Muscle Glucose Uptake Is Effectively Activated by Ischemia in Type 2 Diabetic Subjects

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It has previously been shown that Wortmannin, a phosphatidylinositol 3-kinase inhibitor, inhibits glucose transport activated by insulin but not by ischemia, suggesting the importance of an activating mechanism that bypasses the insulin signal. To evaluate the relevance of this insulin-independent pathway in insulin-resistant subjects, the ability of ischemia to stimulate glucose uptake was investigated in 9 patients with type 2 diabetes and in 9 healthy control subjects (fasting glucose level 9.4 ± 0.8 vs. 5.1 ± 0.1 mmol/l, *P* < 0.001, in type 2 diabetic patients and control subjects, respectively; fasting insulin level insulin 8.1 ± 2.6 vs. 4.5 ± 0.7 mU/l, *P* < 0.05, respectively) matched for sex, age, and BMI. Arterial plasma and interstitial concentrations of glucose and lactate (measured by subcutaneous and muscle microdialysis) were recorded in the forearm before, during, and after ischemia induced locally for 20 min. During ischemia, the muscle interstitial glucose concentration decreased significantly from 7.7 ± 0.6 to 5.4 ± 0.4 mmol/l (*P* < 0.01) and from 4.4 ± 0.3 to 3.6 ± 0.3 mmol/l (*P* < 0.05) in type 2 diabetic patients and control subjects, respectively. The arterial-interstitial (A-I) glucose concentration difference was 1.7 ± 0.6 and 0.7 ± 0.3 mmol/l at basal, and it increased significantly to 3.5 ± 0.7 (*P* < 0.01) and 1.4 ± 0.3 mmol/l (*P* < 0.05) during ischemia in each group, respectively. Interstitial lactate increased significantly during ischemia from 0.8 ± 0.1 to 1.1 ± 0.1 mmol/l (*P* < 0.05) and from 0.5 ± 0.1 to 0.9 ± 0.2 mmol/l (*P* < 0.05), respectively. The A-I glucose concentration difference was abolished immediately postischemia and regained after ~15 min, whereas high interstitial lactate levels remained elevated throughout the study. Subcutaneous interstitial glucose concentrations remained unchanged during ischemia and postischemia in both groups, whereas the interstitial lactate concentration in adipose tissue increased during ischemia from 1.4 ± 0.2 to 2.0 ± 0.2 mmol/l (*P* < 0.05) and from 1.1 ± 0.1 to 1.8 ± 0.3 mmol/l (*P* < 0.05) in type 2 diabetic patients and control subjects, respectively. Plasma glucose and lactate levels were unchanged in both groups during the study period. The results show that in muscle, but not in adipose tissue, glucose uptake is efficiently activated by ischemia in insulin-resistant type 2 diabetic subjects, suggesting the activation of a putative alternative pathway to the insulin signal in muscle cells.
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In skeletal muscle, insulin activates glucose transport through the translocation of GLUT4 from intracellular compartments to the plasma membrane (1). At least 2 distinct signaling pathways for the activation of glucose transport have been detected. In addition to the pathway used by the insulin signal, another pathway activated by contraction or ischemia has been described (2,3). One argument for multiple independent pathways for activation of glucose transport has been based on the observation that a maximal stimulation of glucose uptake by insulin can be further increased by contraction (4–6) or by hypoxia (2). Maximal activation of glucose uptake induced by hypoxia and contraction has been demonstrated to be nonadditive, suggesting activation through the same pathway (2). The increase in muscle glucose transport activity induced by contraction, hypoxia, or both is thought to be partly explained by increased translocation of GLUT4 to the plasma membrane and an increased intrinsic activity of GLUT4 (2,4). Experimental investigations in muscle strips suggest that both contraction and hypoxia mediate activation of glucose transport by a Ca^{2+}-dependent pathway (4,7,8). In contrast, it has been reported that neither the additive effect of insulin and contraction (9) nor that of contraction and hypoxia (10) has been confirmed. Direct evidence for the existence of an alternative signaling pathway is, however, provided by the demonstration that insulin, but not contraction-stimulated glucose transport, is inhibited by Wortmannin, a phosphatidylinositol (PI) 3-kinase inhibitor (11).

