Lack of Effect of α- and β-Adrenergic Inhibition on Forearm Glucose Uptake Despite Differences in Forearm Blood Flow in Healthy Humans

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Insulin has both sympathoexcitatory and vasodilatory actions. It is unclear how these interact to affect muscular glucose uptake. The current study was designed to determine the systemic and local contributions of α- and β-adrenergic activity to muscle glucose uptake. Forearm blood flow (FBF, plethysmography), arterial-venous glucose difference (AV-diff), and forearm glucose uptake (FGU) were measured during a 40-mU/m^2/min insulin infusion with 120 minutes of euglycemia in 6 normal subjects (age, 28.8 ± 4.9 years, mean ± SD). Each subject was studied 5 times, once each with intravenous propranolol (IV PROP, 80 μg/min), intravenous phentolamine (IV PHEN, 500 μg/min), intra-arterial propranolol (IA PROP, 25 μg/min), intra-arterial phentolamine (IA PHEN, 12 μg/min/100 mL forearm tissue), and saline (SAL). FBF did not change during insulin with SAL, IA PROP, or IV PROP, but increased during insulin with IA PHEN and IV PHEN ($P < .05$). Despite the increased glucose delivery during insulin plus IA PHEN and IV PHEN, FGU did not differ between study sessions at any time during the insulin infusion. This was due to the lower AV-diff during insulin with IA PHEN and IV PHEN compared to the other studies ($P < .05$). AV-diff negatively correlated with FBF at the end of the insulin infusion ($P < .001$) for all studies. In normal humans, inhibition of basal sympathetic activity does not alter muscular glucose uptake. The increased insulin-induced vasodilation during α-adrenergic inhibition suggests that insulin-induced sympathetic activation prevents excess vasodilation. This inhibition does not alter glucose uptake because changes in flow are counterbalanced by changes in glucose extraction.

INCREASED SYMPATHETIC nerve activity has been implicated as the cause of insulin resistance.[1-3] Obese, insulin-resistant individuals[4] and lean insulin-resistant hypertensive subjects[5] have increased muscle sympathetic nerve activity. In addition, reflex sympathetic activation[6] and local norepinephrine infusion[7] have been shown to decrease forearm glucose uptake (FGU). The effect of reflex sympathetic activation is more pronounced.[7]

The pathophysiologic mechanisms by which excess sympathetic activity causes insulin resistance are unclear. Muscle glucose uptake depends on the amount of glucose delivered to the tissues and the fraction of glucose extracted.[8] Insulin has been shown to increase both factors.[4,9,10] Proponents of increased sympathetic activity as a cause of insulin resistance suggest that excess sympathetic activity suppresses insulin-induced vasodilation.[7] Another potential mechanism is indicated by the fact that β_2-adrenergic activation increases lipolysis and thus increases free fatty acid (FFA) availability.[11] FFA can be used as an alternate fuel to glucose and can thus decrease glucose use by the tissues.[12,13] Furthermore, insulin has sympathoexcitatory effects that may partially counterbalance its vasodilatory action.[9,14] The sympathetic activation occurs with low doses of insulin that do not cause vasodilation,[15] and although basal sympathetic activity is increased in obese individuals, insulin-induced sympathetic activation and vasodilation are diminished.[4] The role of muscle blood flow in determining glucose uptake is also unclear.

The goal of this study was to determine how basal sympathetic activity affects muscular glucose uptake. We studied the effect of local and systemic α- and β-adrenergic blockade during insulin infusion on forearm glucose uptake. We specifically examined the relationship between insulin-induced vasodilation and glucose extraction during 2-hour euglycemic hyperinsulinemic clamps in 6 normal subjects. Each individual was studied on 5 separate occasions, once each with a concomitant infusion of intrabrachial or intravenous phentolamine, propranolol, or saline.

MATERIALS AND METHODS

Subjects

We prospectively studied 6 healthy adult subjects (5 males, 1 female; age, 28.8 ± 4.9 years; body mass index [BMI], 25.1 ± 4.6 kg/m^2 [mean ± SD] [range, 19.9 to 31.0]) who were free of medication. The Institutional Human Use Committee of the University of Iowa approved the studies, and written informed consent was obtained from all subjects before the study.

Measurements

Heart rate was measured continuously by electrocardiography. Forearm blood flow (FBF) was measured in the dominant arm by venous occlusion plethysmography (EC4; DE Hokanson Inc, Bellevue WA). Arterial pressure was measured continuously from a standard radial artery catheter placed in the nondominant arm. Respirations were monitored continuously with a strain-gauge pneumotachometer.

Protocol

Subjects were admitted to the Clinical Research Center at the University of Iowa and studied in the Human Cardiovascular Physiology Laboratory at similar times each morning on 5 separate study days after an overnight fast. Study sessions were separated by a minimum of 2

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weeks and a maximum of 2 months. For each study, an intravenous catheter was placed in each arm. The catheter in the dominant arm was placed in the antecubital fossa with the tip nonpalpable so that blood sampled from this catheter reflects forearm drainage.[18] The catheter in the nondominant arm was placed to infuse insulin and 20% dextrose. A 27-gauge steel needle attached to a 16-gauge epidural catheter was placed in the brachial artery of the dominant study arm to infuse propranolol, phentolamine, or saline, all at a rate of 1 mL/min. This method of intra-arterial infusion has been shown not to interfere with blood flow measurements or changes.[19] Plethysmographic cuffs and a mercury-in-silastic strain gauge were placed to measure FBF in the dominant arm. In the nondominant arm, a standard radial artery catheter was placed for blood sampling and measuring arterial pressure.

