Abstract—The vasodilation induced by systemic insulin infusion is mediated by nitric oxide and is impaired both in obese subjects and patients with essential hypertension. Whether this vascular defect explains the metabolic resistance to insulin action is uncertain. In 8 overweight male patients with essential hypertension, we used the double forearm (ie, infused versus control) technique, combined with the euglycemic hyperinsulinemic clamp, to test whether sustained vasodilation (induced by intra-arterial sodium nitroprusside infusion) improves insulin-mediated glucose uptake. During the clamp, whole-body glucose disposal rose to 24.4±2.9 μmol·min⁻¹·kg⁻¹. Forearm blood flow in the control forearm was stable (3.1±0.4 versus 2.9±0.3 mL·min⁻¹·dL⁻¹), while in the infused forearm it increased from 3.4±0.5 to 10.6±1.3 mL·min⁻¹·dL⁻¹ in response to sodium nitroprusside. During insulin administration, tissue glucose extraction rose from 2±1% to 21±4% (P<.001) in the control forearm and from 2±1% to 8±3% in the infused forearm (P<.02 versus baseline for both); the calculated net glucose uptake reached similar plateaus in the two forearms (3.5±0.7 versus 3.7±0.6 μmol·min⁻¹·kg⁻¹, control versus infused, P=.6). We conclude that in overweight male patients with essential hypertension skeletal muscle insulin resistance could be overcome by circumventing the functional defect of the forearm vasculature.

Methods

Subjects

Eight male patients with essential hypertension were recruited from the Hypertension Clinic (Table). Each patient had a complete clinical workup to exclude secondary forms of hypertension, diabetes, and hepatic, renal or other endocrine diseases. All subjects were consuming a weight-maintaining diet, and their antihypertensive treatment was discontinued 3 weeks before the study. The purpose, nature, and potential risks of the study were explained to all patients, and their informed consent was obtained at recruitment. The study protocol was approved by the Institutional Review Board.

Experimental Protocol

The study began at 8:30 AM after an overnight (12 to 14 hours) fast, with the subject lying supine in a quiet room with a constant temperature of 21°C to 24°C. A Teflon catheter (20-gauge, 2-in) was inserted retrogradely into a deep vein of each forearm. The cannula was considered to be correctly placed if its tip could not be palpated and if it sampled blood with an oxygen saturation <75%. Another Teflon cannula (20-gauge) was inserted percutaneously into the brachial artery under local anesthesia (2% xylocaine). Hereinafter, the forearm instrumented with the arterial catheter will be called the infused forearm and the contralateral forearm the control forearm. Another catheter (20-gauge, 2-in) was inserted into an antecubital...
vein of the control forearm anterogradely for the infusion of insulin and glucose.

The study consisted of two periods, basal and clamp; during the basal period, four sets of blood samples were drawn from the artery and from the deep vein of both forearms for the determination of blood gases and plasma glucose. Total FBF was measured in both forearms by strain-gauge plethysmography (Vasculab Strain-Gauge Plethysmograph SPG 16, Meda Sonics) immediately after each blood sampling. Each blood flow determination was the mean of at least three consecutive measurements. Blood pressure was measured by means of a mercury sphygmomanometer immediately after each blood flow measurement. Heart rate was measured over 20-second periods by counting the arterial pulses recorded by the plethysmograph. Before each blood sampling and during blood flow measurement, blood circulation to the hand was interrupted for 2 to 3 minutes by inflation of a pediatric cuff around the wrist at suprasystolic pressure. After baseline determinations, a primed (230 pmol • kg⁻¹ over 7 minutes) and continuous (10 pmol • min⁻¹ • kg⁻¹) infusion of regular insulin was started through the superficial antecubital vein, while plasma glucose was maintained constant at basal values by means of a variable 20% glucose infusion. After the insulin prime, sodium nitroprusside (Schlavo SpA) was infused into the brachial artery at an initial rate of 1 μg • min⁻¹. The infusion rate was then adjusted to achieve at least a doubling of basal FBF; the titration period lasted 5 to 15 minutes, and the final infusion rate ranged from 3.9 to 12.0 μg • min⁻¹. During the subsequent period, which lasted for the entire duration of the clamp, minor adjustments (≤1 μg • min⁻¹) of the infusion rate were made to maintain the achieved FBF as constant as possible.

