Reflex Sympathetic Activation Induces Acute Insulin Resistance in the Human Forearm

Kenneth A. Jamerson, Stevo Julius, Thorkell Gudbrandsson, Ove Andersson, and David O. Brant

Inferences about the association between sympathetic overactivity and insulin resistance have been drawn from the infusion of sympathomimetic amines in supraphysiological doses. We used the isolated perfused human forearm to investigate the effect of reflex-induced sympathetic nervous system activation on the peripheral utilization of glucose in the skeletal muscles of 14 healthy men. Local hyperinsulinemia in the forearm (132 ± 25 microunits/mL for 90 minutes) induced a significant increase in the utilization of glucose from baseline (16.4 ± 3.1 mg \cdot dL^{-1} \cdot min^{-1} \cdot 100 \text{ mL forearm volume}) to a plateau (85.7 ± 15.1 mg \cdot dL^{-1} \cdot min^{-1} \cdot 100 \text{ mL forearm volume}) between 40 and 60 minutes of insulin infusion but did not alter the utilization of oxygen. Reflex sympathetic nervous system activation was elicited by unloading of cardiopulmonary receptors with bilateral thigh cuff inflation to 40 mm Hg between 60 and 90 minutes of insulin infusion. Blood flow in the forearm was significantly decreased with inflation of thigh cuffs (average decrease of 19%, \( p < 0.0001 \)). As a result of thigh cuff inflation, there was a reduction in the utilization of glucose (a decrease of 23%, \( p < 0.02 \)), whereas oxygen utilization was unchanged. We find that an increase in sympathetic nervous system activation (within the normal range of physiological responses) can cause acute insulin resistance in the forearm of healthy volunteers. The reflex caused no change in oxygen utilization, but the same stimulus elicited a decrease in the utilization of glucose. The decrease in utilization of glucose in skeletal muscle may be caused by both the decrease in blood flow and by an adrenergic receptor-mediated resistance. The relative contributions of each of these mechanisms to insulin resistance deserves further investigation. (Hypertension 1993;21:618-623)

Key WORDS • insulin resistance • hemodynamics • sympathetic nervous system • hypertension, essential

Insulin secretion and tissue insulin sensitivity are major factors in maintaining the homeostasis of glucose. Abnormalities in glucose utilization are estimated to exist in 25% of the general population\(^1\) and in up to 80% of subjects with hypertension.\(^2\) Insulin resistance is characterized by an inadequate glucose uptake in peripheral tissues at a given concentration of plasma insulin. Insulin resistance has been observed in obesity,\(^2\)-\(^5\) non-insulin-dependent diabetes mellitus,\(^6\)-\(^7\) and hypertension.\(^2\)-\(^4\),\(^8\) The pervasive association between hypertension and insulin resistance has been described in both lean and obese subjects when compared with weight-matched controls.\(^2\)-\(^5\),\(^8\) With direct infusion of insulin into the forearm of hypertensive subjects, Natali et al\(^8\) have shown that the site of insulin resistance in hypertension is skeletal muscle.

There is evidence that insulin resistance may be related to the basic homeostasis of blood pressure: 1) the impairment of glucose utilization has been shown by Ferrannini et al\(^9\) to directly correlate with the severity of hypertension; 2) a higher incidence of markers of insulin resistance has been shown to be associated with borderline hypertension in subjects in Tecumseh, Mich., when compared with that in normotensive subjects;\(^10\) and 3) a correlation has been found between blood pressure and insulin levels in healthy school children.\(^11\),\(^12\)

The underlying mechanisms of insulin resistance include defects in insulin secretion or production (rare), circulating insulin antagonists (growth hormone, glucagon, catecholamines), and defects in the target organs, e.g., insulin and glucose membrane transport, as well as post-receptor defects in the handling of glucose.\(^13\)-\(^15\) Recently, Baron et al\(^16\) have suggested a novel mechanism that may contribute to the genesis of insulin resistance, i.e., that inadequate delivery of glucose from reduced blood flow contributes to the decreased utilization of glucose in the peripheral tissues. They have shown a blunted rise in the postprandial increase in blood flow to the skeletal muscles of subjects with insulin resistance from obesity, and in non-insulin-dependent diabetes mellitus when compared with that of control subjects.\(^16\)-\(^19\) The blunted rise in blood flow after an oral glucose load was accompanied by a decrease in insulin-mediated glucose uptake. Impaired blood flow to tissues secondary to chronic vasoconstriction...
from hypertension has not been previously considered as causative of insulin resistance. The purpose of the present study is to assess the effect of reflex-induced acute vasoconstriction on glucose utilization of skeletal muscle. The hypothesis tested is that acute vasoconstriction within the normal physiological response range can induce changes in glucose uptake in the perfused forearm of normotensive volunteers. If positive, the results would support the conceptual feasibility of the hypothesis that chronic vasoconstriction in hypertension might lead to insulin resistance.19