Arterial pressure, heart rate, respirations, and FBF were recorded for 5 out of every 10 minutes. Arterial and venous blood samples were drawn for measurement of plasma glucose concentration every five minutes. Five minutes of baseline data were collected and then, in a randomized fashion, an, intravenous propranolol (80 μg/min, IV PROP), intravenous phentolamine (500 μg/min, IV PHEN),[16] intra-arterial propranolol (25 μg/min, IA PROP), intra-arterial phentolamine (12 μg/min/100 mL forearm tissue, IA PHEN), or saline (SAL) infusion was initiated and then continued throughout the remainder of the study (Fig 1). The dose of IA PHEN has been shown to decrease forearm vasoconstriction during norepinephrine infusion from 67% to 15% without affecting systemic blood pressure or heart rate.[17] The dose of IA PROP blocks isoproterenol-induced vasodilation without systemic effects (unpublished data). The infusions were started 20 minutes before beginning the insulin clamp.

A primed insulin infusion was begun (40 $mU \cdot m^{-2} \cdot min^{-1}$ after the first 10 minutes) and plasma glucose maintained at 5 mmol/L with a 20% dextrose infusion for the next 120 minutes. During the clamp, arterial pressure, heart rate, respirations, and FBF were recorded in a similar fashion. Just prior to and after each 5-minute measurement, arterial and venous blood samples were drawn for plasma insulin and glucose levels. Just prior to the clamp, at 60 minutes, and at 120 minutes, arterial and venous effluent blood samples were drawn for measurement of plasma lactate, FFA, and catecholamine concentrations.

Data Analysis

Electrocardiogram, FBF, respirations, and arterial pressure were measured simultaneously with a computerized data acquisition system (MacLab, AD Instruments, Grand Junction, CO) and Macintosh Quadra 950 Computer (Apple Computer, Cupertino, CA). FBF was measured as mL/min/100 mL forearm volume. FGU was measured using the Frick principle; FGU = FBF [arterial glucose − venous glucose (AV diff)][1 − hematocrit]).

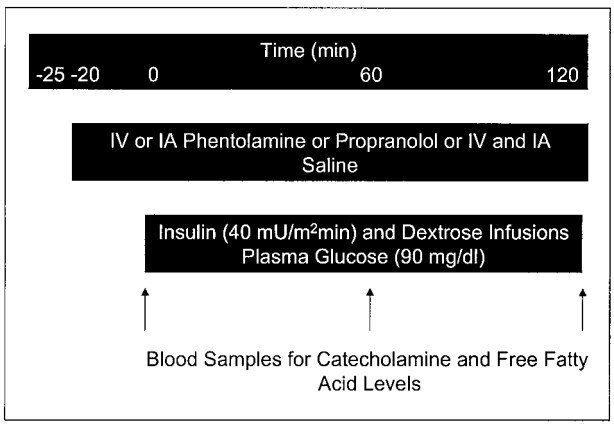

Fig 1. Study protocol.

Assays

Plasma glucose was measured immediately using the glucose oxidase technique with 1 of 2 YSI 2300 Stat Glucose Analyzers (Yellow Springs Instruments, Yellow Springs, OH). Arterial and venous samples were alternately measured on each machine; lactate was measured on one of the analyzers that also had a lactate probe. Catecholamines were determined by adding 1 mL of centrifuged plasma to a glass extraction vial containing 20 mg of acid-washed alumina (AAO; Bioanalytical Systems, West Lafayette, IN), 20 μL of a solution containing the internal standard (3,4 dihydroxy-benzylamine [DHBA] in 0.01N HCl), 1 mL of phosphate buffer (0.1 mol/L, pH 7.0, plus 0.05 mol/L EDTA), and 1 mL Tris buffer (1.5 mol/L, pH 8.6, plus 0.05 mol/L NaEDTA). After immediate gentle shaking for 10 minutes, the alumina was allowed to settle and the supernatant was aspirated to waste. Following 2 washes with water, catecholamines were eluted from the aluminia with 200 μL of 4% acetic acid. After centrifugal microfiltration using individual 0.2-μm regenerated cellulose membranes, each sample was chromatographed on a Catecholamine Column (Keystone Scientific, Bellefonte, PA; 3 μm particle size, 100 × 4.6 mm, reverse-phase, C-18 ODS, 10% carbon, end-capped) using a mobile phase of 75 mmol/L monobasic sodium phosphate, 0.12 mmol/L NaEDTA, 10 mmol/L citric acid, 15% acetonitrile, 10% methanol, and 1.5 mmol/L sodium dodecyl sulfate as the ion pairing agent. The catecholamines were detected with a Coulochem II Dual Potentiostat Electrochemical Detector (ESA, Chelmsford, MA). Peaks were quantitated on a Shimadzu CR5-A integrator (Shimadzu Scientific Instruments, Columbia, MD). A standard curve for extracted catecholamines (0, 125, 250, 500, 750, 1,000, 1,500, and 2,000 pg of each catecholamine) was prepared using "blank" human plasma (dialyzed to remove endogenous catecholamines) and linear regression analysis was used to determine sample plasma concentrations. The assay has interassay and intra-assay coefficients of variation of 3.4% and 3.1%, respectively, and a lower limit of detection of 20 pg/mL. Plasma FFA were measured by an enzymatic colorimetric method (Wako NEFA C Kit; Biochemical Diagnostics, Edgewood, NY). The low-, middle-, and high-range coefficients of variation were 2.7%, 1.1%, and 1.1%, respectively. Plasma insulin was measured by double-antibody radioimmunoassay with interassay and intra-assay coefficients of 9.4% and 5.3%, respectively.

