Insulin-Mediated Vasodilation and Glucose Uptake Are Functionally Linked in Humans

Stephen J. Cleland, John R. Petrie, Shinichiro Ueda, Henry L. Elliott, John M.C. Connell

Abstract—Intra-arterial infusion of insulin in physiological doses causes forearm vasodilation which is augmented by co-infusion of D-glucose, leading us to speculate that local insulin-mediated vasodilation may depend on insulin-mediated glucose uptake. We have examined the relationship between whole-body insulin sensitivity and forearm vasodilation in response to local infusion of insulin/glucose, thus avoiding any confounding effects of sympathetic stimulation on peripheral blood flow. Eighteen healthy, normotensive male volunteers (age, 26±5.4 years) attended on two separate occasions for measurement of: (1) whole-body insulin sensitivity with use of the hyperinsulinemic euglycemic clamp; (2) forearm vasodilation in response to an intra-arterial infusion of insulin/glucose with use of bilateral venous occlusion plethysmography. Insulin-mediated glucose uptake (M) for the group (mean±SD) was 10.0±2.2 mg·kg⁻¹·min⁻¹, and the percentage change in forearm blood flow ratio (%FBFR) for the group (median, interquartile range) was 28.2% (13.6, 48.6). In univariate analysis, M was significantly correlated with %FBFR (r=0.60, P<0.05), but not with body mass index (BMI) (r=−0.42), age (r=−0.39) or mean arterial pressure (r=0.13). In multiple regression analysis, %FBFR remained a significant independent predictor of M (R²(adj)=0.48, t=3.23, P<0.01) in a model involving BMI, age, and blood pressure. These data support the concept of a significant functional relationship between insulin’s metabolic and vascular actions, possibly at an endothelial level. (Hypertension. 1999;33(part II):554-558.)

Key Words: insulin resistance ■ endothelial function ■ forearm blood flow ■ human study ■ euglycemic clamp ■ plethysmography

Epidemiological studies have demonstrated a link between metabolic disorders (such as obesity and noninsulin-dependent diabetes mellitus [NIDDM]), and cardiovascular diseases such as essential hypertension, congestive cardiac failure, and atherosclerosis. These disorders share two important pathophysiological features, namely relative resistance to insulin-mediated glucose uptake and vascular endothelial dysfunction, characterized by reduced basal and stimulated endothelial nitric oxide production. However, the underlying mechanism(s) and significance of this association remain unclear.

Recently, the concept of crosstalk between insulin’s vascular and metabolic actions has been emerging. Specifically, it is proposed that insulin may redirect nutrient blood flow to nutritive capillary beds in skeletal muscle. Given that insulin largely appears to be an endothelium-dependent vasodilator, it seems likely that this linkage between glucose metabolism and blood flow distribution occurs at an endothelial level. Indeed, there is considerable in vitro evidence demonstrating interactions between insulin and nitric oxide synthase as well as emerging evidence that metabolic and vascular control pathways interact in other tissues. However, physiological studies in humans in relation to insulin and regulation of blood flow have been less clear; although some studies have demonstrated a relationship between insulin resistance and endothelial dysfunction, others have not. In addition, there is considerable doubt concerning whether insulin sensitivity and insulin-mediated vasodilation are functionally associated. Those studies that have shown a relationship have used higher doses of insulin and the results are thus of questionable physiological significance.

We hypothesized that the discrepancy between in vitro and in vivo data on the relevance of insulin-mediated vasodilation to insulin-mediated glucose uptake might be attributable to the confounding effect of sympathetic nervous system activation during systemic hyperinsulinemia. In this setting, local vasodilator effects are likely to be influenced by centrally mediated mechanisms. Although these effects are physiologically relevant, we chose to examine forearm responses to local intrabrachial insulin infusions to focus on local actions of the hormone. Until recently, local insulin infusions were thought to have either no effect on limb blood flow or only a very weak influence. However, we have demonstrated in humans that forearm vasodilation during local physiological insulin infusions is significantly...
augmented by co-infusion of intra-arterial D-glucose. This was not simply an osmotic effect because co-infusing L-glucose had no effect. This result led us to hypothesize that insulin-stimulated tissue glucose uptake is a determinant of insulin-mediated vasodilation. In the present study, we set out to examine the relationship at physiological insulin concentrations between local insulin/glucose forearm vasodilation and whole-body glucose uptake during systemic hyperinsulinemia.

