Evidence for physiological coupling of insulin-mediated glucose metabolism and limb blood flow

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The vasodilatory effects of insulin are now well recognized (5, 13, 31) and have been observed with a variety of techniques in many vascular beds with insulin concentrations ranging from physiological to pharmacological (3, 4, 13, 40). Less well appreciated is a similar effect of increased glucose concentrations, in the setting of fixed insulin concentrations, to augment blood flow (6, 36). Glucose does not have this effect in the absence of hyperinsulinemia (18), and the effect is seen with D-glucose but not with L-glucose (36). These findings suggest that the rate of cellular metabolism of glucose is coupled with the changes in blood flow (substrate delivery) and that insulin action has an important role in this coupling.

Couples of insulin-mediated glucose metabolism and blood flow have been previously investigated with conflicting results. Broadly, in situations where vasodilatory effects of insulin have been clearly evident, concurrent changes in metabolism (measured as glucose uptake, oxidation rate, or both) have been present (as reviewed in Ref. 12). In studies where vasodilatory effects of insulin were not apparent, not surprisingly, such parallel changes could not be demonstrated (27, 38). The complementary approach of enhancing glucose uptake through mass action, i.e., by increasing the prevailing concentrations of glucose in the presence of insulin (6, 36), has also shown parallel changes in rates of blood flow and glucose metabolism. No comparisons of glucose- and insulin-driven glucose metabolism and blood flow have been reported.

The question of physiological coupling of blood flow and metabolism is important but remains unresolved (13). If blood flow rates are proportional to rates of metabolism, a given rate of metabolism (achieved through manipulations of applied insulin and glucose) should be accompanied by a physiologically matched rate of blood flow. This should apply in each individual, but moreover, if the coupling between glucose metabolism and blood flow is robust, it should extend across subjects exhibiting a range of insulin sensitivity. To this end, we compared rates of leg blood flow (LBF) in insulin-sensitive lean (L), insulin-resistant obese nondiabetic (OB), and obese diabetic (Type 2 DM) subjects at a single rate of glucose disposal achieved with various combinations of steady-state insulin and glucose concentrations utilizing the glucose clamp technique.

METHODS

Data from two previously published studies (6, 24) were reexamined for the current analysis. These studies had equivalent recruitment criteria and definitions of lean [body...
mass index (BMI) ≤ 27], obese (BMI > 27), and obese type 2 diabetes (National Diabetes Data Group criteria). Both studies applied the leg-balance technique, using a thermodilution catheter to measure blood flow rates, as previously described (24). Parallel study designs were employed using stepped clamp protocols. In the first study, subjects underwent systemic euglycemic hyperinsulinemic clamps (EH) at increasing insulin doses (L, 10–300 mU·m⁻²·min⁻¹; OB, 20–600 mU·m⁻²·min⁻¹; Type 2 DM, 40–1,200 mU·m⁻²·min⁻¹) (24). In the second study, subjects underwent graded systemic hyperglycemic clamps (4, 7, 12, and 20 mmol/l, with a fixed insulin dose of 120 mU·m⁻²·min⁻¹, HH) (6) and concurrent somatostatin (SRIF) infusion to suppress endogenous insulin secretion. For both studies, the first clamp stage lasted 240 min, with measurements performed during the last 30 min. Each subsequent stage lasted 90 min, with measurements performed 60 min after the increase in insulin or glucose infusion rate. Therefore, a range of glucose disposal rates (GDRs) was established for each individual in these studies, by raising either the insulin level during fixed euglycemia or the glucose level during fixed hyperinsulinemia. The HH protocol was designed to provide sufficient insulin to suppress endogenous glucose production and near-maximally increase tissue permeability to glucose. Glucose- and insulin-driven increases in GDR are not physiologically comparable, so all analyses were carried out in parallel on both studies rather than on combined data.

The GDR was calculated under steady-state conditions from the glucose infusion rates at each stage in the study designs. In the HH study, these calculations were corrected for rates of urinary glucose loss. No measure of hepatic glucose output (HGO) was employed in the HH study, because the 120-mU·m⁻²·min⁻¹ doses of insulin in conjunction with hyperglycemia were assumed to completely suppress HGO (23). In the EH study, the GDR was derived isotopically by use of the variable tracer technique (13-H)glucose to assess basal and overall rates of glucose appearance (19). With use of the Fick principle, limb glucose uptake was calculated from the leg arteriovenous glucose balance and the measured rate of blood flow (41). Importantly, the measurements of flow and whole body glucose uptake are completely independent; therefore, comparisons and correlations can be investigated. GDR was expressed in relation to body surface area to normalize for the different degrees of obesity between study groups. More detailed measures of body composition were not available for the subjects in these studies.

