Estimations of muscle interstitial insulin, glucose, and lactate in type 2 diabetic subjects

MIKAELA SJÖSTRAND,1 AGMETA HOLMÄNG,2 LENA STRINDBERG,1 AND PETER LÖNNROTH1
1Lundberg Laboratory for Diabetes Research and 2Wallenberg Laboratory, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden

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Sjöstrand, Mikaela, Agneta Holmäng, Lena Strindberg, and Peter Lönnroth. Estimations of muscle interstitial insulin, glucose, and lactate in type 2 diabetic subjects. Am J Physiol Endocrinol Metab 279: E1097–E1103, 2000.—Previous measurement of insulin in human muscle has shown that interstitial muscle insulin and glucose concentrations are ~30–50% lower than in plasma during hyperinsulinemia in normal subjects. The aims of this study were to measure interstitial muscle insulin and glucose in patients with type 2 diabetes to evaluate whether transcapillary transport is part of the peripheral insulin resistance. Ten patients with type 2 diabetes and ten healthy controls matched for sex, age, and body mass index were investigated. Plasma and interstitial insulin, glucose, and lactate (measured by intramuscular in situ-calibrated microdialysis) in the medial quadriceps femoris muscle were analyzed during a hyperinsulimemic euglycemic clamp. Blood flow in the contralateral calf was measured by vein plethysmography. At steady-state clamping, at 60–120 min, the interstitial insulin concentration was significantly lower than arterial insulin in both groups (409 ± 86 vs. 1,071 ± 99 pmol/l, P < 0.05, in controls and 584 ± 165 vs. 1,253 ± 82 pmol/l, P < 0.05, in diabetic subjects, respectively). Interstitial insulin concentrations did not differ significantly between diabetic subjects and controls. Leg blood flow was significantly higher in controls (8.1 ± 1.2 vs. 4.4 ± 0.7 ml·100 g−1·min−1 in diabetics, P < 0.05). Calculated glucose uptake was less in diabetic patients compared with controls (7.0 ± 1.2 vs. 10.8 ± 1.2 μmol·100 g−1·min−1, P < 0.05, respectively). Arterial and interstitial lactate concentrations were both higher in the control group (1.7 ± 0.1 vs. 1.2 ± 0.1, P < 0.01, and 1.8 ± 0.1 vs. 1.2 ± 0.2 mmol/l, P < 0.05, in controls and diabetics, respectively). We conclude that, during hyperinsulinemia, muscle interstitial insulin and glucose concentrations did not differ between patients with type 2 diabetes and healthy controls despite a significantly lower leg blood flow in diabetic subjects. It is suggested that decreased glucose uptake in type 2 diabetes is caused by insulin resistance at the cellular level rather than by a deficient access of insulin and glucose surrounding the muscle cell.

insulin resistance; skeletal muscle; insulin uptake; glucose uptake; microdialysis

IN TYPE 2 DIABETES, the ability of insulin to stimulate glucose uptake in skeletal muscle is attenuated (10). The mechanisms behind the reduced insulin action are complex and include defects at the cellular level as well as decreased skeletal muscle perfusion. Muscle cells from non-insulin-dependent diabetes (NIDDM) subjects show insulin receptor and postreceptor defects (22) such as a diminished number of glucose transporter proteins and defective activation of tyrosine (insulin receptor) kinase (33). In addition, individuals with type 2 diabetes have a decreased capillary density in skeletal muscle (2, 28), and the insulin-mediated increase in blood flow is impaired (24). This, in turn, could theoretically result in an insufficient distribution of insulin and nutrients to the muscle cell. However, the pathophysiological importance of the decreased blood flow is not clear, and the concept has been challenged by investigators who have found that blood flow has only a minor influence on insulin-stimulated glucose uptake (32), skeletal muscle interstitial glucose (30), and insulin levels (16). To get a clearer picture of the pathophysiological relevance of the reduced blood flow in type 2 diabetes, we also need to evaluate the regulation of muscle insulin uptake and its putative dependence on the blood flow rate.