Methods

Subjects and Protocol

Healthy subjects aged 18–65 years were recruited by advertisement in the local newspaper. Studies were conducted with the approval of the Institutional Review Board of the University of Michigan Medical School (UMMS). Eighteen male volunteers and one female responded. Four studies (three male subjects and the one female) were terminated for technical reasons, e.g., loss of arterial lines. Studies were conducted in the morning, after a 12-hour fast, in the General Clinical Research Center of the UMMS. Subjects had no dietary restrictions before the study; however, they were asked to refrain from tobacco and any nonprescription/prescription medications. Studies were done with subjects in the supine position. After anthropometric data were obtained and the brachial artery was cannulated with a 21G angiocatheter. Venous samples were obtained by retrograde cannulation of the ipsilateral forearm vein. When the catheter was properly placed, the tip was not palpable, and the oxygen saturation of blood samples from the catheter was less than 60%. Arterial and venous blood gases, plasma glucose, and plasma insulin were measured at 15-minute intervals over a 30-minute baseline, at 20-minute intervals during a 60-minute insulin infusion alone, and at 10-minute intervals during an additional uninterrupted 30 minutes of insulin infusion with thigh cuff inflation (total infusion time of insulin was 90 minutes). Forearm blood flow measurements were done every 10 minutes throughout the study. Intra-arterial blood pressure was recorded, and electrocardiographic measurements were made continuously. Details of hemodynamic measurements performed in our laboratory have been documented elsewhere.20

Insulin Infusion

Based on the average of three resting forearm blood flow values, an intra-arterial insulin infusion was begun at a rate calculated to raise forearm plasma insulin levels by 100 microunits/mL over basal levels. Forearm blood flow (ml·min⁻¹·dL⁻¹)×forearm volume (dL)×(100 microunits/mL) (60 min/1 hr)=insulin infusion rate in microunits per hour.9 The infusion consisted of 0.01 unit/mL regular insulin and saline with 1% human albumin to avoid adherence of insulin to tubing. The infusion was begun after a 30-minute baseline and continued for the duration of the study (90 minutes).

Forearm Plethysmography

Total forearm blood flow was measured by a Hokanson (Bellevue, Wash.) venous occlusion plethysmograph with mercury-in-Silastic strain gauge apparatus.21 Blood flow measurements were obtained every 10 minutes for the duration of the study. The room temperature was 25.3°C and maintained within ±0.2°C for each study.

Reflex Sympathetic Vasoconstriction

After a 60-minute insulin infusion, bilateral thigh cuffs were inflated to 40 mm Hg for up to 30 minutes. This elicits a decreased venous filling of the heart and thereby an unloading of cardiopulmonary receptors, which has been shown to induce a rise in sympathetic outflow from the vasomotor centers.22

Biochemical Analyses

Plasma glucose and insulin samples were immediately centrifuged at 3,000 rpm and plasma samples analyzed at the Michigan Diabetes Research and Training Center at the UMMS.10 Complete gas analyses were done with an Instrumentation Laboratory System 1302 and a 282 CO-Oxymeter (Lexington, Mass.).

Glucose and Oxygen Balance

The forearm balance of glucose and oxygen was expressed in two ways: as the arteriovenous gradient (arteriovenous difference in glucose and oxygen) and as the product of the arteriovenous gradient and flow (arteriovenous difference)×(forearm blood flow).

Statistics

All data are expressed as mean±SEM. The values were averaged over the three conditions (baseline, insulin infusion, and insulin infusion plus thigh cuffs). Arteriovenous glucose values were averaged during 40 and 60 minutes of insulin infusion when plasma insulin levels were steady. The 20-minute time point was chosen during thigh cuff inflation for calculation of glucose utilization during this condition. Changes in glucose utilization between conditions were tested with paired t tests.

