reduction in risk for the development of cardiovascular disease (9). Thus, the relative contribution of insulin and glucose to the vascular dysfunction noted in diabetes mellitus is not well understood. Our hypothesis was that in patients with T1DM, hyperglycemia would have greater deleterious consequences on myocardial vascular function than that mitigated by concomitant hyperinsulinemia. Accordingly, myocardial vasodilator function in patients with T1DM was compared under conditions of hyperinsulinemia and hyperglycemia with hyperinsulinemia and euglycemia.
Abbreviations and Acronyms

- HbA1c = hemoglobin A
- HE = hyperinsulinemic-euglycemic clamp
- HH = hyperinsulinemic-hyperglycemic clamp
- MFB = myocardial blood flow
- MBF = myocardial blood flow at rest
- MBFr = myocardial blood flow during adenosine
- MPR = myocardial perfusion reserve
- RPP = rate-pressure product
- T1DM = type 1 diabetes mellitus
- T2DM = type 2 diabetes mellitus

METHODS

Patients. Twenty T1DM patients were enrolled who met the inclusion criteria of a hemoglobin A1c (HbA1c) level of 7% to 10%, no active retinopathy nor clinically significant autonomic neuropathy, serum creatinine of <1.5 mg/dl, sedentary lifestyle, no known cardiac disease, and no other risk factors for coronary artery disease. The choice of studying patients with T1DM instead of patients with T2DM was made to reduce the confounding effects on myocardial vasodilator function of concomitant obesity, hypertension, and hyperlipidemia typically present in patients with T2DM. Non-diabetic controls were not studied because our working hypothesis was that the detrimental effects of hyperglycemia on myocardial hyperemia induced by adenosine would outweigh the potential effects of hyperinsulinemia in T1DM patients. All patients had a normal rest/stress echocardiogram at screening. The study was approved by the Human Studies and the Radioactive Drug Research Committees at Washington University School of Medicine. Written informed consent was obtained from all patients before enrollment into the study.

Study protocol. Patients were admitted to the General Clinical Research Center at Washington University the evening before the study for stabilization of metabolic parameters with an insulin drip at physiologic replacement doses (1 to 2 U/h) to maintain blood glucose levels of 5 to 7 mmol/l or a hyperinsulinemic-hyperglycemic clamp (HH) (n = 10; insulin 0.1 U/kg/h with target glucose of 11 to 14 mmol/l) (11). The clamps were instituted for 90 min, and then the measurements of MBF at rest (MBFr) and during adenosine (MBFa) were repeated. Plasma insulin and glucose levels were measured throughout the imaging study.

Image analysis. To generate myocardial time-activity curves, regions of interest encompassing the anterior, lateral, inferior, and septal walls were placed on three to four mid-ventricular short-axis slices of composite 15O-water images as previously described (12). To generate blood time-activity curves, for each tracer, a small region of interest (1 cm3) was placed within the left atrial cavity on a mid-ventricular slice in the horizontal long-axis orientation of each composite image. Subsequently, blood and myocardial time-activity curves were used in conjunction with a well-established kinetic model to measure MBF (in ml/g/min) in each myocardial region analyzed and averaged to obtain one value per patient per study for MBFr, MBFa, and myocardial perfusion reserve (MPR) (MBFa/MBFr).

Statistical analysis. Differences in plasma insulin, plasma glucose, MBF, and MPR were analyzed by a three-way analysis of variance for repeated measurements, with patient groups (HE vs. HH) as the grouping factor and clamp (vs. baseline) as the repeated measurements factors. Subsequent post-hoc tests using the Bonferroni method were performed on those factors, and factor interactions with p < 0.05 were considered significant. Similarly, differences in MPR were analyzed by a two-way analysis of variance for repeated measurements with patient groups (HE vs. HH) as the grouping factor and clamp (vs. baseline) as the repeated measurement factor. Subsequent post-hoc tests using the Bonferroni method were performed. To determine whether glucose blood levels contributed significantly to the decline in MPR, backward stepwise regression analysis was performed using MPR measured during the clamps as the dependent variable and age, gender, and HbA1c and plasma insulin and glucose levels during the clamps as the independent variables. All average data are presented as mean and standard deviation of the mean.

