Effects of Insulin on Vascular Responses to Mental Stress and Norepinephrine in Human Forearm

Sverker Jern

Abstract Essential hypertension is frequently associated with insulin resistance and hyperinsulinemia. In vitro, insulin has vasodilator actions, but its possible hemodynamic effect on muscular vascular beds in humans is a matter of controversy. We investigated the effects of local hyperinsulinemia on the vascular responses to norepinephrine and physiological vasodilation during mental stress in the perfused-forearm model. Nine glucose-tolerant, normotensive, nonobese men (aged 22 to 36 years) participated. Forearm perfusion studies (venous occlusion plethysmography) were performed during randomized, double-blind intrabrachial artery infusions of insulin (to raise plasma insulin 100 μU/mL) or placebo for 2 hours. A mental stress test and stepwise intra-arterial infusion of norepinephrine (6 to 1200 ng/min) were performed during each infusion. Insulin infusion increased venous plasma insulin to 98.4 μU/mL and increased net glucose uptake threefold. Insulin had a gradual vasodilator effect (P<.05 by ANOVA), and after 90 minutes blood flow was 36 percent units higher relative to the control arm than during placebo (P=.0005). During mental stress, forearm blood flow increased by 81% (t test, P=.006) and 92% (P=.01) in the study arm during insulin and placebo infusions, respectively (insulin versus placebo, P=NS). An increased forearm blood flow was maintained throughout the mental stress test during insulin infusion (ANOVA, P=.03). Forearm glucose uptake increased during stress, reflecting forearm hyperperfusion since fractional glucose extraction was unaffected by stress. The increased blood flow was maintained throughout the five norepinephrine dose steps (ANOVA, P<.04). The highest norepinephrine dose (1200 ng/min) decreased blood flow by 0.81 (ANOVA, P=.01) and 0.83 (P=.001) mL·min⁻¹·100 mL⁻¹ from baseline after insulin and placebo, respectively (P=NS). Slopes of individual dose-response curves were similar during the two conditions. Thus, high physiological levels of insulin exert a tonic vasodilator effect with a rightward shift of the dose-response curve to norepinephrine, but insulin does not interfere with the physiological increase in forearm perfusion during mental arousal. (Hypertension. 1994;24:686-694.)

Key Words • insulin • forearm • stress, psychological • norepinephrine • hemodynamics • hypertension, stress-related • blood flow

Essential hypertension is associated with a defect in insulin-stimulated glucose uptake and peripheral insulin resistance.1-5 Patients with hypertension are hyperinsulinemic compared with normotensive control subjects. Hemodynamically, established hypertension both with and without disturbances of glucose metabolism is characterized by increased systemic vascular resistance and normal (or subnormal) cardiac output.6 Fasting insulin levels have been shown to be directly correlated with systolic and diastolic blood pressures.7-9 It has recently been shown that the rate of insulin-mediated glucose uptake is inversely related to the basal mean arterial pressure, suggesting that attenuated insulin-induced skeletal muscle vasodilation is a major cause of insulin resistance.10 It has therefore been hypothesized that chronic hyperinsulinemia may be implicated in the development of hypertension and more specifically in the etiology of the elevated vascular resistance associated with the hypertensive state.11 In keeping with this hypothesis, a recent study has demonstrated that hyperinsulinemia increases the cardiovascular responsiveness to norepinephrine on the whole-body level.12 However, in vitro and animal studies have shown that insulin has vasodilating properties, and insulin attenuates the vasoconstrictor and inotropic responses to various agonists.13-18 Whether the vasodilator effect is present also in human muscular vascular beds, which constitute the major source of the increase in systemic vascular resistance in essential hypertension, is a matter of some controversy. Increased forearm blood flow (FBF) and/or leg blood flow has been observed in response to both supraphysiological and physiological levels of insulin.19,20-23 However, in a recent study no effect on FBF could be demonstrated in normotensive subjects when physiological doses of insulin were infused locally into the brachial artery.24 Also, in a previous study by our group, we observed no increase in FBF by glucose administration despite a considerable fall in systemic vascular resistance.25 However, a tonic vasodilator action of insulin does not necessarily preclude any enhancement of reactivity to vasoconstrictor hormones.

