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 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, r²=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.¹ These disorders share two important pathophysiological features, namely relative resistance to insulin-mediated glucose uptake²–⁵ and vascular endothelial dysfunction, characterized by reduced basal and stimulated endothelial nitric oxide production.⁶–⁹ 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.¹⁰ 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,¹¹,¹² 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,¹³–¹⁵ as well as emerging evidence that metabolic and vascular control pathways interact in other tissues.¹⁶,¹⁷ 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,¹⁸,¹⁹ others have not.²⁰ In addition, there is considerable doubt concerning whether insulin sensitivity and insulin-mediated vasodilation are functionally associated.²¹–²² Those studies that have shown a relationship²³,²⁴ 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.²⁵,²⁶ 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 flow²⁷–²⁹ or only a very weak influence.³⁰,³¹ However, we have demonstrated in humans that forearm vasodilation during local physiological insulin infusions is significantly

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augmented by co-infusion of intra-arterial D-glucose.32 This
was not simply an osmotic effect because co-infusing
D-glucose had no effect.32 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 vasodi-
lation 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.35 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 manu-
ally to maintain serum glucose at 5.2 mmol/L on the basis of
strenuous physical activity for 24 hours and from food and caffeine-
containing drinks overnight before the 2 study days, which were at
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Local Insulin/Glucose-Mediated Vasodilation
Forearm blood flow was measured by venous occlusion plethysmog-
raphy with electrically calibrated mercury-in-silicone elastometer (Si-
lastic) strain gauges (Hokanson set, PMS instruments).34 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 measure-
ment 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
depth 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.32 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 nonin-
fused arm as a concurrent control.34 To avoid multiple comparisons,
a summary measure was calculated which was the mean value of the
last 3 readings (100 to 120 minutes).35 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 logarithmi-
tically 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,
depth 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 concentra-
tions 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
significant 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

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

<table>
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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 supraphysiological 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 Nω-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). However, 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 stimulated to similar degrees at different systemic insulin concentrations, ranging from 100 to 400 pmol/L. In addition, augmentation of forearm blood flow by 100% with use of intra-arterial adenosine and 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 observed 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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