Statistical Analyses

Differences in measured parameters were determined using a 1-way repeated-measures analysis of variance (ANOVA). Planned contrasts were used to determine differences between groups for changes with IA or IV infusions of phentolamine or propranolol alone and for changes with insulin infusion. Linear regression analysis was used to study the relationships between variables. Statistical significance was defined as $P < .05$. Results are reported as the mean ± SE.

RESULTS

Effect of Local and Systemic Phentolamine

Glucose and insulin. Arterial plasma glucose levels and venous plasma insulin levels did not differ between the IA PHEN, IV PHEN, and SAL at baseline or at any time during the study (Fig 2). Glucose infusion rates over the last 30 minutes of the clamp also did not differ between sessions and were 6.1 ± 0.8 mg/kg/min for IA PHEN, 6.2 ± 0.9 mg/kg/min for IV PHEN, and 7.4 ± 1.2 mg/kg/min for SAL.

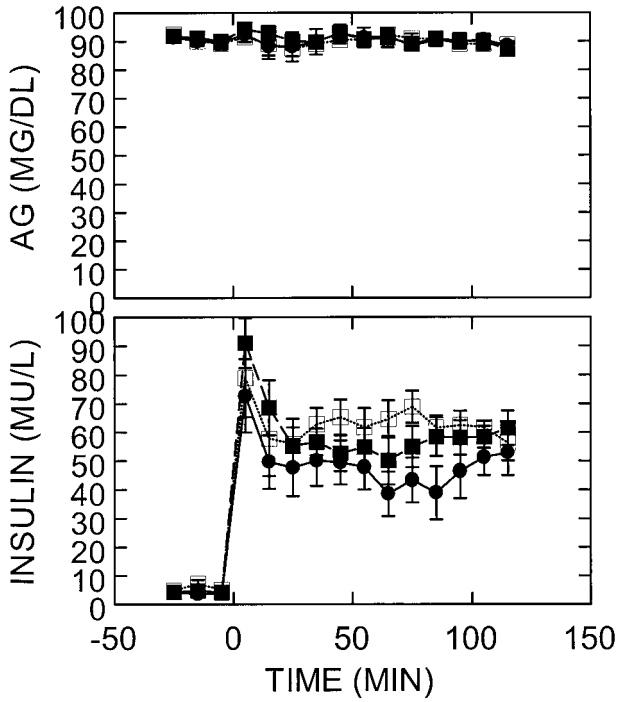

Fig 2. Plasma arterial glucose levels (AG, top) and venous insulin levels (bottom) before and during hyperinsulinemic clamp beginning at 0 minutes. Infusions of intra-arterial (solid) or intra-venous (open) phentolamine (squares) or control saline (circles) infusions were begun 20 minutes before insulin infusion; −25-minute time point is before phentolamine infusions.

Hemodynamics. Heart rate changes significantly varied between groups (time by session interaction, $P = .006$). Heart rate was not affected by infusion of IA PHEN or IV PHEN alone. Heart rate increased significantly during insulin infusion with SAL ($P = .022$), IA PHEN ($P = .002$), and IV PHEN ($P = .008$), but did not differ at any time between IA PHEN, IV PHEN, or SAL (Table 1).

Mean arterial pressure levels varied over time (time by session interaction, $P = .001$), but the responses did not differ between sessions. Mean arterial pressure fell with IV PHEN ($P = .010$) and tended to fall with IA PHEN ($P = .057$). Interestingly, mean arterial pressure increased with insulin during IA PHEN ($P = .001$) and SAL ($P = .002$), but not with IV PHEN. The increase in pressure over 120 minutes of insulin infusion with IA PHEN was greater than the lack of change with IV PHEN ($P = .041$). Mean arterial pressure levels did not differ between sessions at any time (Table 1).

FBF, AV-diff, and FGU. FBF changes also varied between sessions (time by session interaction, $P < .001$) (Fig 3). FBF increased significantly with IA PHEN ($P = .006$) alone. FBF was increased over preinsulin levels 5 minutes after starting insulin ($P = .030$) with IV PHEN and 10 minutes after starting insulin with IA PHEN ($P = .037$). FBF did not change during insulin with SAL. FBF with IA PHEN tended to be greater ($P < .1$) than FBF with SAL beginning 25 minutes after starting insulin, although the difference was not statistically significant until 105 minutes after starting insulin ($P = .031$). After 115 minutes of insulin, FBF with IV PHEN tended to greater than with SAL ($P = .091$). FBF with IA and IV PHEN did not differ. The percent increase in FBF during insulin did not differ between IA PHEN (44.9% ± 10%) and IV PHEN (53.4% ± 21.9%) and both were significantly greater than the lack of change during SAL (−6.4% ± 6.4%; $P = .023$ and $P = .010$, respectively).