In view of the high prevalence of hyperinsulinemic states in hypertension, the potential effect of insulin on vascular tone and/or vascular responses to physiological vasoconstrictors is an issue of considerable pathophysiological importance. The aim of the present study was to investigate the effects of local hyperinsulinemia in the human forearm vascular bed and its possible interaction...
with vascular responses to norepinephrine and physiological vasodilation during mental stress. To minimize the influence of systemic neurohormonal and metabolic changes on regional hemodynamics, we used the perfused-forearm technique to study the effects on muscular blood flow and vascular resistance of insulin infused intra-arterially into the brachial artery. Furthermore, we wanted to investigate the effect of two essential physiological modulators of vascular tone: mental arousal, which together with physical exercise is an important source of the physiological variation in muscular blood flow, and the physiological vasoconstrictor norepinephrine.

**Methods**

**Subjects**

Nine glucose-tolerant, normotensive, nonobese subjects participated in the study. The subjects were recruited from medical students and hospital employees. They were all apparently healthy men without any medication or history of cardiovascular disease, diabetes mellitus, or essential hypertension. All subjects had normal fasting blood glucose values and negative family histories for diabetes mellitus. One of the subjects was a smoker. Table 1 shows clinical and hemodynamic characteristics of the nine investigated subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>27.7</td>
<td>22-36</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.81</td>
<td>1.72-1.93</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.0</td>
<td>61-90</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.7</td>
<td>19.6-25.5</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.82</td>
<td>0.76-0.88</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.56</td>
<td>4.61-6.34</td>
</tr>
</tbody>
</table>

TABLE 1. Clinical and Hemodynamic Characteristics of the Nine Investigated Subjects

The nature, purpose, and potential risks of the study were carefully explained to each subject before informed consent to participate was obtained. The protocol was approved by the Ethics Committee of the University of Göteborg, and the study was conducted according to the Declaration of Helsinki.

**Experimental Protocol**

Forearm perfusion studies were begun in the morning with subjects in the postabsorptive state after an overnight fast (10 to 12 hours) and followed a highly standardized procedure. Each participant was instructed to refrain from heavy exercise and avoid emotional excitement before the experiment. According to a randomized, double-blind schedule, they received local intra-arterial infusions of either insulin or placebo for approximately 2 hours during the morning and afternoon. During these infusions, a 10-minute mental stress test was performed 1 hour after the start of the infusions. At the end of each infusion period, a stepwise intra-arterial infusion of norepinephrine was given over 25 minutes to assess the vasoconstrictive response to α-stimulation.

**Hemodynamic Recordings**

The subjects reported to the catheterization laboratory at approximately 7:30 AM. An arterial polyethylene catheter (Viggo Products, British Viggo) was introduced percutaneously into the brachial artery of the nondominant arm by the Seldinger technique and advanced some 10 cm in the proximal direction. The catheter was connected to an electrical transducer (EMT 35, Siemens-Elema). Intra-arterial blood pressure was continuously recorded throughout the experiment on a Mingograph 82 (Siemens-Elema). Mean arterial pressure was obtained by electrical damping of the pressure signal. A single-lead electrocardiogram was continuously recorded on the Mingograph for heart rate assessment. A short indwelling cannula (Venflon, Viggo) was introduced retrogradely into a deep antecubital vein of the same arm for venous blood sampling.

FBF was studied by venous occlusion plethysmography with a mercury-in-rubber strain gauge. On each point of measurement, mean resting FBF in milliliters per minute per 100 milliliters was calculated from five to eight separate recordings. Resting forearm vascular resistance (FVR) was calculated as
the ratio of mean arterial blood pressure to FBF. FVR was expressed as resistance units (millimeters of mercury per minute per 100 milliliters per milliliter).

After catheterization and application of all recording devices, the subject was left alone in the recumbent position in a dimly lit, soundproof, and air-conditioned room for 60 minutes before the first baseline recordings were begun. Throughout the nonstress periods of the experiment, great care was taken to maintain a calm and friendly atmosphere to allow the subjects to relax as completely as possible. The subjects were asked to try to relax and to avoid unnecessary communication with the examiner throughout the resting periods of the experiment. The initial resting period was followed by a prestimulation baseline period from -20 to 0 minutes.

**Drugs**

Insulin was infused locally through the intra-arterial line at a rate calculated in each subject to elevate plasma insulin concentration by approximately 100 mU/L according to the following formula:

\[
\text{Insulin Infusion Rate (mU/min)} = \frac{\text{FBF (mL/min)} 	imes 100 \text{ mL}}{100 \text{ mU/L}} \times \text{Forearm Volume (100 mL)} \times 0.1 \text{ mU/L}
\]

Human regular insulin (Humulin Regular, Lilly France SA) was made up in an isotonic saline solution containing 1% human serum albumin (Albumin, Novo Nordisk). A placebo infusate (isotonic saline with 1% albumin) was administered in the same volume for each subject. The mean insulin infusion rate was 2.8 mU/min (range, 1.6 to 4.8 mU/min).