Methods

Subjects

Eighteen healthy, normotensive male volunteers aged 18 to 37 years participated in this study, which was approved by the Ethics Committee of the West Glasgow Hospitals University NHS Trust. All subjects gave informed consent, and the procedures followed were in accordance with Ethics Committee guidelines. No subjects were taking medication, and all abstained from alcohol, tobacco, and strenuous physical activity for 24 hours and from food and caffeine-containing drinks overnight before the 2 study days, which were at least 1 week apart. At a screening visit, physical health was confirmed by history and physical examination and supine blood pressure was measured in triplicate (Dinamap Critikon, Johnson and Johnson Professional Products Ltd). Subject characteristics included age 26±5.4 years, mean arterial pressure (MAP) 89±8.8 mm Hg (mean±SD) and body mass index (BMI) 23.2 (21.9, 26.3) kg/m² (median, interquartile range); 2 subjects were smokers; median alcohol consumption was 20 g/week.

Clinical Procedures

Measurement of Insulin Sensitivity

Subjects attended for measurement of whole-body insulin sensitivity (M) by use of a 180-minute hyperinsulinemic euglycemic clamp. A primed continuous infusion of soluble insulin (10.8 pmol · kg⁻¹ · min⁻¹; Novo-Nordisk UK) was administered along with a variable rate infusion of 20% dextrose (Baxter Health Care) adjusted manually to maintain serum glucose at 5.2 mmol/L on the basis of arterialized samples withdrawn every 5 minutes from an ipsilateral right dorsal hand vein (heated-air box at 55°C, University of Nottingham Department of Physiology and Pharmacology). The M-value was calculated during the last 40 minutes of the study.

Measurement of Insulin-Mediated Vasodilation

Forearm blood flow was measured by venous occlusion plethysmography with electrically calibrated mercury-in-silicone elastomer (Si-lastic) strain gauges (Hokanson set, PMS Instruments). A 27-gauge unmounted steel needle (Cooper’s Needleworks) was inserted under local anesthesia into the brachial artery of the nondominant arm for infusions. A venous catheter was positioned retrogradely in an antecubital vein to enable half-hourly blood sampling for insulin, glucose, and potassium levels. Temperature was maintained at 24 to 26°C. Blood flow was recorded in both forearms during 3-minute periods of wrist cuff inflation at 10-minute intervals; each measurement was the mean of 5 sequential recordings.

After baseline readings had been obtained, subjects received an intra-arterial infusion of low-dose D-glucose (75 μmol/min) for 120 minutes to maintain local venous euglycemia in the forearm vascular bed. After 30 minutes, and for the remaining 90 minutes, soluble human insulin (Actrapid, Novo Nordisk) was co-infused at a dose of 36 pmol/min. This solution was prepared in the sterile unit of the hospital pharmacy with use of glass syringes and bottles, and diluted (with saline and 4 mL of the subject’s blood, 8% vol/vol) at the bedside immediately before each study (mean recovery of insulin was 95%; data not shown). In a previous study, we demonstrated that deep venous glucose levels in the contralateral arm were unchanged by this dose of insulin, confirming that there is unlikely to be any significant systemic action of insulin. A uniform infusion rate (2 mL/min) was used throughout the study.

Statistical Evaluation

For the forearm plethysmography data, percentage change from basal values in the ratio of blood flow between infused and noninfused arms was calculated, with the blood flow in the noninfused arm as a concurrent control. To avoid multiple comparisons, a summary measure was calculated which was the mean value of the last 3 readings (100 to 120 minutes). Data were initially examined by simple correlation. If data were nonparametrically distributed, the rank correlation coefficient was calculated. Multiple regression analysis (Minitab for Windows, Minitab Inc.) was performed to examine potential confounders. Insulin sensitivity, age, and MAP data were normally distributed; insulin vasodilation and BMI data were nonparametrically distributed and were therefore logarithmically transformed before analysis. After identification of the best subsets in the initial model, parameters were added in a forward stepwise fashion.