Selection of GDR for analysis. In both studies, differential sensitivity to the effects of glucose and/or insulin was observed across the three subject groups. This necessitated careful selection of the GDR for analysis to choose a single GDR common to all patient groups, one that fell on the steep portion of each group’s dose-response curve, rather than near the plateau, to avoid the confounding effect of differences in maximum velocity (6). With this in mind, a GDR of ~2,000 μmol·m⁻²·min⁻¹ was selected for evaluation. The majority of subjects achieved GDRs near this value with one of the clamp stages, and for each subject category, this GDR was on the linear portion of the dose-response curve. For each subject, the observations recorded during this clamp stage were included for analysis. The rate of blood flow observed under the conditions that produced this GDR was the primary endpoint for statistical analysis.

In the EH study, baseline data (including HGO as above) were collected at each subject’s ambient fasting glucose and insulin concentrations, and this steady state was also compared between groups. Furthermore, to confirm the extension of this relationship observed at a single GDR across the full range of GDRs, we analyzed and compared the continuous relationships between blood flow and GDR among all patient groups using the complete EH and HH datasets.

Statistics. Within each study, ANOVA was used to compare flow responses across the three study groups. Continuous relationships were compared between groups and between studies using multivariate ANOVA. Two-tailed P values < 0.05 were taken as statistically significant.

RESULTS

Study subjects. The characteristics of the study subjects are presented in Table 1. Of note, the two studies included very similar patients within each group, and in both studies, the OB and Type 2 DM subjects were well matched. For two lean subjects in the EH study, the lowest stimulated GDRs were >3,000 μmol·m⁻²·min⁻¹, and data from these two subjects were not included in the main analysis. Otherwise, data were available under conditions providing a GDR of ~2,000 μmol·m⁻²·min⁻¹ from each patient in both studies, and these data were selected for analysis. These results are presented in Fig. 1 and Table 2.

EH clamps. The target GDR of ~2,000 μmol·m⁻²·min⁻¹ was observed at different hyperinsulinemic levels during the euglycemic (4 mmol/l) clamps across the study population: for L subjects, 40 (n = 5) and 300 (n = 1) mU·m⁻²·min⁻¹ were required; OB subjects achieved this GDR under 100 (n = 5) and 300 (n = 1) mU·m⁻²·min⁻¹; and Type 2 DM subjects required 300 (n = 2) or 1,200 (n = 4) mU·m⁻²·min⁻¹ (Fig. 1 and Table 2). Under these conditions, the observed rate of LBF was ~0.4 l/min, without a difference among the three groups (P = 0.72). These conditions also produced matched rates of leg glucose uptake (LGU, calculated as LBF × arteriovenous glucose difference): L, 64.4 ± 4.5 μmol/min; OB, 57.1 ± 5.1; Type 2 DM, 49.2 ± 8.0, P = 0.22. A parallel analysis of the LBF

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>Type 2 DM</th>
</tr>
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<tbody>
<tr>
<td>Euglycemic hyperinsulinemic clamp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>33 ± 2</td>
<td>37 ± 2</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68 ± 3</td>
<td>94 ± 3</td>
<td>103 ± 9</td>
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<td>Body mass index, kg/m²</td>
<td>22 ± 1</td>
<td>30 ± 1</td>
<td>33 ± 3</td>
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<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>4.5 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>35 ± 6</td>
<td>92 ± 24</td>
<td>231 ± 58</td>
</tr>
<tr>
<td>%Glycohemoglobin</td>
<td>12.5 ± 1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemic hyperinsulinemic clamp</td>
<td></td>
<td></td>
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<tr>
<td>Age, yr</td>
<td>36 ± 2</td>
<td>39 ± 2</td>
<td>44 ± 1</td>
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<td>Weight, kg</td>
<td>67 ± 2</td>
<td>100 ± 7</td>
<td>109 ± 8</td>
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<td>Body mass index, kg/m²</td>
<td>22 ± 1</td>
<td>33 ± 2</td>
<td>36 ± 2</td>
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<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>5.0 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>13.0 ± 1.0</td>
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<tr>
<td>Fasting insulin, pmol/l</td>
<td>36 ± 7</td>
<td>131 ± 23</td>
<td>244 ± 67</td>
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<tr>
<td>%Glycohemoglobin</td>
<td>12.0 ± 1.2</td>
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Results are presented as means ± SE. For euglycemic hyperinsulinemic clamp, n = 6/group; for hyperglycemic hyperinsulinemic clamp, n = 7/group. DM, diabetes mellitus.
response was carried out using GDR data expressed per kilogram (matching to \(-50 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)), which resulted in a similar but not identical selection of data for analysis from each patient and produced identical results (data not shown).