The norepinephrine infusate was prepared by diluting 0.5 mg norepinephrine tartrate (Noradrenalin, Apoteksbolaget) in 50 mL isotonic 5% glucose (Glukos, Kabi Pharmacia) solution. Norepinephrine was given in five dose steps of 6, 30, 150, 600, and 1200 ng/min. Each dose step was infused during 5 minutes with a constant infusion rate of 1.5 mL/min.

**Mental Stress Test**

The mental stress test was performed 1 hour after the start of the intra-arterial insulin/placebo infusion and followed the highly standardized procedure we have previously described in detail. Reproducibility data for the mental arithmetic has been published previously. The experiment began with a 10-minute prestress baseline period with the subject lying relaxed in the quiet and dimly lit room. Then the room was fully lit and the investigator gave an approximately 30-second oral instruction after which the stress test was started. The subject performed forced mental arithmetic for 10 minutes with serial subtractions of 7 from 700 trying to keep pace with a metronome at a rate of approximately 90 beats per minute. After a positive and reassuring comment, the investigator reduced the light and left the room. Each experiment was concluded with a 20-minute poststress baseline period.

**Blood Sampling and Biochemical Assays**

Arterial and venous blood samples were obtained simultaneously at preinfusion baseline, after 60 and 90 minutes after the start of insulin/placebo infusion, after 10 minutes of mental stress, and at the highest norepinephrine dose step. The first 3 to 4 mL of blood was always discarded. Blood samples were collected in sodium fluoride/sodium heparin or sodium heparin tubes. Within 2 minutes the samples were centrifuged at +4°C and 2000g for 5 minutes, and aliquots were stored at -70°C. Arterial and venous sample pairs were assayed in the same analytic run. Plasma glucose was determined in duplicate with the glucose oxidase technique using an automatic analyzer (Greiner G-400, Schweiz). Plasma insulin and arterial plasma C-peptide were determined by radioimmunoassay (Diagnostic Products Corp).

**Statistical Analysis**

Standard statistical methods were used. Unless otherwise stated, data are given as mean and SEM. The ratio of blood flow in the infused arm compared with that in the control arm was calculated for each measurement according to the method of Greenfield and Patterson to control for the effects on FBF of external systemic factors such as the level of arousal. Hemodynamic changes in response to the two treatments during basal conditions, mental stress, and intra-arterial norepinephrine infusions were evaluated by one- and two-way ANOVA for repeated measures with subject as random factor. For the repeated measures ANOVA, degrees of freedom were corrected according to the conservative Greenhouse and Geisser procedure for possible violation of the assumption of sphericity. The tests were considered significant at a value of P<0.05 (two-tailed test).

Predefined contrasts were calculated for hemodynamic or metabolic changes during the two conditions when ANOVA indicated a significant main effect of treatment or period/dose or a significant interaction effect. The following contrasts were computed: (1) hemodynamic or metabolic effects of insulin versus placebo by comparison of levels during the two conditions at 90 minutes after the start of insulin infusion and the changes (baseline versus 50-minute levels) induced by the treatments and (2) the changes in FBF and FVR induced by norepinephrine infusion (prenorepinephrine infusion baseline versus 1200 ng/min levels) during the two conditions. In all cases, contrast analyses were performed on means calculated for each point of measurement.

The relation between FBF and fractional glucose extraction was evaluated by univariate linear regression analysis.

**Results**

**Hemodynamic Effects of Regional Hyperinsulinemia**

Group means of basal FBF were somewhat lower in the study arm compared with the control arm before the insulin infusion (2.38 versus 2.72 mL·min⁻¹·100
mL⁻¹, $P=NS$ by $t$ test) but were similar before placebo infusion. This difference in basal perfusion was controlled for by using relative infusion FBF and FVR values for each experimental session as the difference between changes from preinfusion baseline levels of the infused arm in relation to the control arm.