Table 1. Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>HE Group</th>
<th>HH Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (#)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Gender (females #)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>±40</td>
<td>±44</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>±75</td>
<td>±76</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>±7.8</td>
<td>±7.7</td>
</tr>
<tr>
<td>Duration of DM (yrs)</td>
<td>±26</td>
<td>±24</td>
</tr>
<tr>
<td>Cholesterol level (mg/dl)</td>
<td>±173</td>
<td>±179</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>±96</td>
<td>±107</td>
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</table>

DM = diabetes mellitus; HE = hyperinsulinemic-euglycemic clamp; HH = hyperinsulinemic-hyperglycemic clamp; LDL = low-density lipoprotein.
RESULTS

Clinical characteristics. The HE and HH groups were similar with respect to age, gender composition, duration of T1DM, cholesterol profile, and level of glycemic control (Table 1). All patients were taking insulin for glycemic control; only one patient was additionally on glucophage. Three patients in each group were on thyroid supplementation.

Plasma substrates and insulin. The plasma insulin levels under baseline metabolic conditions were similar in the groups both at rest (HE, 27.4 ± 8.0 mU/l; HH, 30.5 ± 8.4 mU/l; p = NS) and during adenosine (HE, 22.8 ± 5.3 mU/l; HH, 20 ± 13.7 mU/l; p = NS) (Fig. 1). Under clamp conditions, plasma insulin levels were also similar between the groups both at rest (HE, 95.8 ± 13.1 mU/l; HH, 100.9 ± 15.3 mU/l; p = NS) and during adenosine (HE, 85.8 ± 11.7 mU/l; HH, 65.2 ± 8.5 mU/l; p = NS). Insulin levels increased during hyperinsulinemic clamp in both groups as expected (p < 0.0001) compared with baseline metabolic conditions. Under baseline metabolic conditions, plasma glucose levels were similar between the groups both at rest (HE, 6.4 ± 0.2 mmol/l; HH, 6.1 ± 0.3 mmol/l; p = NS) and during adenosine (HE, 6.2 ± 0.2 mmol/l; HH, 5.7 ± 0.5 mmol/l; p = NS) (Fig. 2). As anticipated, plasma glucose levels during clamp conditions were higher in the HH group compared with the HE group both at rest (13.7 ± 0.7 mmol/l vs. 5.8 ± 0.2 mmol/l, p < 0.0001) and during adenosine (12.8 ± 1.0 mmol/l vs. 5.0 ± 0.2 mmol/ml, p < 0.0001).

Hemodynamics. Under baseline and clamp metabolic conditions, heart rate, systolic blood pressure, diastolic pressure, and the rate-pressure product were similar between the two groups at rest (Table 2). During adenosine, the heart rate increased to a similar level in both groups (p < 0.0001 compared with rest). Systolic blood pressure did not change in either group with adenosine. Diastolic blood pressure decreased with adenosine similarly in both groups (p < 0.0001). Because of the increase in heart rate, the rate-pressure product (RPP) increased to a similar extent in both groups with adenosine (p < 0.0001 compared with rest). Similar patterns were noted at rest and during adenosine in the clamp groups. There were no differences in the rest or adenosine values between baseline metabolic and clamp conditions or between the clamp groups.