Results

The procedures were performed without complication and were well tolerated by all subjects. During plethysmography, deep venous potassium concentration was not significantly different from baseline in response to intra-arterial infusion of insulin and glucose.

Insulin Concentrations

During the euglycemic clamps, systemic insulin concentrations were raised to 617±99.7 pmol/L during the last hour. During the local insulin infusions, insulin levels in the forearm vascular bed were raised to 782±279.8 pmol/L during the last hour.

Whole-Body Insulin Sensitivity

M-value (mean±SD) for the group was 10.0±2.2 mg · kg⁻¹ · min⁻¹ (range, 6.8 to 14.7).

Local Insulin/Glucose-Mediated Vasodilation

Percentage change in forearm blood flow ratio (%FBFR) as a summary measure was calculated which was the mean value of the last 3 readings (100 to 120 minutes). %FBFR remained a significant independent predictor of M value after these potentially confounding variables were included in the model (R² [adj]=0.48, t=3.23, P<0.01).

Univariate Analysis

Depending on the distribution of the data, simple (r) or rank correlation (rₛ) analysis was performed. M value was significantly correlated with %FBFR (rₛ=0.60, P<0.05) (Figure), but not with BMI (rₛ=−0.42, P>0.1), age (rₛ=−0.39, P>0.2), or MAP (rₛ=0.13, P>0.2). %FBFR was not significantly correlated with BMI (rₛ=−0.15, age (rₛ=0.16), or MAP (rₛ=0.15) (P>0.2).

Multiple Regression Analysis

The Table displays adjusted R² and t for %FBFR as a predictor of M value, with forward stepwise addition of BMI, age, and MAP. %FBFR remained a significant independent predictor of M value after these potentially confounding variables were included in the model (R² [adj]=0.48, t=3.23, P<0.01).

Discussion

This study is the first to examine the relationship between whole-body insulin sensitivity and insulin-mediated vasodi-
Insulin Vasodilation vs Insulin Sensitivity

finding, however, the same study demonstrated a significant relationship between insulin-induced leg vasodilation and whole-body insulin sensitivity during systemic hyperinsulinemia,22 and the overall conclusion from these studies remains uncertain. In a more recent study in which higher resolution methods were used, the same group was able to detect co-localization of insulin-stimulated muscle blood flow with regional glucose uptake, consistent with redirection of flow to areas where insulin stimulates glucose uptake.36 Our data, which demonstrate a close correlation between direct insulin/glucose-induced arterial vasodilation and whole-body insulin-stimulated glucose uptake, also support this hypothesis.

We have observed that local insulin-mediated vasodilation is significantly augmented by co-infusion of n-glucose and have hypothesized that insulin-stimulated glucose uptake may determine muscle blood flow via crosstalk with nitric oxide pathways at an endothelial level. For example, an increase in ATP production caused by aerobic glycolysis could activate membrane ion pumps (for example Na\(^+-K^+\) ATPase resulting in hyperpolarization and changes in Ca\(^{2+}\) fluxes in both endothelium and vascular smooth muscle cells.37 In support of this notion we, and others, have recently shown that insulin-mediated vasodilation can be inhibited by ouabain, an inhibitor of Na\(^+-K^+\) ATPase.38,39 Furthermore, clamping glucose at 20 mmol/L during systemic hyperinsulinemia (and hence increasing tissue glucose uptake) causes 50% augmentation of insulin-stimulated blood flow in humans.40