At rest, study arm FBF fell slightly compared with that of the control arm during the placebo condition but increased during active insulin infusion (Fig 1). This gradual vasodilator effect of insulin was confirmed by a significant treatment $\times$ time interaction by ANOVA ($P<.05$). Ninety minutes after the start of the insulin infusion, relative blood flow was 36 percent units higher during the insulin than the control condition ($P=.0005$ by ANOVA contrast analysis). The changes of absolute FBF during insulin infusion were significantly different in the infused versus the control arm (ANOVA, arm $\times$ period interaction, $P=.03$; data not shown). Relative FVR fell to a level 30.6 percent units lower than during the placebo condition ($F=.01$). During this period, mean arterial pressure and heart rate did not vary significantly.

Insulin infusion increased venous plasma insulin to 98.4 $\mu$U/mL, which was accompanied by a fall in venous plasma glucose concentration from 5.56 to 4.50 mmol/L ($P=.0001$ by ANOVA contrasts). Arterial plasma glucose and C-peptide levels did not change during the insulin infusion. The arteriovenous glucose concentration gradient increased from 0.17 to 0.76 mmol/L (ANOVA contrasts, $P=.02$). Fractional glucose extraction increased from a basal ratio of 2.9% to 14.2% after 90 minutes of insulin infusion (ANOVA contrasts, $P=.01$). Glucose uptake across forearm tissues increased more than threefold from 0.42 to 1.44 $\mu$mol $\cdot$ min⁻¹ $\cdot$ 100 mL⁻¹ (ANOVA contrasts, $P=.07$).

Responses to Mental Stress Test

Responses to the mental stress test are shown in Fig 2 and Table 2. Mental stress induced highly significant increases of mean intra-arterial blood pressure and heart rate during both insulin and placebo treatments ($P<.001$ by one-way ANOVAs throughout). There were no significant differences in either levels or patterns of the blood pressure and heart rate responses during the two conditions (Fig 2).

In response to the mental challenge, FBF increased in the infusional and contralateral arms during both conditions ($P<.03$ by one-way ANOVAs). The absolute increase in blood flow was nonsignificantly greater during local insulin infusion, but because of the baseline elevation of flow before stress, the relative increases were actually slightly lower compared with those observed during placebo treatment. FBF increased by 81% ($t$ test, $P=.006$) and 92% ($t$ test, $P=.01$) in the study arm during insulin and placebo infusions, respectively. The difference in vascular responses between the two conditions was not statistically significant. Vasodilator responses were somewhat attenuated in the control arm, but the differences were not significant compared with the infusion arm. No effect of the insulin infusion on the stress-induced forearm vasodilation was observed in the control arm.

The relative FBF in the infusion over the control arm was maintained on a significantly higher level throughout the mental stress test during insulin treatment ($P=.03$ by ANOVA) (Fig 2, top right). Accordingly, FVR also tended to be lower during the insulin condition ($P=.06$ by ANOVA) (Fig 2, bottom right). Whereas mental stress induced significant changes in relative FBF and FVR during placebo treatment ($P<.05$ by one-way ANOVA).
TABLE 2. Effects of Mental Stress on Forearm Blood Flow, Mean intra-arterial Pressure, and Heart Rate During Local Intra-arterial Infusion of Insulin and Placebo

<table>
<thead>
<tr>
<th>Hemodynamic Variable</th>
<th>Insulin</th>
<th>Placebo</th>
<th>P (Insulin vs Placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm blood flow, study arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔFBF, mL·min⁻¹·100 mL⁻¹</td>
<td>+1.58 (0.53)</td>
<td>+1.31 (0.43)</td>
<td>.016</td>
</tr>
<tr>
<td>ΔFBF, %</td>
<td>+81.0 (21.8)</td>
<td>+92.0 (27.8)</td>
<td>.011</td>
</tr>
<tr>
<td>Forearm blood flow, control arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔFBF, mL·min⁻¹·100 mL⁻¹</td>
<td>+1.17 (0.36)</td>
<td>+0.99 (0.33)</td>
<td>.017</td>
</tr>
<tr>
<td>ΔFBF, %</td>
<td>+61.0 (15.9)</td>
<td>+54.3 (15.4)</td>
<td>.0076</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔMAP, mm Hg</td>
<td>+12.1 (2.2)</td>
<td>+11.2 (1.8)</td>
<td>.003</td>
</tr>
<tr>
<td>ΔMAP, %</td>
<td>+16.1 (2.7)</td>
<td>+14.7 (2.4)</td>
<td>.002</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔHR, bpm</td>
<td>+14.2 (3.4)</td>
<td>+16.0 (2.3)</td>
<td>.001</td>
</tr>
<tr>
<td>ΔHR, %</td>
<td>+26.7 (7.1)</td>
<td>+30.3 (4.8)</td>
<td>.002</td>
</tr>
</tbody>
</table>

Effects are shown as change from prestress baseline to mean level during stress. Values are mean (SEM). Significance levels by ANOVA contrast analysis.