When FBF, exogenous glucose infusion rate, and arterial plasma glucose concentration were all judged to be relatively stable (70 to 100 minutes after the start of the insulin infusion), another four sets of blood samples were collected, and FBF and blood pressure were measured at 5- to 10-minute intervals. To avoid systematic differences, the infused forearm was the dominant arm in 4 patients and the nondominant arm in the other 4 patients.

Blood and Plasma Determinations
Each blood sample was divided into 3 aliquots: (1) 1 mL was collected in heparinized syringes for the determination of plasma glucose; (2) 2 mL was collected in chilled tubes containing sodium EDTA for the determination of insulin (Insik 5, Sorin Biomedica); and (3) 1.5 mL was collected in heparinized syringes for immediate blood gas determination and oximetry (Instrumentation Laboratory [IL] System 1302 and IL 282 CO-Oxymeter).

Calculations
All forearm data are presented in four different ways: (1) arterio–deep venous (A-V) concentration difference, (2) extraction ratio (ie, (A-V)/A); (3) standard net balance calculation (total FBF × A-V concentration difference); and (4) net substrate balance divided by the oxygen balance measured in the same blood sample pair. Calculation 2 provides an index of the intrinsic efficiency with which a substrate is handled when total FBF and tissue flow partition are constant. During vasodilation, if the increased FBF results from an increased flow velocity through already perfused tissue, substrate content in a draining deep vein will increase in exact proportion to the rise in blood flow, thereby reducing the extraction ratio. Alternatively, if all of the increase in FBF results from capillary recruitment in previously unperfused or underperfused tissue, the substrate content in the deep vein will remain unchanged. Between these two extremes, variable combinations of faster blood flow and capillary recruitment will determine the actual substrate content of deep venous blood. By comparing the changes in extraction ratios with the concomitant changes in blood flow, the extent of capillary recruitment can be estimated. Calculation 3 is the standard way of expressing balance data for comparison with previous results. The rationale for calculation 4 is as follows. The greater part of forearm oxygen consumption (V˙O₂) is contributed by muscle oxidations (and is therefore reflected in deep venous oxygen content) because of the predominantly glycolytic metabolism of superficial tissues. The ratio of a substrate balance to the concomitant oxygen balance is a fully flow-independent measure (FBB cancels out in the quotient). It relates the changes in substrate handling to the concomitant level of oxygen metabolism in muscle (ie, the mass of metabolically active tissue). Thus, the substrate-to-oxygen ratio takes into account any recruitment phenomenon and corrects for basal differences in forearm muscularity and deep vein drainage.

Whole-body glucose disposal (M) was estimated by averaging the glucose infusion rates every 20 minutes and then adjusting for changes in the body glucose pool (assuming a distribution volume of 0.25 L • kg⁻¹).

Statistical Analysis
For each four sets of measurements within each study period, ANOVA for repeated measures was first performed to assess intra-individual variability. When ANOVA indicated statistically insignificant changes, the four sets of values were averaged, and paired t test analyses were then used to compare basal versus clamp and infused versus control values. ANOVA for doubly repeated measures (over the two study periods and the two forearms) was also carried out on the mean values; with this design, the effect of sodium nitroprusside was evaluated as an interaction term (forearm × study period).

Results
During the clamp, both systolic and diastolic blood pressure decreased from baseline (154±4/101±3 to 141±5/94±3 mm Hg, P<.05 for both), whereas heart rate showed a small but significant increase (from 60±3 to 63±4 bpm, P<.05). In response to the primed-continuous insulin infusion, which produced a stable plateau of 890±118 pmol • L⁻¹, whole-body glucose disposal increased as a function of time, approaching a steady state after 70 minutes (Fig 1). The M value of the last 40 minutes averaged 24.4±2.9 μmol • min⁻¹ • kg⁻¹. At baseline, blood flow in the control forearm was slightly lower compared with that in the con-