AV-diff responses differed between sessions (time by session interaction, $P < .001$) (Fig 3). IA PHEN and IV PHEN alone did not alter AV-diff. AV-diff significantly increased compared to the preinsulin baseline after 25 minutes of insulin infusion with SAL ($P = .002$). AV-diff was significantly above or tended to be above baseline between 35 minutes ($P = .028$) and 95 minutes of insulin infusion with IV PHEN but was not different from baseline at 105 and 115 minutes. With IA PHEN, AV-diff never significantly increased above baseline. AV-diff with SAL was significantly greater 35 minutes after the initiation of the insulin infusion than with IA PHEN ($P < .05$) and 25 minutes after starting insulin than with IV PHEN.

FGU changed over time in all sessions (time effect, $P < .001$), but did not vary between sessions (Fig 3). FGU significantly increased only with IA PHEN alone ($P = .042$). FGU was further increased after 25 minutes of insulin with IA PHEN ($P = .046$). For the IV PHEN and SAL, FGU was significantly increased 35 minutes after starting insulin ($P = .002$). FGU did not differ at any time between the 3 study sessions.

FFA and Forearm Lactate Production. Arterial FFA levels fell during the insulin infusions with IA PHEN, IV PHEN, and SAL ($P < .001$) and did not differ between the studies at any time (Table 2). Net forearm fat uptake did not differ between the groups and was not altered by insulin infusion. Forearm lactate production increased significantly with insulin infusion during the IA PHEN and IV PHEN treatments ($P < .001$) and tended to increase during SAL ($P = .051$). Forearm lactate production at the end of insulin infusion was thus greater with IA PHEN ($P = .046$) and IV PHEN ($P = .007$) than SAL.

Catecholamines. Venous epinephrine levels did not change throughout the studies. Venous norepinephrine did not differ

Table 1. Hemodynamic Parameters Before and With Intra-arterial or Intravenous Infusion of Phentolamine, Propranolol, or Saline Alone and With Insulin Infusion

	Baseline −25 min	Infusion 0 min	Insulin 60 min	Insulin 120 min
Heart rate (beats/min)				
SAL	64 ± 2	60 ± 1	65 ± 3	67 ± 4†
IA PHEN	60 ± 3	58 ± 3	67 ± 3*	68 ± 3†
IV PHEN	62 ± 4	65 ± 3	74 ± 4*	73 ± 4†
IA PROP	61 ± 3	59 ± 1*	58 ± 2	59 ± 2
IV PROP	61 ± 2	59 ± 2	57 ± 3	58 ± 1
Mean arterial pressure (mm Hg)				
SAL	84 ± 3	84 ± 2	86 ± 3	91 ± 2*
IA PHEN	87 ± 4	84 ± 3	87 ± 2	92 ± 3*
IV PHEN	88 ± 4	84 ± 4*	85 ± 3	86 ± 3
IA PROP	90 ± 7	91 ± 7	94 ± 7	97 ± 8*
IV PROP	81 ± 3	82 ± 3	82 ± 3	84 ± 3

*$P < .05$ *v* previous time period.
†$P < .05$ *v* infusion only.

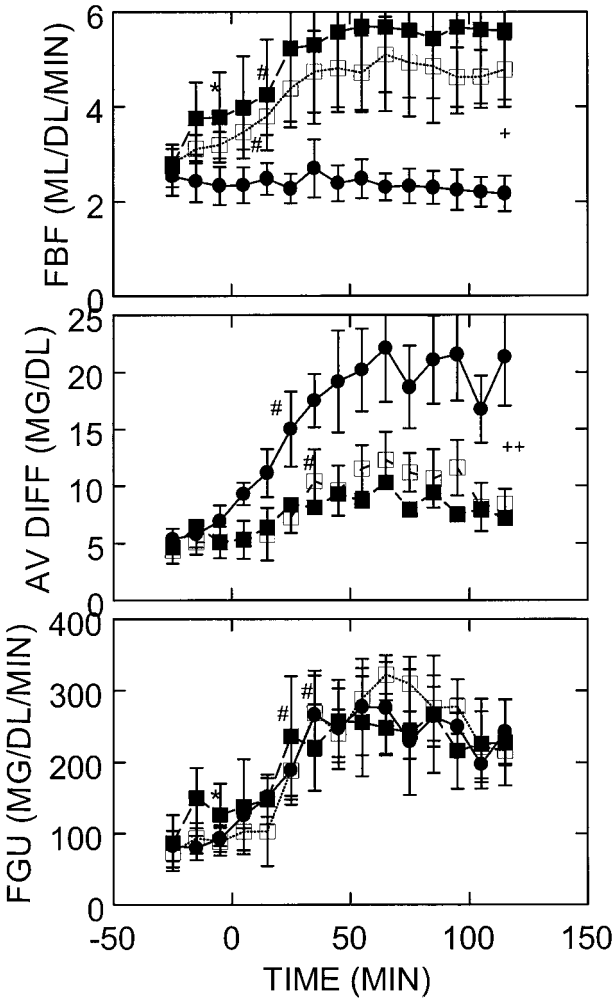

Fig 3. FBF (top), AV DIFF (middle), and FGU (bottom) before and during 120-min hyperinsulinemic clamp beginning at 0 minute. Infusions of intra-arterial (solid) or intravenous (open) phentolamine (squares) or control saline (circles) infusions were begun 20 minutes before insulin infusion; −25-minute time point is before phentolamine infusions. *$P < .05$ *v* −25 mins for same study. #$P < .05$ from this point forward *v* 0-minute time point except for AV-diff at 105 and 115 minutes for IV PHEN.+ $P < .05$, IA PHEN *v* SAL. ++$P < .05$, IA PHEN and IV PHEN *v* SAL.