ANOVA), the variations in relative blood flow and resistance were attenuated and not significant during the insulin infusion.

Changes in forearm glucose metabolism during stress are shown in Table 3. Forearm fractional glucose extraction and arteriovenous glucose concentration gradients were unaffected by stress. However, forearm glucose extraction increased during stress, presumably reflecting the increase in FBF. There was a negative correlation between FBF and fractional glucose extraction at rest during the prestress baseline (r = -0.47 to -0.53) as well as during stress on placebo treatment (r = -0.65). However, insulin infusion disrupted this negative relation between blood flow and glucose extraction (r = 0.07).

TABLE 3. Changes of Forearm Glucose Metabolism in Response to Mental Stress During Local Intra-arterial Infusion of Insulin and Placebo

<table>
<thead>
<tr>
<th>Metabolic Variable</th>
<th>Prestress</th>
<th>Stress</th>
<th>Poststress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma insulin, µU/mL</td>
<td>98.4 (20.7)</td>
<td>85.8 (23.7)</td>
<td>89.9 (23.3)</td>
</tr>
<tr>
<td>Arterial plasma glucose, mmol/L</td>
<td>5.29 (0.16)</td>
<td>5.09 (0.23)</td>
<td>5.26 (0.15)</td>
</tr>
<tr>
<td>Venous plasma glucose, mmol/L</td>
<td>4.54 (0.14)</td>
<td>4.36 (0.13)</td>
<td>4.50 (0.19)</td>
</tr>
<tr>
<td>A-V difference, mmol/L</td>
<td>0.75 (0.19)</td>
<td>0.73 (0.24)</td>
<td>0.76 (0.20)</td>
</tr>
<tr>
<td>Fractional glucose extraction, %</td>
<td>13.7 (3.2)</td>
<td>13.0 (4.1)</td>
<td>14.2 (3.8)</td>
</tr>
<tr>
<td>Glucose uptake, µmol/min · 100 mL</td>
<td>1.30 (0.26)</td>
<td>2.50 (0.69)</td>
<td>1.44 (0.35)</td>
</tr>
<tr>
<td>Placebo infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma insulin, µU/mL</td>
<td>6.51 (0.60)</td>
<td>6.19 (0.56)</td>
<td>6.22 (0.54)</td>
</tr>
<tr>
<td>Arterial plasma glucose, mmol/L</td>
<td>5.36 (0.17)</td>
<td>5.26 (0.15)</td>
<td>5.17 (0.17)</td>
</tr>
<tr>
<td>Venous plasma glucose, mmol/L</td>
<td>4.99 (0.14)</td>
<td>4.87 (0.18)</td>
<td>4.81 (0.17)</td>
</tr>
<tr>
<td>A-V difference, mmol/L</td>
<td>0.37 (0.05)</td>
<td>0.39 (0.10)</td>
<td>0.37 (0.09)</td>
</tr>
<tr>
<td>Fractional glucose extraction, %</td>
<td>6.8 (0.8)</td>
<td>7.5 (2.0)</td>
<td>7.1 (1.7)</td>
</tr>
<tr>
<td>Glucose uptake, µmol/min · 100 mL</td>
<td>0.63 (0.15)</td>
<td>0.93 (0.17)</td>
<td>0.63 (0.15)</td>
</tr>
</tbody>
</table>

A-V indicates arteriovenous. Values are mean (SEM).
however, it is unlikely that plasma epinephrine is involved in the modulatory effect of insulin on stress-induced vasodilation, because we have shown that plasma epinephrine responses to stress are unaltered even during systemic hyperinsulinemia.25

The baseline elevation of FBF and reduction of FVR during insulin infusion were also maintained throughout the stepwise norepinephrine infusion, but the vasocostricter response to norepinephrine was preserved in the hyperinsulinemic situation. Taken together, these findings suggest that insulin has a tonic vasodilator effect with a rightward shift of the dose-response curve to norepinephrine, but insulin does not interfere with the physiological increase in forearm perfusion during mental arousal.