Several in vitro studies provide additional evidence that glucose uptake may be important in determining vascular smooth muscle cell relaxation.13,14,41 Of particular note is a study demonstrating nitric oxide production secondary to insulin stimulation in endothelial cell culture and subsequent blocking of this in the presence of an inhibitor of phosphatidylinositol 3-kinase.15 The delayed time course of insulin-mediated vasodilation would also support this hypothesis: the vasodilator effect of the hormone is only evident after 30 to 40 minutes, consistent with primary effects to increase glucose uptake and metabolism. One pathophysiological implication of this hypothesis would be that a primary derangement of insulin-mediated glucose uptake would lead to reduced insulin-mediated vasodilation (and, possibly, effects on other aspects of vascular endothelial function), so providing a mechanism for vascular dysfunction in insulin-resistant subjects. The absence of a change in blood flow

### Multiple Regression Models With Insulin Sensitivity (M) as Dependent Variable

<table>
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<th>Model</th>
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<th>Age</th>
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<th>MAP</th>
<th>Adjusted (R^2)</th>
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Prediction of insulin sensitivity (M) from percentage change in forearm blood flow ratio in response to local insulin infusion (insulin vasodilation), age, BMI, and MAP. Changes are shown as adjusted \(R^2\) and \(t\) for insulin vasodilation, with addition of age, BMI, and MAP into the model. Predictor variables: model 1, insulin vasodilation; model 2, insulin vasodilation + age; model 3, insulin vasodilation + age + BMI; model 4, insulin vasodilation + BMI + age + MAP.
during a systemic infusion of fructose could be cited as an argument against this hypothesis, but does not rule out the possibility that the coupling signal for promotion of blood flow is unique to intracellular glucose metabolism.

The alternative physiological hypothesis, originally advanced by Baron, is that insulin-mediated vasodilation promotes insulin-mediated glucose uptake. This has the pathophysiological corollary that endothelial dysfunction, resulting in a relative inability of mediators including insulin to stimulate muscle blood flow, could account for resistance to insulin-mediated glucose uptake in some disorders. However, this hypothesis assumes that substrate delivery can be rate-limiting for glucose disposal, which may only be the case at supraphysiologic insulin levels maintained for many hours. Results of studies in which glucose uptake has been measured during hyperinsulinemia and manipulation of limb blood flow are conflicting. For example, it has been reported that infusion of N\(^-\)monomethyl-l-arginine (L-NMMA) into the femoral artery during hyperinsulinemic euglycemia reduces insulin-mediated stimulation of blood flow (via inhibition of endothelial nitric oxide production) and decreases limb glucose uptake by 25\% (despite a 50\% increase in arteriovenous fractional glucose extraction). Other investigations have failed to support the notion that tissue glucose uptake is dependent on skeletal muscle blood flow.

Dissociation of insulin-mediated glucose uptake from blood flow was observed in a study in which both limb blood flow and muscle sympathetic nerve activity were increased by 100\% with use of intra-arterial bradykinin has no detectable effect on forearm glucose uptake. However, it has been argued that these vasodilators (in contrast to insulin) may not recruit areas of tissue that were previously involved in predominantly anaerobic metabolism.

Because correlation is not proof of causality, the possibility remains that insulin’s metabolic and vascular actions are not causally related in either direction but are influenced by a third factor. For example, obesity could impair insulin-mediated glucose uptake and vascular endothelial function by two separate mechanisms. It has been shown that obese insulin-resistant subjects are characterized by endothelial dysfunction and resistance to endothelium-dependent insulin-mediated vasodilation, and that there is no difference in these variables in a group of patients with NIDDM, matched for age and BMI. These results suggest that obesity may play a primary role in the link between insulin resistance and endothelial dysfunction, and it may help to explain why results obtained in obese NIDDM and hypertensive patient groups cannot necessarily be extrapolated to patients with a normal BMI. However, the relationship between insulin-mediated vasodilation and glucose uptake was not confirmed by BMI in the current study.

In summary, we report in healthy male volunteers a significant functional relationship between insulin sensitivity and local insulin-mediated vasodilation in an experimental setting that avoids the confounding hemodynamic effects of systemic hyperinsulinemia. Further mechanistic studies are required to tease out the nature of the crosstalk between insulin signaling, glucose transport, and the endothelial nitric oxide pathway. These studies may elucidate the relationship between insulin resistance and endothelial dysfunction in the early stages of cardiovascular and metabolic disorders before the development of complications.

Acknowledgments

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