between studies after 15 minutes of IA PHEN, IV PHEN, or SAL. Norepinephrine levels were increased 60 minutes after starting insulin with IA PHEN ($P = .001$) and IV PHEN ($P < .001$), and further increased after 120 minutes of insulin with IA PHEN ($P < .003$) but not with IV PHEN. No change was seen with insulin during SAL infusion. Venous norepinephrine levels were significantly greater after 60 and 120 minutes of insulin with IA PHEN and IV PHEN than with SAL ($P < .005$) (Table 3).

Effect of Local and Systemic Propranolol

Glucose and insulin. Arterial plasma glucose levels and venous plasma insulin levels did not differ between the IA PROP, IV PROP, and SAL infusions at any time during the study (Fig 4). Glucose infusion rates over the last 30 minutes of insulin infusion did not differ (IA PROP, 5.5 ± 0.4 mg/kg/min; IV PROP, 6.3 ± 1.1 mg/kg/min).

Hemodynamics. Heart rate varied over time for all sessions (time effect, $P = .015$) and the responses tended to vary between groups (time by group interaction, $F_{28\text{-}210} = 1.6$; $P = .054$) (Table 1). Heart rate fell with IA PROP ($P = .031$) alone. No changes were seen in heart rate during insulin infusion with either IA or IV PROP.

Mean arterial pressure also varied over time (time effect, $P < .001$), but the responses did not differ between sessions (Table 1). Mean arterial pressure did not change with either IA PROP or IV PROP alone, but increased during insulin infusion with IA PROP ($P = .023$). There were no significant differences at any time between sessions.

FBF, AV-diff, and FGU. FBF changed over time in both sessions (time effect, $P < .001$), but the responses did not differ between IA and IV PROP (Fig 5). FBF fell with IA PROP ($P = .033$) alone. FBF fell after 75 minutes of insulin infusion with IV PROP and remained below preinsulin baseline levels throughout the rest of the study. FBF did not change during hyperinsulinemia with IA PROP. FBF did not differ between IA PROP and IV PROP.

AV-diff also varied with time across all sessions (time effect, $P < .001$), but the responses did not differ between sessions (Fig 5). AV-diff increased with IA PROP ($P = .014$) alone and tended to increase with IV PROP alone ($P = .063$). AV-diff significantly increased after 35 minutes of insulin infusion for both IA PROP ($P = .004$) and IV PROP ($P = .014$) and remained above baseline levels throughout the study. There were no differences in AV-diff between IA PROP, IV PROP, or SAL.

Since FBF and AV-diff responses did not differ between IA PROP, IV PROP, or SAL, FGU by necessity followed the same pattern (Fig 5). FGU did change over time in all sessions (time effect, $F_{14\text{-}210} = 12.3$; $P < .001$), but the responses did not vary between sessions. FGU significantly increased after 35 minutes of insulin infusion for IA PROP ($P = .018$) and 45 minutes of insulin infusion for IA PROP ($P = .043$). As with FBF and AV-diff, there were no differences at any time between sessions.

FFA and forearm lactate production. Arterial plasma FFA levels decreased with insulin infusion ($P = .001$) during both IA PROP and IV PROP and did differ between the 2 at any time (Table 2). Arterial FFA levels tended to be lower at the end of insulin with IV PROP than at the end of IV PHEN ($P = .092$). There were no differences between IA PROP and IA PHEN. Net forearm fat uptake did not change with insulin infusion during either studies and was not different between the 2 sessions. Forearm lactate production increased during insulin infusion during both IA PROP and IV PROP but did not differ between the 2 or between them and SAL.

Catecholamines. Venous epinephrine did not change during insulin infusion with IA PROP or IV PROP (Table 3). Venous norepinephrine levels were increased after 120 minutes of insulin with IA PROP, but did not change with IV PROP. Norepinephrine levels at the end insulin infusion with IV PROP were lower than with IV PHEN ($P = .016$) and with IA PROP than with IA PHEN ($P = .004$).

Table 2. Arterial FFA Levels and Forearm Fat Use and Lactate Production With Intra-arterial or Intravenous Infusion of Phentolamine, Propranolol, or Saline Alone and With Insulin Infusion

	Infusion 0 min	Insulin 60 min	Insulin 120 min
Arterial FFA (mmol/L)			
SAL	0.43 ± 0.06	0.15 ± 0.03*	0.11 ± 0.04†
IA PHEN	0.61 ± 0.09	0.10 ± 0.04*	0.10 ± 0.03†
IV PHEN	0.50 ± 0.11	0.23 ± 0.09*	0.15 ± 0.05†
IA PROP	0.43 ± 0.05	0.13 ± 0.02*	0.08 ± 0.03†
IV PROP	0.45 ± 0.08	0.05 ± 0.02*	0.06 ± 0.02†
Forearm fat uptake (μmol/100 mL/min)			
SAL	0.074 ± 0.079	0.139 ± 0.066	0.046 ± 0.073
IA PHEN	−0.005 ± 0.131	−0.212 ± 0.133	0.038 ± 0.235
IV PHEN	−0.324 ± 0.169	−0.030 ± 0.138	−0.141 ± 0.099
IA PROP	0.009 ± 0.037	−0.109 ± 0.058	0.053 ± 0.044
IV PROP	−0.034 ± 0.056	−0.083 ± 0.082	0.008 ± 0.027
Forearm lactate output (μEg/100 mL/min)			
SAL	0.033 ± 0.013	0.047 ± 0.013	0.055 ± 0.014†
IA PHEN	0.014 ± 0.007	0.103 ± 0.0.038*	0.094 ± 0.014†
IV PHEN	0.015 ± 0.007	0.133 ± 0.028*	0.111 ± 0.011†
IA PROP	0.014 ± 0.009	0.041 ± 0.014	0.046 ± 0.011†
IV PROP	0.024 ± 0.010	0.049 ± 0.009*	0.056 ± 0.010†