The net uptake of glucose across the forearm tissues increased more than threefold in response to local insulin infusion. This increase is similar to the fourfold increase reported by the Ferrannini group (Natali et al24) in healthy humans using a similar perfused-forearm study design. Forearm glucose extraction was approximately doubled during stress, presumably reflecting the large increase in FBF because fractional glucose extraction was unaffected by stress. There was a negative correlation between FBF and fractional glucose extraction at rest during the prestress baseline as well as during stress on placebo treatment. For unknown reasons, insulin infusion disrupted this inverse relation between blood flow and glucose extraction.

Short-term oral carbohydrate administration reduces systemic vascular resistance and increases cardiac output,26 an effect that probably could be attributed to hyperinsulinemia because other studies have shown similar hemodynamic effects of insulin when changes of glucose levels are prevented. In canines, infusion with the euglycemic hyperinsulinemic clamp technique was shown to lower total peripheral resistance but to increase cardiac output, heart rate, and left ventricular contractility.27 It appears that the major mechanism behind the elevated cardiac output is increased sympathetic activity.37 Insulin administration is known to raise circulating norepinephrine,22,34 and microelectrode nerve recordings have shown that insulin increases muscle sympathetic outflow.23,35 However, although these effects on sympathetic activity have been interpreted to reflect a direct stimulatory action of insulin on the central nervous system,33,36 it is equally likely that the increased sympathetic drive is a secondary, reflex phenomenon to compensate for an insulin-mediated fall in systemic vascular resistance.

Vasodilator effects of insulin have been observed in both laboratory animals and humans.16,19-21,23,32,36 Despite a few previous negative studies,24 including one from our group,25 some investigators have observed vasodilator responses to insulin in the human forearm.19-23 In the classic studies by Zierler's group (Andres et al19), forearm perfusion increased during and after short-term (26-minute) intrabrachial artery infusions of 0.1 mU · kg⁻¹ · min⁻¹ insulin, a dose that is two to three times higher than the one used in the present study. During incremental forearm infusions of insulin, a dose-dependent vasodilation has been documented.20 This effect was present also when hypoglycemia was prevented by euglycemic glucose clamping. The vasodilator response could be blocked by pretreatment with

**Discussion**

The results of the present study show that insulin has a gradual vasodilator effect when infused at physiological levels into the human forearm. Ninety minutes after the start of the intra-arterial insulin infusion, blood flow was 36 percent units higher relative to the control arm than during placebo treatment. However, local insulin infusion had no effect on systemic blood pressure or heart rate, and there were no signs of any effect on systemic glucose/insulin homeostasis as arterial plasma glucose and C-peptide levels were unaffected by local insulin infusion. In response to the mental stress test, FBF increased on average 72%, a figure very similar to the 74% increase in forearm perfusion we observed in a previous study during physiological conditions.24 The insulin-induced increase in resting forearm perfusion was maintained throughout the mental stress test. The relative increments in blood flow elicited by stress during local insulin infusion were similar to those observed during placebo treatment. The patterns of the blood pressure and heart rate responses during the two conditions were almost identical. The peripheral vasodilatation induced by mental stress may in part be brought about by increases in circulating plasma epinephrine. However, it is unlikely that plasma epinephrine is involved in the modulatory effect of insulin on stress-induced vasodilation, because we have shown that plasma epinephrine responses to stress are unaltered even during systemic hyperinsulinemia.25

The baseline elevation of FBF and reduction of FVR during insulin infusion were also maintained throughout the stepwise norepinephrine infusion, but the vasocostricter response to norepinephrine was preserved in the hyperinsulinemic situation. Taken together, these findings suggest that insulin has a tonic vasodilator effect with a rightward shift of the dose-response curve to norepinephrine, but insulin does not interfere with the physiological increase in forearm perfusion during mental arousal.

The net uptake of glucose across the forearm tissues increased more than threefold in response to local insulin infusion. This increase is similar to the fourfold increase reported by the Ferrannini group (Natali et al24) in healthy humans using a similar perfused-forearm study design. Forearm glucose extraction was approximately doubled during stress, presumably reflecting the large increase in FBF because fractional glucose extraction was unaffected by stress. There was a negative correlation between FBF and fractional glucose extraction at rest during the prestress baseline as well as during stress on placebo treatment. For unknown reasons, insulin infusion disrupted this inverse relation between blood flow and glucose extraction.