MBF measurements. Under baseline metabolic conditions both MBFr (HE, 1.19 ± 0.19 ml/g/min; HH, 1.20 ± 0.24 ml/g/min; p = NS) and MBFa (HE, 3.70 ± 0.48 ml/g/min; HH, 3.05 ± 0.26 ml/g/min; p = NS) were similar between the groups (Fig. 3). With institution of the clamp, the MBFr in the HE group did not change (1.06 ± 0.04 ml/g/min, p = NS), although it increased (1.53 ± 0.11 ml/g/min, p < 0.005) in the HH group compared with baseline resting conditions. In response to adenosine, no significant differences were noted in MBFa in the HE group (3.99 ± 0.37 ml/g/min) compared with baseline metabolic conditions (p = NS). However, in the HH group, MBFa was significantly lower during the clamp (2.81 ± 0.26 ml/g/min) when compared with baseline metabolic conditions (p < 0.05). The MPR under baseline metabolic conditions was similar between the HE (3.14 ± 0.40) and HH (2.69 ± 0.41) groups (p = NS) (Fig. 4). Under clamp conditions, the MPR did not change in the HE group (3.79 ± 0.36, p = NS). In the HH group, the MPR tended to decrease with institution of the clamp (1.92 ± 0.21, p < 0.1 compared with baseline metabolic conditions). Moreover, the MPR in the HH group was significantly lower than that observed in the HE group (p < 0.001).

Impact of plasma insulin and glucose on MPR. Backward stepwise regression analysis identified plasma glucose levels and HbA1c as significant negative predictors of MPR measured during the clamps. Glucose was a stronger predictor (F = 20.3) than HbA1c (F = 4.93). Gender (F = 1.81 [+]), plasma insulin (F = 0.32 [+]), and age (F = 0.00) were not significant predictors of MPR.

Figure 1. Plasma insulin levels under baseline metabolic conditions and during hyperinsulinemic-euglycemic (HE) and hyperinsulinemic-hyperglycemic clamp (HH).
DISCUSSION

The results of this study show that at least in the short term, hyperglycemia negates the salutary effects of hyperinsulinemia on myocardial vasodilator function in patients with T1DM. Thus, in patients with T1DM, the presence of hyperglycemia may be a significant contributor to the development of abnormal myocardial vascular reactivity observed in these patients.

Vasodilator function in diabetes mellitus. Diabetes mellitus is associated with a state of endothelial dysfunction (13) in both animal models (14) and human tissue (15), resulting in impaired myocardial vasodilatory reserve (5). The values for MPR obtained under baseline metabolic conditions (Fig. 4) are lower than those reported for healthy individuals without diabetes mellitus (16–18), suggesting that vasodilator function was reduced in our patients. Possible mechanisms for the impairment in vasodilatory reserve include effects of reduced capillary surface density, autonomic neuropathy, hyperlipidemia, hyperglycemia, and hypoinsulinemia (13,19–22).

Effects of glucose. Potential mechanisms mediating the detrimental effects of hyperglycemia on vascular function include oxidative stress, reduced nitric oxide production, and an increase in vasoconstrictive prostanoids (7). Under hyperglycemic conditions, as much as one-third of the metabolism of glucose is shunted through the polyol pathway. Increased activity of the polyol pathway leads to oxidative stress by virtue of the fact that aldose activity requires and may deplete nicotine-adenine dinucleotide phosphate. Free-radical scavengers such as superoxide dismutase (23) and aldose reductase inhibitors (24) reverse the effects of hyperglycemia on vascular responsiveness. Many enzymes, including nitric oxide synthase, the enzyme responsible for the generation of endothelial-dependent relaxing factor, nitric oxide, require reduced nicotine-adenine dinucleotide phosphate. The nitric oxide-mediated component of acetylcholine-induced relaxation is impaired in streptozotocin diabetic rats (25). Hyperglycemia also leads to increased production of endothelial vasoconstrictor prostanoids, especially prostaglandin H2, and this can impair the acetylcholine-mediated vasodilator response (26). Cultured porcine aortic cells exposed to elevated glucose levels produce significantly more prostanoids when stimulated with bradykinin (27). Increased prostanoid production is mediated by protein kinase C activation, and effects on vasoconstriction might be mediated by free radicals (26). Finally, when plasma and cell membrane proteins are exposed to chronic high glucose concentrations, they un-