*$P < .05$ *v* previous time period.
†$P < .05$ *v* infusion only.

Determinants of AV-diff. To determine potential causes of the differences in AV-diff between the sessions, we examined the relationship between AV-diff and FBF and arterial FFA levels across all study sessions. Only the relationship between AV-diff and FBF was found to be significant ($r = -0.61$, $P < .001$). The relationship between AV-diff and FBF^{-1} was even more significant ($r = .72$, $P < .001$) (Fig 6).

DISCUSSION

The lack of effect of sympathetic inhibition on FGU during a euglycemic hyperinsulinemic clamp indicates that basal sympathetic tone plays little role in determining muscular insulin sensitivity in normal individuals either through direct or systemic actions. Our results also indicate that glucose delivery plays little role in determining glucose uptake, since marked increases in flow during local phentolamine administration were counterbalanced by decreases in glucose extraction.

Table 3. Venous Forearm Effluent Epinephrine and Norepinephrine Levels and Forearm Fat Use and Lactate Production With Intra-arterial or Intravenous Infusion of Phentolamine, Propranolol, or Saline Alone and With Insulin Infusion

	Infusion 0 min	Insulin 60 min	Insulin 120 min
Venous epinephrine (pg/mL)			
SAL	25 ± 5	20 ± 0	20 ± 0
IA PHEN	25 ± 6	30 ± 6	32 ± 7
IV PHEN	24 ± 4	36 ± 13	27 ± 7
IA PROP	20 ± 0	28 ± 6	29 ± 8
IV PROP	25 ± 4	21 ± 0.5	31 ± 8
Venous norepinephrine (pg/mL)			
SAL	94 ± 36	130 ± 24†‡	123 ± 32‡
IA PHEN	152 ± 23	351 ± 54*	539 ± 66*
IV PHEN	181 ± 32	461 ± 55*	523 ± 80†
IA PROP	128 ± 17	148 ± 15‡	225 ± 62†‡
IV PROP	138 ± 19	142 ± 17‡	170 ± 20‡

*$P < .05$ *v* previous time period.
†$P < .05$ *v* infusion only.
‡$P < .05$ *v* IA PHEN and IV PHEN.

Our results are in contrast to studies by Jamerson et al[6] in which reflex sympathetic activation in the forearm, induced by occlusion of venous blood flow return in the legs, produces a fall in forearm glucose uptake. In a separate study, local norepinephrine administration that caused a similar decrease in FBF produced a smaller decrease in FGU. According to their data, reflex sympathetic activation causes insulin resistance through decreased glucose delivery and some other undetermined action.[7] The differences between our findings and theirs may be due to the differences in how the interaction between sympathetic activity and insulin resistance was evaluated. Specifically, our study addresses the question: does inhibition of basal sympathetic activity increase insulin sensitivity? Their studies ask the question: do increases in basal sympathetic activity decrease insulin sensitivity? The combination of our data and theirs suggests that in healthy individuals the maintenance of a set basal sympathetic tone is not a prerequisite for determining insulin sensitivity but when sympathetic activity increases above a pre-existing level insulin sensitivity falls.

Somewhat surprisingly, during a 2-hour euglycemic hyperinsulinemic clamp with intra-arterial saline infusion we found no increase in FBF. There are 2 potential reasons for this. First, insulin-induced vasodilation is a dose-dependent process.[20] Using similar techniques, we found increased FBF during a hyperinsulinemic clamp when plasma insulin concentrations were increased to 71 mU/L[9] but not when levels were 25 mU/L.[15] The levels in our study were intermediate to these. Utriainen et al[21] also did not find increased FBF, with levels similar to ours.