Short-term oral carbohydrate administration reduces systemic vascular resistance and increases cardiac output,26 an effect that probably could be attributed to hyperinsulinemia because other studies have shown similar hemodynamic effects of insulin when changes of glucose levels are prevented. In canines, insulin infusion with the euglycemic hyperinsulinemic clamp technique was shown to lower total peripheral resistance but to increase cardiac output, heart rate, and left ventricular contractility.27 It appears that the major mechanism behind the elevated cardiac output is increased sympathetic activity.37 Insulin administration is known to raise circulating norepinephrine,22,34 and microelectrode nerve recordings have shown that insulin increases muscle sympathetic outflow.23,35 However, although these effects on sympathetic activity have been interpreted to reflect a direct stimulatory action of insulin on the central nervous system,33,36 it is equally likely that the increased sympathetic drive is a secondary, reflex phenomenon to compensate for an insulin-mediated fall in systemic vascular resistance.

Vasodilator effects of insulin have been observed in both laboratory animals and humans.16,19-21,23,32,36 Despite a few previous negative studies,24 including one from our group,25 some investigators have observed vasodilator responses to insulin in the human forearm.19-23 In the classic studies by Zierler's group (Andres et al19), forearm perfusion increased during and after short-term (26-minute) intrabrachial artery infusions of 0.1 mU · kg⁻¹ · min⁻¹ insulin, a dose that is two to three times higher than the one used in the present study. During incremental forearm infusions of insulin, a dose-dependent vasodilation has been documented.20 This effect was present also when hypoglycemia was prevented by euglycemic glucose clamping. The vasodi-

<table>
<thead>
<tr>
<th>Hemodynamic Variable</th>
<th>Insulin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study Arm</td>
<td>Control Arm</td>
</tr>
<tr>
<td></td>
<td>Study Arm</td>
<td>Control Arm</td>
</tr>
<tr>
<td>Forearm blood flow, mL • min⁻¹ • 100 mL⁻¹</td>
<td>2.10 (0.26)</td>
<td>1.94 (0.29)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>6 ng/min</td>
<td>2.00 (0.27)</td>
</tr>
<tr>
<td></td>
<td>30 ng/min</td>
<td>1.81 (0.22)</td>
</tr>
<tr>
<td></td>
<td>150 ng/min</td>
<td>1.63 (0.20)</td>
</tr>
<tr>
<td></td>
<td>600 ng/min</td>
<td>1.50 (0.13)</td>
</tr>
<tr>
<td></td>
<td>1200 ng/min</td>
<td>1.29 (0.12)</td>
</tr>
<tr>
<td>Forearm vascular resistance, U</td>
<td>40.9 (5.3)</td>
<td>44.2 (4.3)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>46.4 (7.9)</td>
<td>46.0 (5.5)</td>
</tr>
<tr>
<td></td>
<td>49.6 (7.3)</td>
<td>45.4 (6.3)</td>
</tr>
<tr>
<td></td>
<td>53.4 (5.7)</td>
<td>46.6 (5.9)</td>
</tr>
<tr>
<td></td>
<td>57.4 (7.0)</td>
<td>41.5 (5.9)</td>
</tr>
<tr>
<td></td>
<td>68.5 (8.1)</td>
<td>42.3 (4.0)</td>
</tr>
<tr>
<td>Mean intra-arterial pressure, mm Hg</td>
<td>77.0 (3.2)</td>
<td>...</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>79.3 (3.6)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>79.4 (3.5)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>79.9 (3.7)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>79.8 (3.5)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>81.0 (3.2)</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are mean (SEM).

propranolol, suggesting that β-adrenergic mechanisms are involved in the vasodilator response. In the calf, Laakso and coworkers have observed rather pronounced increases in blood flow in response to high-rate insulin infusion. Taken together with the present observations, these data support the notion that even if there may be differences between vascular districts, insulin has vasodilator properties.

Only a few previous in vitro studies have investigated the effects of insulin on the vasoconstrictor responses to norepinephrine. Alexander and Oake reported that insulin caused a significant attenuation of the vasoconstriction induced by norepinephrine in isolated perfused rat tails. This effect was observed with both pharmacological (120 mU/mL) and high physiological (150 µU/mL) doses of insulin. Also, insulin was found to have a dose-dependent inhibitory effect on norepinephrine-and angiotensin II–induced contraction in the perfused rabbit femoral artery and vein. Using the euglycemic hyperinsulinemic clamp technique, Scott and coworkers studied forearm vascular responses to graded hypovolemia elicited by lower body negative pressure. Again, hyperinsulinemia was accompanied by forearm vasodilation in the basal state, and forearm perfusion was higher at each level of lower body negative pressure than during control conditions. The forearm vasoconstrictor response to lower body negative pressure was preserved during hyperinsulinemia.