Table 2. Hemodynamics During Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Rate (beats/min)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>RPP (mm Hg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>HE</td>
<td>73 ± 4</td>
<td>114 ± 5</td>
<td>67 ± 2</td>
</tr>
<tr>
<td></td>
<td>HH</td>
<td>72 ± 3</td>
<td>129 ± 7</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>Adenosine*</td>
<td>HE</td>
<td>100 ± 3</td>
<td>118 ± 4</td>
<td>64 ± 2</td>
</tr>
<tr>
<td></td>
<td>HH</td>
<td>103 ± 4</td>
<td>115 ± 6</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>Clamp</td>
<td>HE</td>
<td>76 ± 5</td>
<td>121 ± 7</td>
<td>69 ± 2</td>
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<tr>
<td></td>
<td>HH</td>
<td>83 ± 5</td>
<td>126 ± 6</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>Clamp adenosine*</td>
<td>HE</td>
<td>98 ± 3</td>
<td>123 ± 4</td>
<td>62 ± 3</td>
</tr>
<tr>
<td></td>
<td>HH</td>
<td>111 ± 4</td>
<td>124 ± 5</td>
<td>63 ± 3</td>
</tr>
</tbody>
</table>

*p < 0.0001 effect of adenosine vs. respective rest conditions (baseline metabolic or clamp) for HR, DBP, and RPP.

DBP = diastolic blood pressure; HE = hyperinsulinemic-euglycemic clamp; HH = hyperinsulinemic-hyperglycemic clamp; RPP = rate-pressure product; SBP = systolic blood pressure.
dergo nonenzymatic glycosylation and cross-linking; the resultant advanced glycosylation end products can inhibit nitric oxide (28).

The results of this study confirm these observations in humans with T1DM. Indeed, our results are consistent with results of studies in patients with either T1DM or T2DM, in which hyperglycemia has been shown to be an independent predictor of abnormal myocardial vasodilator function (29,30). Of note, Sundell et al. (31) performed a similar type of study but observed that hyperglycemia in the setting of hyperinsulinemia did not reduce MPR in patients with T1DM. However, they exclusively studied men with a mean age of 33 years, whereas our population was 80% female with a mean age of 44 years (Table 1). Our population had T1DM for a longer duration, and was sedentary. To our knowledge there is no information about gender or age differences in myocardial vasodilator capacity in diabetes mellitus, particularly as a function of plasma glucose levels. However, it is becoming increasingly apparent that both the incidence and the manifestations of coronary artery disease are more pronounced in female patients with diabetes mellitus compared with their male counterparts (32,33). Although requiring further study, an increased sensitivity in myocardial vasodilator capacity to plasma glucose levels may partially explain this phenomenon.

**Effects of insulin.** Insulin is a skeletal muscle vasodilator that acts via nitric oxide-dependent mechanisms leading to an increase in capillary recruitment (34) in both healthy patients and those with T1DM (35). In rat mesenteric arteries it has been shown that insulin-mediated vasodilation is blocked by nonsteroidal antiinflammatory agents and potassium channel antagonists, suggesting that insulin acts
by cyclooxygenase-dependent mechanisms that involve activation of adenosine triphosphate-dependent potassium channels to cause vasodilation (36). In alloxan-induced diabetic sheep, coronary artery sensitivity to adenosine was normalized by acute insulin administration, suggesting that reduction in adenosine’s vasodilator effect was caused by hypoinsulinemia (8). Recently it has been reported that insulin directly affects myocardial perfusion (37) and increases adenosine-stimulated coronary flow in a dose-dependent manner in healthy middle-aged men (6). Plasma free fatty acids have been shown to impair endothelial-dependent vasodilation (38). Thus, the beneficial effects of insulin on myocardial vasodilation may in part be related to a lowering in plasma fatty acid levels that occurs with insulin administration. It has been reported that myocardial energy transduction is decreased in transgenic models of diabetes mellitus, most likely because of the increase in myocardial oxygen consumption caused by the overdependence on fatty acid oxidation (39). Consequently, a further increase in myocardial oxygen consumption with insulin could lead to an increase in myocardial blood flow. However, the myocardial oxygen demands did not increase with either clamp compared with baseline metabolic conditions (Table 2). Moreover, insulin infusion should shift myocardial substrate metabolism toward glucose use and improve efficiency, not decrease it. Thus, it is unlikely that alterations in myocardial energy transduction were responsible for the observed changes in MBF with insulin in the current study.