**HH clamps.** The HH conditions that produced a GDR of \(\sim 2,000 \, \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}\) were achieved at various glycemic levels during the 120-mU \cdot \text{m}^{-2} \cdot \text{min}^{-1} \) insulin infusion. For L, they were \(4 \, (n = 6)\) and \(7 \, (n = 1)\) mmol/l glucose; for OB, \(4 \, (n = 4)\) and \(7 \, (n = 3)\) mmol/l glucose; and for Type 2 DM, \(7 \, (n = 8)\), \(12 \, (n = 2)\), and \(20 \, (n = 1)\) mmol/l glucose. A GDR of \(\sim 2,000 \, \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}\) was associated with a LBF rate of \(\sim 0.4 \, \text{l/min}\) in all groups \((P = 0.71; \text{Fig. 1 and Table 2})\). LGU was also matched under these conditions \((L, 65.9 \pm 11.3; \text{OB}, 49.5 \pm 13.8; \text{Type 2 DM}, 65.2 \pm 12.8, P = 0.66)\). Similar to the EH study data, expressing the GDR per kilogram before data selection yielded a similar selection of data and identical results (data not shown).

**Fasting steady-state GDR and LBF.** To confirm the existence of this relationship under other metabolic conditions, two further analyses were undertaken. First, the relationship between LBF and GDR was compared under basal (unstimulated) fasting steady-state conditions. These data were available from the baseline period of the EH study. The spontaneous insulin and glucose concentrations were markedly different across groups \((\text{insulin: L, } 30 \pm 5; \text{OB, } 76 \pm 21; \text{Type 2 DM, } 254 \pm 47 \, \text{pmol/l}; \text{glucose: L, } 5.2 \pm 0.2; \text{OB, } 5.1 \pm 0.2; \text{Type 2 DM, } 10.4 \pm 1.4 \, \text{mmol/l})\), consistent with the known physiology of states of insulin resistance. Under these endogenous basal conditions, GDRs were well matched \((L, 486 \pm 28; \text{OB, } 472 \pm 41; \text{Type 2 DM, } 494 \pm 56 \, \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}, P = 0.93)\), and the corresponding basal rates of LBF were also well matched \((L, 0.22 \pm 0.03; \text{OB, } 0.25 \pm 0.02; \text{Type 2 DM, } 0.27 \pm 0.02 \, \text{l/min}, P = 0.36)\).

**Continuous relationship between GDR and LBF.** Second, in both complete data sets, the continuous relationship of GDR and LBF was compared among the study groups \((\text{Fig. 2})\). The slope of this relationship reflects the coupling of blood flow and metabolism, and the observed slopes are presented in Table 3. Within each study, there was no statistical difference between the slopes observed in the three study groups \([\text{EH, } -8.5 \times \text{min}^{-1}]\).

| Table 2. Observations under conditions providing a GDR of \(\sim 2,000 \, \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}\) |  |  |  |
|---|---|---|---|---|---|---|
|  | Euglycemic Hyperinsulinemic Clamp |  | Hyperglycemic Hyperinsulinemic Clamp |  |  |
|  | Lean | Obese | Type 2 DM | \(P\) Value | Lean | Obese | Type 2 DM | \(P\) Value |
| GDR, \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}\) | 1,892 \pm 141 | 2,076 \pm 104 | 1,852 \pm 129 | 0.42 | 1,901 \pm 64 | 1,963 \pm 156 | 2,100 \pm 103 | 0.41 |
| Insulin, pmol/l | 1,443 \pm 805 | 11,050 \pm 5,771 | 39,418 \pm 2,879 | 0.038 | 1,364 \pm 240 | 2,106 \pm 249 | 2,445 \pm 213 | 0.27 |
| Glucose, mmol/l | 4.99 \pm 0.17 | 4.96 \pm 0.17 | 5.00 \pm 0.09 | 0.98 | 4.99 \pm 0.52 | 5.82 \pm 0.84 | 11.19 \pm 1.53 | 0.005 |
| LBF, l/min | 0.42 \pm 0.03 | 0.43 \pm 0.03 | 0.38 \pm 0.07 | 0.72 | 0.44 \pm 0.04 | 0.39 \pm 0.05 | 0.41 \pm 0.04 | 0.71 |

Results are presented as means \pm SE. GDR, glucose disposal rate; LBF, leg blood flow.
this coupling should be evident across the range of insulin sensitivity. In subjects with widely varying insulin sensitivity to the vascular and metabolic actions of insulin, we found that, when conditions were applied that resulted in equivalent rates of glucose metabolism, equivalent rates of LBF were, in fact, evident across all subject groups. These data provide additional evidence for functional coupling between insulin-mediated glucose metabolism rate and vasodilation.