Results

Table 1 shows the baseline characteristics of the group. Subjects were all young, healthy men within 20% of ideal body weight.

Hemodynamic Effects of Insulin

Baseline blood pressure, heart rate, weight, and glucose and insulin levels were well within normal limits.
TABLE 2. Hemodynamic Effects of Intra-arterial Insulin

<table>
<thead>
<tr>
<th>Study conditions</th>
<th>Insulin (μM/mL)</th>
<th>FABF (ml/dL forearm volume per minute)</th>
<th>HR (bpm)</th>
<th>MABP (mm Hg)</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10.8±1.4</td>
<td>3.8±0.26</td>
<td>61.53±1.60</td>
<td>90.86±1.4</td>
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<tr>
<td>Insulin</td>
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<td></td>
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<tr>
<td>(average at 40–60 minutes of infusion)</td>
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<tr>
<td>Insulin+thigh cuffs</td>
<td>132.6±24.7*</td>
<td>5.05±0.4†</td>
<td>61.49±1.2</td>
<td>91.90±1.0</td>
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FABF, forearm blood flow; HR, heart rate; bpm, beats per minute; MABP, mean arterial blood pressure. n = 14 subjects. Values are mean±SEM.

*p<0.0004, †p<0.03, change from baseline to insulin infusion.

Figure 1 and Table 3 demonstrate the effects of insulin on glucose and oxygen balance at baseline, during insulin infusion, and during insulin infusion plus thigh cuffs. The basal utilization of oxygen and glucose across the skeletal muscles of the forearm was 27.0±2.4 vol%·dL⁻¹·min⁻¹ and 16.4±3.1 mg·dL⁻¹·min⁻¹, respectively. During insulin infusion there was an increase in the extraction of glucose in proportion to the rise in insulin concentration with both plasma insulin values and glucose utilization reaching a steady state after 40 minutes of insulin infusion. In Figure 1, glucose extraction appears to rise during the entire insulin infusion period, but there was no significant change in the glucose utilization values from 40 to 50 or 60 minutes of insulin infusion. Also, from our preliminary work and from Ferrannini's work, we know that glucose utilization plateaus at about 40 minutes during the infusion of insulin and remains at that plateau for about 200 minutes before there is any evidence of systemic spillover. There was no evidence in our study of systemic spillover of insulin during or after 90 minutes of insulin infusion since plasma arterial glucose remained steady at 92 mg/dL while the arteriovenous glucose gradient widened from 4.1±0.6 to 17.2±2.8 mg·dL⁻¹·mL⁻¹ (p<0.0005). The utilization of glucose achieved a steady state (85.7 mg·dL⁻¹·min⁻¹ per 100 cc forearm volume) after 40 minutes of insulin infusion and remained stable over the remainder of the infusion until intervention with reflex sympathetic activation by thigh cuff inflation. On the contrary, there was no effect of insulin on the arteriovenous gradient or utilization of oxygen.

Metabolic and Hemodynamic Effects of Reflex Sympathetic Activation With Thigh Cuffs

After 60 minutes of insulin infusion and achievement of a new steady state for glucose utilization, bilateral
The major finding in the present study is that reflex sympathetic activation can elicit acute insulin resistance in normotensive humans. It has been previously speculated that sympathetic overactivity can cause insulin resistance, but the supporting evidence for this notion came from ex vivo studies or from catecholamine infusion studies in humans. Epinephrine causes hyperglycemia and decreases the insulin-mediated glucose uptake in skeletal muscle cells. During glucose clamp studies in humans, Epinephrine causes hyperglycemia and decreases the insulin-mediated glucose uptake. However, the supporting evidence for this notion is neither uniform nor long-lasting.