It should be noted that during the clamps, plasma insulin levels were lower during adenosine administration (Fig. 1). Although the insulin levels during adenosine were on average lower in the HH group than in the HE group, the difference was not statistically significant. The decline in plasma insulin levels during adenosine administration likely reflects the potentiation of insulin-stimulated glucose transport in adipose tissue, cardiac muscle, and possibly skeletal muscle (40). However, the multivariable analysis suggests that any decrease in plasma insulin had little impact on myocardial vasodilator function because only plasma glucose levels and HbA1c levels were significant predictors of the difference in MPR observed during the clamp studies.

Adenosine-induced myocardial vasodilation is mediated by both endothelial-dependent and –independent mechanisms, and has been used as an integrated measure of coronary reactivity (6). The results of this study suggest that the effects of insulin on adenosine-induced hyperemia are less pronounced in patients with T1DM (Fig. 3) and are counteracted by the effects of hyperglycemia. This is in contrast to results of the study performed by Sundell et al. (31), in which insulin significantly increased hyperemic blood flow and MPR in patients with T1DM. The reasons for these differences are unclear, although, as mentioned above, gender differences could potentially be contributory.

Study limitations. The impairment in vasodilator reserve caused by acute elevations in glucose might not mechanistically reflect the impairments noted with chronic elevations in glucose observed in the diabetic state. Although we studied patients with T1DM, the relevance of these findings in those with T2DM remains to be defined. Diabetic autonomic neuropathy is associated with impaired coronary vasodilator response to sympathetic stimulation, which is related to the degree of sympathetic dysfunction (41). Although 20% to 40% of patients with diabetes show abnormal autonomic function by clinical testing, even in the absence of apparent autonomic neuropathy many have alterations in cardiac sympathetic pathways as determined by myocardial scintigraphy. Testing for autonomic dysfunction was not performed; however, none of the patients had significant peripheral neuropathy or clinically apparent autonomic neuropathy. The increase by ~30 beats/min during adenosine administration (Table 2) is higher than what is typically observed with conventional vasodilator stress perfusion imaging with this drug. The reason for the higher heart rate is not clear. For example, women have a greater reflex tachycardia than do men in during adenosine infusion. However, patients with diabetes mellitus have an attenuated heart rate response (42). Regardless, there were no differences in the heart rate levels during adenosine at baseline or during the clamp intervention between the two clamp groups.

Clinical implications. It is possible that attenuation of coronary vasodilation by hyperglycemia during periods of increased myocardial demand leads to repeated episodes of myocardial ischemia. Indeed, the value for MPR in the HH group (1.92 ± 0.21) is nearly identical to the positron emission tomography-measured MPR values in myocardium subtended by coronary arteries with >75% diameter stenosis (1.9 ± 0.7) (18). The relationship between coronary microvascular dysfunction and diabetic cardiomyopathy is supported by observation of similar microvascular abnormalities in both diabetes and dilated cardiomyopathy (4). Endothelial dysfunction not only precedes and predicts clinical macrovascular disease, but also is an independent prognostic marker of adverse long-term cardiovascular outcomes (43). The results provide a partial explanation for the salutary effects of tight glycemic control on reducing the incidence and severity of cardiac complications in patients with T1DM (9). Identifying the mechanisms by which hyperglycemia leads to impaired vasodilatory capacity and determining strategies to prevent it from occurring may provide adjunctive therapy for diabetic patients, in whom control of hyperglycemia is elusive. Such therapy instituted early enough may have the potential to prevent or reduce the manifestations of atherosclerosis and delay diabetes-associated cardiomyopathy.

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REFERENCES