The specific clamp conditions that produced the target GDR differed across and within subject groups, as expected. Importantly, the stepped sequential nature of the study design is such that the responses observed at low insulin infusion rates were at earlier time points in the studies. Therefore, the recognized interaction of time and insulin dose for vascular and metabolic effects is not a confounder and, if anything, biased the studies against finding equal responses. This supports the focus on insulin effect (metabolic and/or vascular), rather than insulin concentration, as a physiological endpoint.

It is interesting that this apparent relationship between insulin-mediated glucose metabolism rate and vasodilation also held true under basal conditions, where, presumably, each patient satisfies his or her basal metabolic needs through endogenous homeostatic adjustments of serum insulin and glucose. Furthermore, when data across the range of GDRs observed under both EH and HH conditions were analyzed, the linear relationships between LBF and GDR did not differ between groups. Together, these argue that these observations are not likely to reflect an artifact of the particular clamp conditions chosen for analysis and that insulin-mediated increments in blood flow and GDR are closely and precisely coupled. The observation of this relationship across subjects exhibiting a range of insulin sensitivity is novel, and strengthens the previous literature suggesting the existence of this link.

**Metabolism as a physiological endpoint.** The focus on insulin-mediated metabolism rate, rather than on insulin concentration, as a major determinant of the rate of limb (skeletal muscle) blood flow is useful in the interpretation of the previous literature exploring the vascular aspects of insulin action. In particular, this focus provides insight into reports which at first seem to argue against physiological coupling. Dela et al. (15), utilizing the isoglycemic clamp technique, studied in-

![Fig. 2. Continuous relationships of metabolism and blood flow. A: EH clamp data. B: HH clamp data. Within each data set, the slopes of this relationship are not statistically different among the 3 patient categories (P = not significant, by ANOVA).](image)

<table>
<thead>
<tr>
<th>Table 3. Regression analysis of relationship between blood flow and GDR</th>
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<tbody>
<tr>
<td><strong>Euglycemic Hyperinsulinemic Clamp</strong></td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Lean</td>
</tr>
<tr>
<td>Obese</td>
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<tr>
<td>Type 2 DM</td>
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</table>

Values are means ± SE. *Significant correlation for each subject group. Within each study, the slopes of this relationship did not differ between patient groups, but the slopes were different between the 2 studies ($P < 0.001$). Flow was expressed in l/min and GDR in $\mu$mol·m$^{-2}$·min$^{-1}$. 

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**DISCUSSION**

We hypothesized that, if insulin-mediated glucose metabolism and blood flow are coupled, equivalent rates of blood flow should be observed at a given rate of glucose metabolism regardless of the particular combination of insulin and glucose applied. Furthermore,
controls on fasting glucose concentrations (Type 2 DM) and nondiabetic controls at their spontaneous insulin-mediated LBF in type 2 diabetic subjects (Type 2 DM). As suggested by the authors, this approach allows for the elevation of glucose levels to compensate for resistance to insulin-mediated glucose uptake, tending to equalize rates of glucose uptake in insulin-sensitive tissues in diabetic and control subjects. Under these conditions, there was no difference between the groups in the LBF response to insulin infusion. The baseline data of the EH protocol in our study, where subjects of varying insulin sensitivity exhibited matched rates of basal glucose uptake, reflects conditions analogous to those reported by Dela et al. Under these conditions, we also observed matched rates of LBF across the three subject groups. Thus a focus on insulin-mediated glucose metabolism as a primary determinant of insulin-mediated vasodilation may reconcile, at least partially, some of the conflicting reports in this area.

Importantly, other reports exist that argue against physiological coupling of insulin-mediated glucose metabolism and blood flow (25, 37) for which the focus on metabolism does not provide an explanation. These differences could be due to variations in technique, methodology, pharmacology, and/or biology, but the present study was not designed to explore these aspects.

Positron emission tomography (PET) would seem to be an ideal technique for addressing this issue, and some work along these lines has been reported (26, 29, 38, 39). With the use of different isotopes, simultaneous independent measures of blood flow and tissue glucose fluxes can be made. Some of these studies show insulin-induced increases in blood flow but a lack of commensurate insulin-mediated changes in whole leg glucose uptake (26, 29). Unanticipated changes in the "lumped constant," which corrects for differential cellular transport of D-glucose and [18F]fluorodeoxyglucose and is now known to be affected by insulin and other interventions (10, 17), may have confounded the measures of glucose uptake. Another possible explanation is suggested by a PET study that found that insulin-stimulated blood flow colocalized with glucose uptake (39); in other words, the coupling of flow and metabolism is evident only when regions of the leg are studied.