Earlier measurements of insulin in leg lymph (5, 39), as well as in the interstitial fluid in human subcutaneous tissue (19) and skeletal muscle (36), have shown that the interstitial insulin concentration is ~50% lower here than in arterial plasma. These data, together with the previously demonstrated existence of a receptor-mediated endocytosis route for insulin transport through endothelial cells, suggest an endothelial barrier for insulin transport, which could be rate limiting for the insulin-mediated glucose uptake in peripheral tissues. In a microdialysis study in rat muscle, a limited transcapillary transport of insulin was found in normal but not in insulin-resistant rats (14).

To our knowledge, insulin measurements in the interstitial fluid in skeletal muscle in type 2 diabetic subjects have not been performed previously. The aim of the present study was to combine blood flow measurements with microdialysis to further investigate the relation between blood flow and leg muscle interstitial insulin, glucose, and lactate levels in type 2

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diabetes. These parameters give us the opportunity to estimate and compare the apparent insulin and glucose uptake in skeletal muscle in non-insulin-dependent diabetes mellitus (NIDDM) patients and controls.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Ten volunteers with NIDDM (five males and five females) and 10 group-matched control subjects who did not take any regular medication were investigated. The clinical characteristics of the participants are listed in Table 1. Among the NIDDM patients, one was being treated with insulin, eight had oral hypoglycemic agents, and one had a combination of insulin and oral medication. They did not take their insulin or oral hypoglycemic agents on the morning of the investigation. All subjects gave their informed consent, and the study was approved by the Ethical Committee of the University of Göteborg.

**Study protocol.** The investigations started at 0800 after an overnight fast. The subjects were rested in the supine position in a room kept at 25°C. The forearm was heated with electrical pads to arterialize the venous blood (3). A polyethylene catheter was placed in a forearm vein for blood sampling. Fasting plasma glucose, plasma lactate, and plasma insulin were taken. Inulin (Inutest; Kemiflor, Stockholm, Sweden) was given as a intravenous bolus injection, followed by a constant infusion (24 ml/h) for 360 min to reach steady-state plasma inulin after 240 min (35). Thirty minutes after the inulin infusion was initiated, a euglycemic hyperinsulinemic clamp was started as described previously (12). The insulin infusion started with a primed infusion for 10 min, followed by a constant infusion rate of 120 mU·m⁻²·min⁻¹ for 120 min paralleled with glucose infusion to maintain euglycemia. Potassium chloride (0.1 mmol/l) was infused at a rate of 10 mmol/h during the clamp procedure to prevent hypokalemia. Arterial blood samples were taken every 5 min for glucose and every 30 min for lactate, insulin, and inulin. Each sample was immediately centrifuged at 4°C and stored at −18°C before analysis.

The principle of muscle microdialysis has been described in detail previously (26, 36). Briefly, two microdialysis catheters (12 × 0.5 mm, 100-kDa molecular cut-off, and 16 × 0.5 mm, 40-kDa molecular cut-off; CMA microdialysis, Stockholm, Sweden) were inserted at an angle of 45° (through the steel mandrin of a 20-gauge cannula) in the medial quadriceps muscle. The inlet of the catheters was connected to a microinjection pump (CMA 100; CMA), and the perfusion fluid was isotonic saline with addition (for glucose and lactate measurements) of 1.5 mmol/l glucose, 200 μmol/l lactate, [³H]glucose, and [¹⁴C]lactate, and for insulin and inulin measurements 1% albumin and 1.5 mmol/l glucose. The flow rate was 2.5 μl/min (in the catheter for glucose and lactate measurements) and 1.0 μl/min (in the catheter measuring insulin and inulin). Insulin and inulin, being larger molecules than glucose and lactate, were perfused at a lower rate to allow a greater exchange between the perfusate and the interstitial fluid. After a 30-min equilibration, the dialysates for glucose and lactate measurements were collected at 15-min intervals and for insulin and inulin during the last 60-min interval during the clamp. The clamp continued for 360 min to assure steady state for plasma inulin levels.