The change in glucose and oxygen extraction from the insulin infusion baseline (the average of 40–60 minutes of infusion) to reflex sympathetic activation with thigh cuff inflation is shown in Table 3. The arteriovenous glucose gradient was blunted from 17.2±2.8 mg/dL during insulin infusion alone to 13.8±2.4 mg/dL with inflation of thigh cuffs. The same stimulus had no effect on the arteriovenous gradient of oxygen. There was a decrease in glucose utilization of 19 mg·DL⁻¹·min⁻¹ (23%) with inflation of thigh cuffs (p<0.02). The oxygen utilization was essentially unchanged (a decrease of 4.4 vol%·DL⁻¹·min⁻¹, p=NS). In Table 3, the utilization of oxygen and glucose was calculated at the point of the maximal decrease in blood flow because reflex-induced vasoconstriction in response to thigh cuff inflation is neither uniform nor long-lasting. We do, however, find a temporal correlation in our index of reflex sympathetic nervous system activation (a decrease in forearm blood flow) and the decrease in glucose utilization. In 11 of 14 subjects, their maximal decrease in blood flow occurred at 20 minutes, and the flow returned to baseline by 30 minutes of thigh cuff inflation. Only one subject had a maximal decrease in blood flow at 30 minutes of cuff inflation; in this subject the lowest glucose utilization occurred at the 30-minute mark. Two subjects had no change in their forearm blood flow nor change in their glucose utilization in response to inflation of thigh cuffs. Figure 1 demonstrates that the maximal decrease in glucose utilization for the whole group occurred at 20 minutes. Thereafter, the effect of the reflex dissipated. Blood flow returned to preintervention levels for the group, and the glucose utilization returned toward pre-cuff inflation values.

**Discussion**

The major finding in the present study is that reflex sympathetic activation can elicit acute insulin resistance in normotensive humans. It has been previously speculated that sympathetic overactivity can cause insulin resistance, but the supporting evidence for this notion came from ex vivo studies or from catecholamine infusion studies in humans. Epinephrine causes hyperglycemia and decreases the insulin-mediated glucose uptake in skeletal muscle cells. During glucose clamp studies Deibert and DeFronzo infused epinephrine to reproduce plasma epinephrine levels similar to the ones observed during stress and in diabetic ketoacidosis. Epinephrine decreased the glucose metabolism by 41%, and this reduction could be abolished with β-adrenergic receptor blockade. This study proved that adrenergic stimulation can cause a β-receptor-mediated insulin resistance, and the authors speculated that the site of the resistance is in the skeletal muscle. However, under physiological and pathophysiological conditions the sympathetic activation is a mix of β- and α-adrenergic influences. Consequently the epinephrine infusion studies do not fully mimic the possible physiological role of sympathetics in the glucose regulation. The present study suggests that reflex changes in sympathetic tone that are in the normal range of human physiological responses can induce insulin resistance in human subjects.

Possible mechanisms by which the reflex activation of the sympathetic drive to the forearm could have decreased the insulin-stimulated glucose utilization include β-receptor-mediated reduction and a decrease in glucose uptake mediated through the reduction of the blood flow in the forearm. The relative contribution of receptor-mediated insulin resistance versus vasoconstriction during reflex-induced increases in sympathetic tone are not clearly delineated by the present study. The observed hemodynamic response in the forearm was vasoconstriction, i.e., the prevalent influence was α-adrenergic. Although α-adrenergic stimulation decreases the insulin release by the pancreas, there are no indications α-receptors directly affect glucose uptake in the skeletal muscle. Therefore, the hemodynamic effect of α-adrenergic vasoconstriction could be responsible for the observed decrease in the glucose uptake in this study. Some lines of evidence support this view. Recently, Baron et al have suggested that changes in glucose uptake may follow the Fick principle and that inadequate blood flow may lead to decreased glucose extraction. They have shown a blunted rise in the postprandial increase in blood flow to skeletal muscles of subjects with insulin resistance from obesity and in non-insulin-dependent diabetes mellitus when compared with that of control subjects. The converse also appears to be true: insulin resistance in subjects with hypertension is ameliorated in response to vasodilator therapy. Antihypertensive treatment with such diverse vasodilators as captopril, prazosin, and some calcium antagonists results in improvement of
glucose utilization. Some of our findings indirectly support the possible importance of hemodynamic factors for the decreased glucose utilization in our experiment. The thigh cuff inflation induced vasoconstriction and a decrease of the forearm blood flow. Under such conditions the diffusion distance between the fewer open capillaries and the metabolically active cells is likely to increase, and the arteriovenous extraction of larger molecules may be more affected than that of freely diffusible substances. This indeed was the case in our experiments. We observed during reflex vasoconstriction that the utilization of oxygen was not reduced, but both the extraction and the utilization of glucose actually decreased.