Physiological and pharmacological changes in metabolism and blood flow. The present findings suggest an association or coupling of insulin-mediated blood flow and glucose metabolism. Other evidence, both physiological and pharmacological, supports this coupling as well. After both an oral glucose tolerance test (7) and a more physiological standardized mixed meal (20), concordant changes in blood flow and glucose uptake have been reported, including parallel impairment in both measures in obese insulin-resistant subjects (7). In animal models (9) and in humans (1, 16), a marked synergistic effect of exercise and insulin to stimulate blood flow has been described, arguing for a contributory role of insulin in meeting the attendant increased metabolic demands. The opposite situation, bed rest, produced decreases in rates of both glucose metabolism and limb blood flow in lean healthy men (14). Coincident with the reduction in limb blood flow is a rest-induced reduction in muscle insulin sensitivity (34).

Pharmacological alterations in insulin-mediated glucose metabolism also result in concordant changes in blood flow. Impairment of local tissue sensitivity to insulin for glucose uptake, using either exogenous increases in free fatty acids (in healthy human subjects) (11, 33) or glucosamine (in wild-type rats) (21), has been shown to reduce the associated rates of insulin-stimulated blood flow. Pharmacologically improving insulin sensitivity in insulin-resistant women with the polycystic ovarian syndrome with troglitazone, a peroxisome proliferator-activated receptor-γ agonist, produced increases in both the rates of glucose uptake and LBF (28). Conversely, blocking insulin-mediated vasodilation with Nω-monomethyl-L-arginine (32) caused a reduction of both LBF and glucose uptake (8). Furthermore, decreasing blood flow with α-Met 5-hydroxytryptamine (a serotonin analog) has recently revealed a relationship of insulin-mediated changes in capillary flow distribution with muscle glucose metabolism (30). Together with the present findings, these data support an intrinsic coupling of insulin-mediated blood flow and metabolism.

These findings are specific to the insulin-stimulated state and cannot necessarily be extended to interactions of flow and metabolism under other conditions. For example, α-adrenergic blockade produced decrements in flow without appreciable change in metabolism (22), and a dissociation of flow and metabolism has been described after unilateral denervation (35). This does not detract, however, from the relevance of the coupling described in the present paper under physiological conditions.

Glucose-driven metabolism and blood flow. The conditions applied in the HH study included here provide a unique window on the physiology of the coupling of flow and metabolism. On the background of hyperinsulinemia, progressive hyperglycemia allows glucose metabolism to be driven through both insulin-dependent and insulin-independent pathways. The latter are not subject to the tight physiological control of the classical insulin-dependent pathways, and this was likely responsible for the greater variability in rates of blood flow observed with increasing glucose concentrations (Fig. 2). Also, the slopes of the relationship between GDR and blood flow differed between the EH and HH studies (Table 3), suggesting that the nature of the coupling is different under the different clamp conditions. From this, we suggest that the more tightly regulated insulin-mediated glucose uptake is reflected in a better correlation with rates of blood flow, whereas non-insulin-mediated glucose uptake is less tightly regulated, resulting in more variable rates of blood flow. These observations suggest an important specific role of insulin action in the regulated coupling of blood flow and glucose metabolism.
**Limitations.** In addition to the relationship between whole body glucose uptake and blood flow, the relationship of LGU and LBF is also of interest. Unfortunately, the parameters we used to calculate LGU include LBF itself (LGU = atreiovenous glucose difference × flow). Therefore, the current data do not allow an independent assessment of this relationship. Similarly, the question of coupling between leg muscle blood flow and leg muscle glucose uptake would be of interest, but the data collected did not include measures of body composition to allow these calculations. Given that, under insulin-stimulated conditions, the vast majority of glucose uptake is into skeletal muscle, the current finding of a coupling of independent measures of whole body glucose uptake and LBF are all the more robust.

In conclusion, we have found a significant relationship between rates of whole body glucose uptake and rates of leg blood flow under spontaneous, basal conditions and under stimulated conditions designed to increase insulin-mediated glucose metabolism. This relationship existed across populations exhibiting a wide range of insulin sensitivity, and the continuous relationships between glucose uptake and leg blood flow did not differ across subject groups despite marked differences in insulin sensitivity. Together, these data further support the notion of metabolic coupling between skeletal muscle blood flow (substrate delivery) and insulin-mediated metabolic requirements of insulin-sensitive tissues.

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