<table>
<thead>
<tr>
<th>Characteristics of the Study Population</th>
<th>Mean±SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50±3</td>
<td>34-64</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177±3</td>
<td>167-190</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>86±3</td>
<td>65-106</td>
</tr>
<tr>
<td>Body mass index, kg • m⁻²</td>
<td>27.3±0.9</td>
<td>23.3-31.3</td>
</tr>
<tr>
<td>Waist to hip ratio, cm • cm</td>
<td>0.96±0.03</td>
<td>0.92-0.99</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>153±4</td>
<td>138-164</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>102±3</td>
<td>92-114</td>
</tr>
<tr>
<td>Duration of hypertension, y</td>
<td>4.2±1.0</td>
<td>0.1-10</td>
</tr>
<tr>
<td>Serum cholesterol, mmol • L⁻¹</td>
<td>5.7±0.4</td>
<td>3.7-7.4</td>
</tr>
<tr>
<td>Serum triglycerides, mmol • L⁻¹</td>
<td>1.6±0.1</td>
<td>1.3-2.3</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol • L⁻¹</td>
<td>5.7±0.4</td>
<td>3.7-7.4</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol • L⁻¹</td>
<td>72±9</td>
<td>38-118</td>
</tr>
<tr>
<td>Clamp insulin, pmol • L⁻¹</td>
<td>890±118</td>
<td>567-1512</td>
</tr>
<tr>
<td>M value, μmol • min⁻¹ • kg⁻¹</td>
<td>24.4±2.9</td>
<td>13.6-34.8</td>
</tr>
</tbody>
</table>

*For definition of M value, see "Methods."
which remained stable throughout the clamp (except for a 10% increase from 0 to 60 minutes, during which blood flow was measured without excluding circulation to the hand). In the infused forearm, after the titration period of sodium nitroprusside (0 to 20 minutes), blood flow was maintained relatively stable throughout the remainder of the study. Over the last 40 minutes, sodium nitroprusside infusion rates ranged from 3.9 to 12.0 \( \text{mg} \cdot \text{min}^{-1} \) (mean, 9 ± 0.9 \( \text{mg} \cdot \text{min}^{-1} \)) and elicited a mean 28.6% rise in FBF with respect to baseline (Fig 1). Arterial and deep venous plasma glucose concentrations were stable during both the baseline period and the final 30 minutes of the clamp. At baseline, deep venous hemoglobin oxygen saturation in the control forearm was lower than in the infused forearm (57.7 ± 3.3% versus 69.1 ± 2.9%, \( P < .03 \)); this resulted in a greater oxygen extraction (40 ± 4 versus 29 ± 3%, \( P < .04 \)), which coupled with the lower blood flow rate yielded similar rates of oxygen consumption in the two forearms (10.2 ± 1.3 versus 9.2 ± 1.4 \( \text{\mu mol} \cdot \text{min}^{-1} \cdot \text{dL}^{-1} \)). During the clamp, oxygen consumption rose in both the control and infused forearms (to 12.7 ± 2.2 and 11.5 ± 1.8 \( \text{\mu mol} \cdot \text{min}^{-1} \cdot \text{dL}^{-1} \), respectively; \( P < .05 \) for both), with no significant difference between the two. Since neither blood flow nor A-V glucose and oxygen gradient changed significantly during either study period (ANOVA for repeated measures), the four determinations of the baseline period and those of the 70- to 100-minute interval were averaged. As depicted in Fig 2, with insulin glucose extraction rose more than 10-fold in the control forearm (from 1.8 ± 0.7% to 21.2 ± 3.9%, \( P < .001 \)) but only 4-fold in the infused forearm (from 1.9 ± 0.5% to 8.4 ± 2.5%, \( P < .02 \)). When glucose extraction was multiplied by the glucose input into the limb (arterial concentration \( \times \text{FBF} \)), glucose uptake rates were superimposable in the two forearms (3.5 ± 0.7 versus 3.7 ± 0.5 \( \text{\mu mol} \cdot \text{min}^{-1} \cdot \text{dL}^{-1} \), control versus infused, \( P = .6 \)). The oxygen to glucose ratios also were similar in the control and infused forearm (Fig 2). Glucose uptake was correlated with whole-body glucose disposal in each forearm (\( r = .79 \), \( P < .03 \) and \( r = .86 \), \( P < .01 \), control and infused forearm, respectively), and the slope of the two regression lines was similar (0.18 ± 0.06 and 0.17 ± 0.06 \( \text{kg} \cdot \text{dL}^{-1} \) in the control and infused forearms, respectively).

**Discussion**

In this study, a relatively high insulin infusion rate (10 \( \text{pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \)) was used to maintain A-V glucose gradients large enough to exceed the measurement error of plasma glucose (ie, 2%) under conditions of vasodilation in insulin-resistant individuals. Mean whole-body glucose disposal (24.4 \( \text{\mu mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \)) in our patients fell below the lower
20th percentile (with individual values between the 2nd and the 40th percentile) of the frequency distribution of M values in nondiabetic, normotensive, lean male subjects (n=373) selected from a large collection of clamp studies performed with use of a 30% lower insulin infusion rate (ie, 7 instead of 10 μmol·min⁻¹·kg⁻¹).16 This marked insulin resistance clearly reflects the selection of patients with moderate to severe hypertension and overweight.