The calibration of the microdialysis probes measuring lactate and glucose was done according to the internal reference or the “hot loss” technique (27). [³H]glucose (5 μCi/ml) and [¹⁴C]lactate (200 μCi/ml) (New England Nuclear, Boston, MA) were added to the perfusate. The concentration of tracer in the perfusate was 0.1 mmol/l for glucose and 20 μmol/l for lactate. In each subject, the amounts of labeled glucose and lactate were analyzed in four microdialyses during the study and were found to be in steady state. The means of these measurements were used, and the percentage of loss of radioactivity over the microdialysis membrane was taken as an index of relative recovery (dialysate concentration/interstitial concentration). In this study, the mean in vivo recovery obtained by means of internal reference calibration was 25 ± 2 and 28 ± 2% for glucose and lactate, respectively. For interstitial insulin measurements, calibration of the microdialysis catheters was done according to the external reference technique described in more detail in Ref. 36. Briefly, it was found in both rodent and human muscle that

\[
\text{recovery of inulin in vitro} = \frac{\text{recovery of inulin in vivo}}{\text{recovery of insulin in vivo}}
\]

**In the present study, the relationship between inulin and insulin recoveries in vitro was 2.3; the recovery of inulin in vivo was 13 ± 0.2% (because interstitial inulin is supposed to equal plasma inulin after 4 h, the recovery is calculated from dialysate inulin/plasma inulin at 5.5 h after initiation of the clamp). The in vivo recovery of insulin was then calculated in each subject according to this formula. The mean calculated in vivo recovery of insulin was 5.6 ± 0.9%. The in vivo recovery factor was used for recalculating steady-state dialysate insulin content to interstitial insulin concentrations. Leg blood flow (LBF) was measured after 90 and 120 min of the clamp in the contralateral leg by strain-gauge plethysmography.**

**Estimation of glucose uptake, lactate release, and insulin uptake.** Fick’s principle was used to estimate the regional rate of glucose uptake and microdialysis for estimation of the apparent extraction fraction (EF). The EF was calculated according to the formula

\[
\text{EF} = \frac{(A - I)(1 - e^{-\text{PS}Q})}{(A - I)}
\]

where A is the arterialized plasma concentration, I is the interstitial concentration, Q is the leg plasma flow, and PS is the permeability surface area product. PS was approximated to be 4 ml·100 g⁻¹·min⁻¹ for glucose and lactate (9). PS for insulin was approximated to be 0.6 ml·100 g⁻¹·min⁻¹ (13).

**Analytical methods.** Glucose and lactate concentrations in plasma and in the dialysate fractions were determined enzymatically using 10-μl samples for simultaneous analysis of glucose and lactate on a YSI 2700 select biochemical analyzer (Yellow Springs Instruments, Yellow Springs, OH). Radioactivity was counted in a liquid scintillation counter using a quenched corrected (external standards), double-isotope program (1900 CA, TRI-CARB; Packard Instruments, Meriden, CT). Plasma insulin was measured with a double-antibody

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Data are means ± SE. Controls are compared with non-insulin-dependent diabetes mellitus (NIDDM) subjects. Student’s t-test for unpaired observations was used. BMI, body mass index. *P < 0.001.
radioimmunoassay (Pharmacia, Uppsala, Sweden). The concentration of insulin in the microdialysates was determined with an enzymatic immunoassay (DAKO Diagnostics, Cambridge, UK). Insulin concentrations in plasma and dialysates were determined photometrically using 20-μl samples (38).