Based on the Fick principle a decrease in blood flow ought to cause an increase in the fractional extraction of the substrate that is being delivered to the tissue. Although the perfused forearm technique is ideal for the study of local metabolism in a limb, a requirement for interpretation of any studies based on the Fick principle is that the arteriovenous difference for a substrate can accurately reflect extraction only under steady-state conditions. It could be questioned whether we had achieved such a steady state after a relatively short period of thigh cuff inflation. However, we believe that the validity of observed decrease in glucose utilization in the present study is not hindered by this limitation. If during the decreased blood flow the utilization of a substrate is determined before achieving a new state of equilibrium, one would expect the extraction to remain the same or be on the rise until a new plateau or steady state was achieved. However, with thigh cuff inflation we observed an actual decrease of the arteriovenous extraction of the glucose. The transit time of glucose in the human limb has been estimated to be 2–4 minutes; thus, reflex vasoconstriction by thigh cuff inflation over a 30-minute interval should allow ample transit time such that the arterial glucose concentration is completely reflected on the venous side during simultaneous sampling. We therefore contend that the decrease in glucose utilization observed during reflex sympathetic activation of the nervous system in our study is a result of physiological vasoconstriction and physiological adrenergic receptor activation.

Although increased vascular resistance is characteristic of hypertension, a decreased forearm blood flow is not characteristic of hypertension. However, structural and functional rarefaction of small blood vessels may lead to less efficient capillary exchange despite an apparently normal flow in the larger vessels. One third of the falloff in blood pressure between the arterial and venous sides of the circulation occurs at the level of arterioles and capillaries; thus significant resistance to flow could occur despite the absence of flow abnormalities in larger vessels. The resulting less homogeneous capillary perfusion and longer distance between the capillaries and the muscle bed could result in less efficient capillary exchange, thereby restricting nutritional blood flow.

There is considerable debate as to the hemodynamic effects of insulin. Recently, hyperinsulinemia as a result of insulin resistance has been increasingly investigated as being important in the genesis of hypertension and atherosclerosis. However, in vitro studies suggest that insulin may attenuate smooth muscle contraction in response to calcium, potassium chloride, serotonin, or sympathetic amines, suggesting a possible vasodilator effect of insulin. Intra-arterial insulin infusion in humans elicits an increase in local forearm blood flow in our experiment. Similarly, Anderson et al found a significant increase in forearm blood flow with intravenous systemic infusion of insulin in physiological doses. However, Natali et al found no effect on blood flow with arterial infusion of insulin in healthy male subjects. The present study was not primarily designed to assess the effect of insulin on forearm blood flow. Accordingly, appropriate controls for such effects on forearm blood flow as room temperature, prolonged bed rest, and the composition of infusants were not addressed. In a study designed to assess the effects of insulin on local forearm blood flow, Creager et al found an incremental rise in forearm blood flow with increasing doses of insulin infused through the brachial artery in seven healthy men. Propranolol attenuated or completely inhibited the changes in forearm blood flow, suggesting that insulin causes forearm vasodilatation primarily by stimulation of β-adrenergic receptors.

The pathogenesis of insulin resistance has previously been characterized by impaired secretion of insulin, decreased sensitivity of insulin receptors, and depletion of glucose transport proteins. We now suggest that hemodynamic abnormalities may contribute to insulin resistance. The acute hemodynamic changes induced by thigh cuff inflation in the present study are not a surrogate for chronic changes induced by long-standing hypertension. Nevertheless our findings do support in principle the hypothesis that vascular sequelae from the increased vascular tone in hypertension may play a role in insulin resistance. If the findings in the present study were negative and sympathetic vasoconstriction had not led to insulin resistance, the basic concept espoused in our recent overview of the possible hemodynamic determinants of the association between insulin resistance, sympathetic overactivity, and hypertension would have been proven unfeasible.

In summary, reflex increase in sympathetic tone in normotensive individuals can lead to acute insulin resistance in the forearm. The data suggest that this acute insulin resistance may be secondary to sympathetic vasoconstriction and the ensuing decrease of blood flow to the forearm. These observations provide the first necessary link to evaluate the possible hemodynamic underpinnings of the association between human hypertension and insulin resistance. However, our experiments did not resolve the relative importance of the vasoconstriction versus the stimulation of insulin resistance–inducing β-adrenergic receptors for the observed decrease of glucose utilization in the forearm. In future studies we intend to concentrate on the relation between sympathetic overactivity, hemodynamic aberrations, and the insulin resistance.

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