Although blood supply is reputed to be a limiting factor for insulin-stimulated glucose uptake in healthy subjects as well,12 we reasoned that insulin-resistant patients would be more likely to benefit from the removal of any restraint to blood flow on account of their impaired insulin-induced vasodilation. In a previous study,9 we showed that increasing forearm perfusion with adenosine did not improve metabolic insulin resistance in overweight patients with essential hypertension. However, different vasodilatory stimuli may act at different sites (deep versus superficial tissues, small versus medium-size arterioles, nutritive versus nonnutritive vessels); in particular, adenosine might not reproduce the vasodilation that occurs during systemic hyperinsulinemia. The mechanism(s) by which insulin relaxes vascular smooth muscle have recently been investigated in a series of elegant studies, which collectively indicate that insulin-induced vasodilation depends on an adequate endothelial NO synthesis.10,11,17 The choice of sodium nitroprusside for the present experiments aimed at mimicking more closely the vasodilation elicited by insulin in normal individuals. We did not use acetylcholine to stimulate NO synthesis because both essential hypertensive patients13 and obese individuals14 have been shown to have a compromised acetylcholine-dependent vasodilation but a normal sodium nitroprusside vascular response. Because this vascular defect results from inadequate endothelial NO synthesis, direct supply of NO through intra-arterial sodium nitroprusside infusion should bypass any dysfunction of NO synthesis. In addition, it is pertinent to recall that in essential hypertension, acetylcholine-induced vasodilation is not entirely abolished by L-NMMA infusion, suggesting that this compound also activates an NO-independent pathway.15

In our experiments, particular attention was given to fulfill the requisites for the measurement of forearm metabolism from A-V balances.18 As shown in Fig 1, blood flow was measured and blood samples were collected under quasi steady state conditions for both local glucose uptake and blood flow. Strain-gauge plethysmography has been criticized because of its low accuracy. However, the good correlation between forearm and whole body glucose uptake in both forearms in our subjects indicates that the method provided sensitive and reliable estimates.

During forced vasodilation with intra-arterial sodium nitroprusside, we did not observe changes either in oxygen or in glucose uptake by forearm tissues. We can thus conclude that at high physiological levels of plasma insulin, blood flow is not rate limiting for oxygen and glucose forearm uptake in insulin-resistant patients with essential hypertension. With use of the experimental data of fractional glucose extraction and blood flow, this degree of vasodilation would be expected to induce an average 9% improvement in forearm glucose uptake according to the A-V gradient dilution effect described by Renkin.19 We failed to observe this difference; however, such a change is within the error of the balance technique, which, with our sample size, cannot detect differences lower than 15%.

From our data it emerges that forearm muscle metabolism is essentially independent of changes in FBF above baseline values: the increased blood flow simply dilutes the forearm gradient so that the product of FBF and glucose (ie, glucose uptake) remains approximately constant. Consequently, the relationship between FBF and glucose extraction, drawn in the same individual and for the same tissue, should take the form of a hyperbola. We indirectly tested this hypothesis by plotting the observed values of FBF and forearm glucose extraction for each patient (relative to the control forearm) (Fig 3). It can be appreciated that despite the between-subject and between-forearm variability, the experimental results fit the expected hyperbolic relationship reasonably well. This test provides further support for the conclusion that forcing vasodilation by directly providing NO to muscle tissues does not affect their intrinsic ability to extract glucose from plasma in response to physiological hyperinsulinemia. We recognize that our study group consisted of only a small number of male, middle-aged, overweight patients; therefore, we cannot exclude that specific subgroups of hypertensive patients with different clinical characteristics may respond differently to sodium nitroprusside infusion. In addition, flooding vascular tissues with NO may not correct the endothelial defect of essential hypertension and may not reproduce the physiological insulin-induced NO synthesis/release. Further experiments using different pharmacological tools and/or experimental designs are needed before the issue is definitely resolved. With this proviso, we conclude that in insulin-resistant subjects, increasing whole-tissue perfusion via an NO donor does not facilitate insulin action.

References


Vasodilation With Sodium Nitroprusside Does Not Improve Insulin Action in Essential Hypertension
Andrea Natali, Alfredo Quiñones Galvan, Neda Pecori, Giovanna Sanna, Elena Toschi and Ele Ferrannini

Hypertension. 1998;31:632-636
doi: 10.1161/01.HYP.31.2.632

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/31/2/632

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/