These observations agree with the effects of norepinephrine reported in the present study. However, Gans and coworkers recently reported that high insulin levels during euglycemic hyperinsulinemic clamping causes cardiovascular hyperresponsiveness to norepinephrine. The reasons for the apparent discrepancy between that and the present study are unclear, but there are some important differences between the two studies. First, the previous study focused on the diastolic blood pressure responses to norepinephrine when insulin was infused systemically into the circulation in contrast to our local insulin infusion. Since the forearm circulation receives only approximately 1% of cardiac output, recirculation of locally infused insulin is negligible when the perfused-forearm model is used. Thus, some systemic effect of insulin (e.g., on the level of the autonomic nervous system) may contribute to the divergent findings. Second, the net diastolic blood pressure response to a vasoconstrictor is subjected to more complex hemodynamic influences than the direct local blood flow measurement used in the present study. Above all, diversity of responses to insulin in various
vascular districts may contribute to substantial differences in net blood pressure effects at the whole-body level.

To our knowledge, our study is the first to investigate the vasoconstrictor response to norepinephrine during local insulin infusion in vivo in humans. In contrast to some of the in vitro data, however, the present findings suggest that there is a parallel rightward shift of the vasoconstrictor response to increasing doses of norepinephrine. Thus, insulin did not appear to compromise the vasoconstrictor response to α-adrenergic receptor stimulation.

The cellular mechanisms behind the vasodilator effect of insulin still remain elusive. One obvious possibility is that the increased perfusion is caused by local vasodilator substances released in response to an increase in tissue glucose utilization and metabolic rates. However, as shown by the Ferrannini group, despite its stimulatory effects on glucose tissue uptake, local hyperinsulinemia does not alter local rates of substrate oxidation and energy expenditure. A more likely mechanism is the interaction of insulin with intracellular cation homeostasis. Recently, Standley et al. have demonstrated that insulin attenuates vascular smooth muscle Ca²⁺ influx by both receptor- and voltage-operated channels. This alteration provides a potential mechanism for a decreased smooth muscle vascular tone of insulin and also for a diminished responsiveness to vasoconstrictive agents. It is possible that the finding that insulin may antagonize the inotropic effect of norepinephrine on cardiac cells may be dependent on similar cellular mechanisms.

In a previous study we observed that a male fat distribution pattern, in contrast to global obesity, was associated with higher systemic vascular resistance and lower cardiac output. Furthermore, abdominal obesity was associated with a vasoconstrictor type of stress response rather than the physiological increase in cardiac output. As expected, there was a significant positive correlation between fasting insulin and the degree of abdominal obesity. This relation probably reflects the presence of peripheral insulin resistance in subjects with a high waist-to-hip ratio. Interestingly, the decrease in FVR in response to stress was inversely correlated to fasting serum insulin levels, suggesting that subjects with higher insulin levels (and probably insulin resistance) had a less effective vasodilation during mental arousal.

If insulin reduces vascular smooth muscle cell contractile responses by attenuating agonist-induced rises in [Ca²⁺], through its effect on calcium channels, subjects with insulin resistance and impaired cellular responses to insulin may have a decreased insulin modulation of vasoconstrictive responses to agonist stimulation. Although the net result could be enhanced [Ca²⁺]-mediated vascular contractility and thereby increased vascular blood flow resistance. Thus, it is conceivable that a defective insulin-mediated vasodilation may contribute to the high-resistance state and the vasoconstrictive response to stress associated with abdominal obesity. If so, a similar mechanism may be postulated for insulin resistance in established hypertension, which is hemodynamically characterized by high peripheral resistance, even in the absence of frank diabetes. In line with this speculation, studies show that the vasodilator action of insulin is blunted in diabetic patients and in alloxan-diabetic lambs.

Acknowledgments

This work was supported by grants from the Swedish Medical Research Council (09046 and 7324), the Swedish Heart-Lung Foundation, and the Hoechst Diabetes Foundation. The author wishes to thank Annika Johansson and Hanneke Korhonen for excellent technical assistance throughout the study.

References


Effects of insulin on vascular responses to mental stress and norepinephrine in human forearm.
S Jern

Hypertension. 1994;24:686-694
doi: 10.1161/01.HYP.24.6.686

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/24/6/686

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/