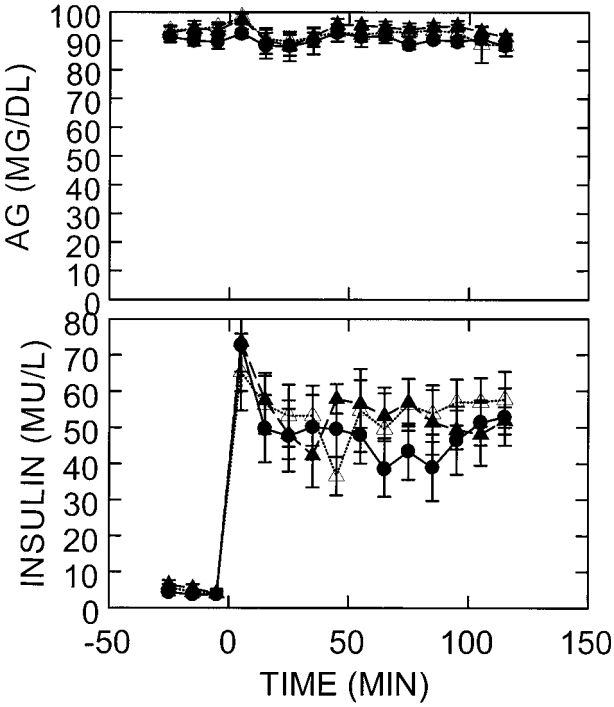

Fig 4. Plasma arterial glucose levels (AG, top) and venous insulin levels before and during hyperinsulinemic clamp beginning at 0 minutes. Infusions of intra-arterial (solid) or intravenous (open) propranolol (triangles) or control saline (circles) infusions were begun 20 minutes before insulin infusion; −25-minutes time point is before propranolol infusion.

A second reason for the difference is that in our previous studies and in most other studies, venous or arterialized venous plasma glucose levels rather than true arterial glucose levels were held constant. In the current study, had we held venous plasma glucose concentrations constant, the arterial glucose level would have increased by approximately 17% based on the arterial-venous glucose difference present at the end of the study. Even minimal hyperglycemia increases blood flow apart from increases in plasma insulin.[22,23]

The failure to find an increase could also be due to the direct mechanical interference of the intra-arterial infusion. The use of bilateral flow measurements might have addressed this question but could not be done because of the radial artery line in the nonstudy arm. Studies that have performed bilateral flow measurements have found that an intra-arterial infusion rate of 1 mL/min does not alter flow measurements.[23]

Our results show that α-adrenergic blockade of hyperinsulinemic sympathetic activation enhances insulin-induced vasodilation. This is in direct contrast to a study by Randin et al[24] who reported that α-adrenergic inhibition does not augment vasodilation since insulin-induced vasodilation was not altered by oral prazosin, an α_1-adrenergic inhibitor. They, like us, found no effect of β-blockade with propranolol. Our use of intra-arterial and intravenous infusions, and of the nonspecific α-adrenergic inhibitor, phentolamine, likely account for the contrasting results. Thus, from our results, the main function of insulin-induced sympathetic activation is to prevent excessive vasodilation. This has also been demonstrated when comparisons are made between sympathetically innervated and denervated limbs.[25]

Our healthy subjects compensated for increased vasodilation during euglycemia with IV PHEN by increasing heart rate, and likely cardiac output, and thus were able to maintain normal blood pressure levels. The cardiac compensation is apparently β-adrenergically mediated since heart rate did not increase with insulin infusion and IV PROP.

There is a great deal of controversy regarding the role of glucose delivery in determining muscular glucose uptake. Hespel et al[26] found that increasing the perfusion rate of isolated rat hindquarter increased muscular glucose uptake and that increasing the perfusate insulin concentration at submaximal levels aug-

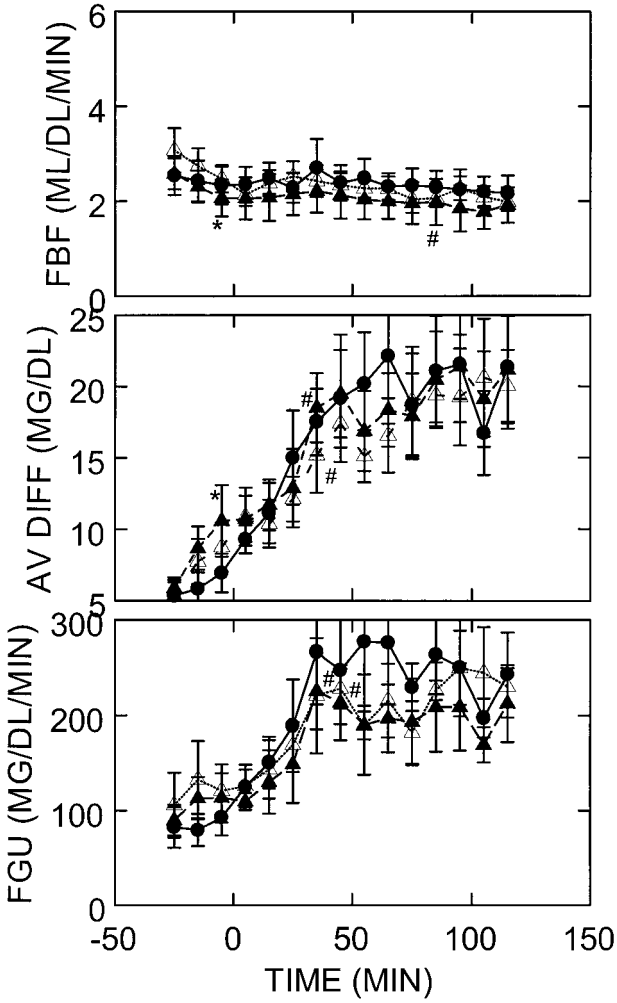

Fig 5. FBF, (top), AV-diff (middle), and FGU (bottom) before and during 120-minute hyperinsulinemic clamp beginning at 0 minutes. Infusions of intra-arterial (solid) or intravenous (open) propranolol (triangles) or control saline (circles) infusions were begun 20 minutes before insulin infusion; −25-minute time point is before propranolol infusion. *$P < .05$ *v* −25 mins for same study IA PROP only. #$P < .05$ from this point forward *v* 0-minute time (for FBF, applies only to IA PROP).