Statistics. Statistical calculations of the means of the four collected microdialysates with lactate and glucose, the microdialysates (one sample) with inulin and insulin, and the two LBF measurements were made using absolute values obtained during the last 60 min of the clamp (steady state). The results are expressed as means ± SE. StatView statistics software for the Macintosh was used for all statistical calculations. Significance of difference was tested with Student’s t-test for paired and unpaired observations when appropriate. For unpaired comparison of the insulin data, the non-parametric Mann-Whitney test was used, and for paired insulin data the Wilcoxon Signed Rank test was applied. A value of P < 0.05 was considered statistically significant. Correlations were calculated according to the linear regression method.

RESULTS

During steady-state hyperinsulinemic euglycemic clamping conditions, the mean glucose infusion rate and the LBF were significantly lower in patients with type 2 diabetes than in normal subjects (Table 2). LBF and glucose infusion rate (GIR) tended to correlate without reaching statistical significance (r² = 0.20, p < 0.07).

During the euglycemic hyperinsulinemic clamp, the interstitial insulin concentration was significantly lower than arterial insulin in both groups (409 ± 86 vs. 1,071 ± 99 pmol/l, P < 0.01, in controls and 584 ± 165 vs. 1,253 ± 82 pmol/l, P < 0.05, in diabetic subjects, respectively). Neither the interstitial insulin nor the arterial-interstitial insulin concentration differences differed significantly between diabetic subjects and controls. Also, the estimated muscle insulin uptake was similar in both groups (Fig. 1).

The arterial-interstitial glucose concentration differences were similar in the two groups. In both groups, the mean arterial glucose concentration was significantly higher than the muscle interstitial fluid concentration of glucose. The calculated glucose uptake rate in diabetic patients was lower than in controls (Table 2). The estimated glucose uptake rate and the GIR tended to have a positive correlation without reaching statistical significance (r² = 0.18, P < 0.09). Estimated glucose uptake rate correlated significantly with blood flow (r² = 0.55, P < 0.01), whereas there was no correlation between interstitial muscle glucose level and blood flow (r² = 0.008, not significant).

During steady-state clamping, the mean arterial and interstitial lactate concentrations were higher in the control group. The arterial-interstitial lactate concentration differences were insignificant and similar in both groups (Table 2).

DISCUSSION

The data in this study demonstrate that, despite a significantly lower LBF in type 2 diabetic subjects (with a significantly reduced glucose infusion rate), the distribution of insulin to the interstitial muscle fluid is similar to that in healthy controls during hyperinsulinemia. Furthermore, it is confirmed that insulin-mediated glucose uptake is impaired in insulin-resistant type 2 diabetic patients. The present data also show significantly lower levels of lactate in plasma and muscle interstitial fluid in the diabetic group compared with controls during the hyperinsulinemic euglycemic clamp.

Estimation of insulin uptake. The interstitial concentrations of insulin in muscle tissue in the present study were ~50% of the level of insulin in plasma, which is in accord with previous studies in lymph (39) as well as in adipose tissue (19) and human skeletal muscle (36). Lower lymph insulin than arterial plasma insulin has also been demonstrated in insulin-resistant obese men during euglycemic hyperinsulinemic clamp, but the lymph insulin in obese subjects was higher than in lean controls, indicating that the transcapillary transport of insulin was not insufficient in insulin-resistant humans (5). Similar findings of higher-than-normal insulin levels in lymph (1) and muscle interstitial fluid (14) in insulin-resistant animals support the view that a defect in insulin penetration to the interstitial fluid is not the cause of insulin resistance.

The muscle interstitial insulin concentrations in this study were obtained during clamping conditions with high plasma insulin levels. Because the microdialysis technique and analytical methods do not allow measurements of interstitial insulin at lower physiological plasma insulin levels, the insulin transport remains unclear under these circumstances. Because the recovery of insulin in the dialysate is low, the method used for calibrating the microdialysis catheters, called the external reference technique, has been validated in Ref. 36. The decreased blood flow during hyperinsulinemia in the diabetic group in this study is in accord with earlier clamp studies, where an impaired insulina
mediated vasodilatation was found in obese (23) and NIDDM subjects (24).