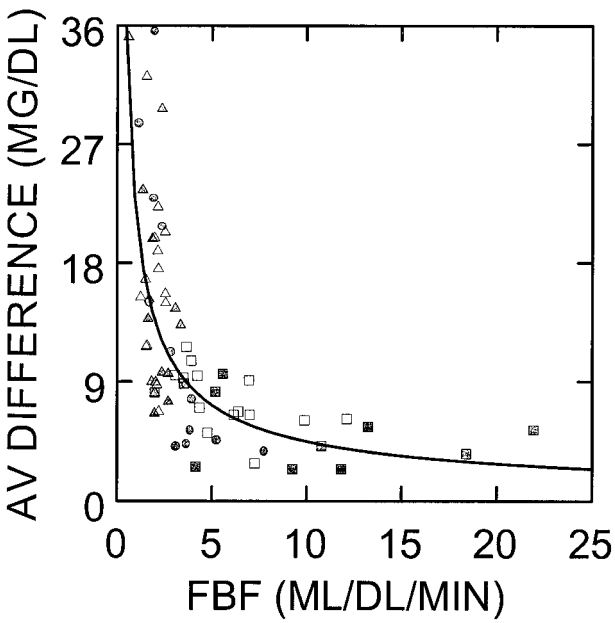

Fig 6. Relationship between AV-diff and FBF ($r = 0.72$, $P < .001$). SAL, circles; PROP, triangles; PHEN, squares; IA, solid: IV, open.

mented the effect of flow on glucose uptake. Baron et al[8] reported that diminished leg blood flow accounted for lower postprandial limb glucose uptake in obese humans. They found no difference in glucose extraction between obese and lean subjects. Laakso et al[27] found that a right-shift in the dose-response curves for both insulin-induced vasodilation and glucose extraction may be responsible for the insulin resistance in obesity. Vollenweider et al[4] also found diminished insulin-induced vasodilation in obese subjects, further supporting the concept that diminished blood flow plays a role in insulin resistance. In non-obese subjects, Jern[28] found that increases in blood flow entirely accounted for the increase in forearm glucose utilization during mental stress compared to baseline, regardless of whether insulin was present or not. Jern also found that insulin disrupts the normal negative relationship present between FBF and glucose extraction.[28] Thus, decreasing glucose extraction did not counterbalance insulin-induced vasodilation.[28]

In contrast to the above studies, several reports have indicated that flow does not play a significant role in determining glucose uptake. Neahring et al[29] found no differences in insulin-induced vasodilation between obese hypertensive insulin-resistant and lean normotensive insulin-sensitive men. Hernandez Mijares and Jensen[30] found large increases in glucose extraction, but minimal increases in leg blood flow in postprandial humans. Rosdahl et al,[31] using microdialysis, challenged whether insulin actually increases muscular blood flow. They found an increase in flow in adipose tissue, and that increased muscle glucose uptake was primarily due to increased extraction. Further, positron emission tomography shows no difference in insulin-induced distribution of muscular flow between men with type 2 diabetes and control subjects, despite the insulin resistance present in the latter.[32] In studies similar to ours, Natali et al[33] found that adenosine-induced increases in FBF led to increased basal FGU, but did not increase insulin-induced glucose uptake in obese insulin-resistant hypertensive men. Our results in healthy subjects clearly support their conclusion that vasodilation in the fasting state modestly increases muscular glucose uptake, but has no effect on glucose utilization during hyperinsulinemia.

Natali et al[33] suggested that the increase in muscular glucose uptake during the basal state is due to recruitment of oxidative muscle tissue. During hyperinsulinism in their study, as in ours, increases in FBF were counterbalanced by decreases in glucose extraction. Furthermore, unlike Jern,[28] we did not find that insulin infusion eliminates the normal negative relationship between flow and glucose extraction. Considering all the data, it is possible that, in contrast to the effect of insulin-induced sympathoexcitation, decreases in FBF may impair muscular glucose uptake, but increases above basal levels do not appear to enhance insulin-induced muscular glucose uptake.

A second possibility is that in healthy subjects flow has little effect but plays a more significant role in abnormal disease states. In support of this possibility, Hirai et al[34] found a negative correlation between the steady-state plasma glucose concentration, a measure of insulin resistance, and flow-mediated vasodilation in smokers and subjects with impaired glucose tolerance but not in control subjects.

Two limitations of our study are the fact that we did not measure bilateral flows, as discussed above, and that we did not specifically test the adequacy of the blockade achieved. A failure to achieve complete blockade might mask differences present between groups. The doses of phentolamine[16,17] and propranolol used were previously tested in other studies.[16] Furthermore the hemodynamic differences present between groups demonstrate that doses were effective. Specifically, in regards to phentolamine, it appears that IA PHEN dose had little systemic effects since changes in mean arterial pressure were not different from SAL. The dose of IA PROP may have been high, since it prevented the insulin-induced increase in heart rate, as did IV PROP.

In conclusion, our studies indicate that inhibition of basal sympathetic activity and enhancement of insulin-induced vasodilation do not increase muscular glucose uptake in normal humans. It remains possible that increased sympathetic activity above normal basal levels may play a role in suppressing insulin-induced vasodilation and insulin sensitivity in human obesity.

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