Even though the type 2 diabetic subjects had 40% lower LBF during hyperinsulinemia, the interstitial insulin levels were similar in both groups. The estimated insulin uptake did not differ between type 2 diabetics and controls. It should be noted that the calculated insulin uptake depends on the blood flow as well as on the PS; therefore, it is of major importance which value of PS is used in the formula described above. In human skin, PS was found to be higher in the diabetic subjects at rest, but after exercise they had the same PS as controls (21). In rat muscle, PS did not differ between insulin-resistant and normal rats (C. Holmäng, M. Niklasson, B. Rippe, and P. Lönroth, unpublished observations). For small molecules, increases with blood flow, whereas PS for larger molecules like insulin/insulin is less dependent on the blood flow rate (9). In a recent microdialysis study, we found that PS for insulin in human skeletal muscle was unchanged when the blood flow increased by 40% (13). Hence, when calculating the insulin uptake, we used the same PS (0.6) in both diabetic subjects and controls.

The finding of a similar insulin uptake in diabetic subjects and controls despite a significantly impaired blood flow in the insulin-resistant state suggests that the insulin uptake in human skeletal muscle during steady-state hyperinsulinemia is not regulated by blood flow. However, the possible existence of a delayed transcapillary insulin transport in type 2 diabetes still remains to be elucidated in direct studies during non-steady-state conditions.

**Glucose metabolism and estimation of glucose uptake.** Previous studies have shown that insulin's ability to stimulate glucose uptake is reduced in insulin-resistant rats (31) and in legs in NIDDM subjects (11). In accord with those previous results, the estimated glucose uptake in this study was significantly lower in insulin-resistant type 2 diabetic subjects than in controls.

When estimating the glucose uptake, we adopted a PS value of 4 ml·100 g⁻¹·min⁻¹ in both groups. It might be argued that a lower PS should have been used in the diabetic group, because they had ∼40% lower blood flow, and PS for small molecules has been found to correlate positively with blood flow (9). However, by use of the same PS in both groups, the apparent differences in glucose uptake rates between diabetic subjects and controls would not be overestimated. In harmony with previous studies (23, 24), we found that blood flow correlated positively with muscle glucose...
uptake, whereas, in the present study, there was no correlation between blood flow and muscle interstitial glucose. Hence, according to our data, it does not seem that blood flow is regulating the apparent glucose uptake by increasing the glucose concentration in the muscle interstitial fluid, and, consequently, blood flow does not seem to be a discriminating factor behind reduced muscle glucose uptake in insulin-resistant muscle. Instead, the apparently decreased glucose uptake in type 2 diabetes seems to be caused by insulin resistance at the cellular level (rather than by blood flow-limited delivery of glucose). Muscle glucose transport is a facilitated diffusion process activated by insulin and is, therefore, dependent on the interstitial concentrations of glucose as well as insulin. In this study, the interstitial muscle concentrations of both glucose and insulin were similar in type 2 diabetes patients and controls despite the decreased muscle blood flow in the diabetic state, suggesting that the defect in muscle glucose uptake is located at the cellular level. The finding of similar interstitial glucose levels and arterial-interstitial glucose level differences in both groups, despite a significant group difference in blood flow, may lead to the speculation that cellular glucose uptake and metabolism might regulate blood flow to keep the arterial-interstitial concentration differences of glucose and insulin constant (15, 16).

In this study, the concentration of lactate in plasma as well as that in muscle interstitial fluid was lower in the diabetic subjects than in the controls during hyperinsulinemia. Earlier studies have shown that fasting plasma lactate is increased in insulin-resistant obese (20, 25) and NIDDM subjects (17, 34) compared with controls. On the other hand, the ability to produce lactate in response to an insulin/glucose infusion (25) or an oral glucose load (20) has been found to be impaired in the insulin-resistant state. However, higher forearm lactate release in hyperglycemic NIDDM subjects during an oral glucose load has also been reported (29), reflecting the concentration dependence of both plasma insulin and glucose for lactate production. The present data demonstrating that the plasma and interstitial muscle lactate levels are decreased in the NIDDM subjects during euglycemic hyperinsulinemia are compatible with data reported in the aforementioned study (25).

In the present study, no significant arterial-interstitial lactate concentration difference was found in either of the groups; therefore, the apparent lactate release rate could not be accurately calculated. A significantly increased lactate production rate may be demonstrated through the increasing interstitial fluid concentrations of lactate. However, the estimation of lactate release should be interpreted with caution, considering the presence of oxidation and local turnover of lactate in the muscle tissue (7, 8). The source of lactate appearing in plasma during hyperinsulinemia is not clear, and it has even been suggested that tissues other than the muscles may be more important because no lactate release was observed from the forearm at high plasma insulin levels (40). In contrast, Consoli et al. (8) have estimated that muscle accounts for ∼40% of systemic lactate appearance both basally and during hyperinsulinemia. In conclusion, the increased plasma lactate levels during insulin infusion should be considered to reflect the rate of Cori cycling of lactate, which may differ depending on the magnitude of insulin resistance.

Regulation of blood flow in insulin-resistant muscle. The finding that insulin-resistant muscle has a reduced capillary density (2, 28) in combination with a decreased insulin-mediated vasodilatory response has formed the basis for the hypothesis that an insufficient distribution of insulin and glucose to the muscle tissue might be the cause of insulin resistance. However, the hypothetical role of reduced blood flow as a causal factor behind insulin-resistant muscle glucose uptake has been debated. Insulin-stimulated vasodilation and glucose uptake in muscle correlate positively in conscious rats (18) as well as in humans (23). Vasodilation during hyperinsulinemia also seems to be accompanied by capillary recruitment in the limb (4). These findings have raised the hypotheses that blood flow (and tissue recruitment) is regulating the glucose uptake in muscle and that the vasodilatory effect of insulin is of major importance for the glucose uptake. However, there seems to be a delay in the effect of insulin on blood flow in relation to its direct effect on glucose uptake (41). In addition, no increase in femoral glucose uptake was found in a study where LBF was doubled by infusing bradykinin (32). Also, microdialysis studies of glucose (30) and insulin (14) in muscle interstitial fluid have not provided evidence that the interstitial concentrations of glucose or insulin are regulated by the blood flow rate, indicating that blood flow is only a minor determinant for insulin-mediated glucose uptake. Interestingly, insulin resistance and reduced muscle glucose uptake in glucosamine-infused rats decrease muscle lactate production and blood flow (16).

This is in accord with the data in the present study and with the view that glucose nonoxidative metabolism may regulate the blood flow. Hence, instead of a causal relationship between blood flow and muscle glucose uptake, we believe that the lower interstitial fluid concentrations of lactate may play a role in the diminished blood flow in insulin-resistant muscle. The suggested mechanisms behind the vasodilatory action of insulin may include both the direct effect to increase endothelium-derived nitric oxide (37) and a secondary effect to enhance lactate production through nonoxidative glucose metabolism (6).

In summary, the data in this study show that, during hyperinsulinemia, muscle interstitial insulin and glucose concentrations did not differ between patients with type 2 diabetes and healthy controls despite significantly lower leg blood flow rates in diabetic subjects. Thus it may be suggested that the apparently decreased glucose uptake in type 2 diabetes is caused by insulin resistance at the cellular level and not by deficient access by insulin and glucose surrounding the muscle cell. Furthermore, lactate levels in plasma and muscle interstitial fluid
were decreased in NIDDM subjects, which might play a role in the impaired insulin-mediated vasodilatory response.

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