In type 2 diabetes, the decrease in whole-body insulin-dependent glucose uptake is directly related to the glucose transport rate in skeletal muscle (12). Impaired insulin-stimulated translocation of GLUT4 to the plasma membrane has been shown in the insulin-resistant obese Zucker rat (13) and in incubated muscle strips from type 2 diabetic subjects (14). In muscles and adipose tissue biopsies from high-fat–fed rats (15) and also in muscles from obese subjects (16), impaired activation of PI 3-kinase has been observed. In a recently published study that investigated the effect of high-fat feeding in normal rats, it was demonstrated that an insulin-mediated but not a contraction-activated pathway for glucose transport was impaired

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A-I, arterial-interstitial concentration difference; DPM, disintegrations per minute; FFA, free fatty acid; HOMA, homeostasis model assessment; PI, phosphatidylinositol.

TABLE 1
Clinical characteristics of the study subjects

	Type 2 diabetic subjects	Control subjects
n	9	9
M/F	5/4	5/4
Age (years)	55 ± 3	52 ± 4
Weight (kg)	79.1 ± 3.6	77.8 ± 4.4
BMI (kg/m^2)	26.3 ± 1.5	25.4 ± 0.8
Fasting glucose (mmol/l)	9.4 ± 0.8*	5.1 ± 0.1
Fasting insulin (mU/l)	8.1 ± 2.6†	4.5 ± 0.7
HOMA index	3.3 ± 1.2*	1.0 ± 0.2
Fasting FFA, mmol/l	0.7 ± 0.1	0.6 ± 0.1

Data are *n* and means ± SE. *P < 0.001 vs. control subjects; †P < 0.05 vs. control subjects.

due to reduced translocation of GLUT4. An acquired defect in insulin activation of the PI 3-kinase was suggested because glucose transport in response to hypoxia and thus the contraction-mediated pathway was intact (17).

Interestingly, direct evidence for the existence of an intact pathway to activate glucose transport in type 2 diabetes was given by the recent observation that glucose transport was stimulated in incubated insulin-resistant human muscle strips after exposure to hypoxia (18). It may thus be suggested that glucose transport activation is functional in type 2 diabetes, provided that pathways alternative to the insulin-dependent signal are activated. To further evaluate this hypothesis in vivo, the ability of ischemia to stimulate glucose uptake in type 2 diabetic subjects was investigated in the present study. The forearm microdialysis technique was used to assess glucose uptake under ischemic conditions in the brachioradialis muscle and in subcutaneous adipose tissue. As estimated from the glucose elimination rate, our results demonstrate that in muscle, but not in adipose tissue, glucose uptake is activated by ischemia in both type 2 diabetic and control subjects.

RESEARCH DESIGN AND METHODS

Subjects. A total of 9 patients with type 2 diabetes and 9 healthy matched control subjects with normal glucose tolerance participated in the study. The subjects were recruited by advertising in a local newspaper. Table 1 lists the clinical characteristics of the subjects. Insulin resistance was estimated by the homeostasis model assessment (HOMA) index, which has been demonstrated to correlate with glucose infusion rates obtained during the euglycemic (r = 0.88, P < 0.0001) and euglycemic-hyperinsulinemic (r = 0.69, P < 0.01) clamp. The following formula (19,20) was used:

$$\text{resistance} = \text{fasting insulin}/(22.5e^{-\ln[\text{fasting glucose}]})$$

Of the 9 subjects, 7 with type 2 diabetes were treated with oral hypoglycemic agents and 1 type 2 diabetic patient was treated with antihypertensive medicine. Drug treatment was discontinued the night before the study. Of the type 2 diabetic patients, 2 were smokers. All subjects gave their informed consent, and the study was approved by the Ethics Committee of Göteborg University.

Microdialysis procedure. The microdialysis probe and the principles of microdialysis have been described in detail previously (21). In the present study, 2 types of microdialysis catheters were used, one for intramuscular measurements and the other for subcutaneous measurements. In muscle, a commercially available custom-made microdialysis catheter (16 × 0.5 mm, 20-kDa molecular wt cutoff) (CMA-10; CMA, Stockholm) was used. Another catheter made of a single dialysis tubing (30 × 0.3 mm, 3-kDa molecular wt cutoff) glued to a 50-mm nylon tubing was used in the subcutaneous tissue. The catheter was inserted without anesthetics into the brachioradialis muscle and into the subcutaneous tissue by puncturing the surface of the disinfected skin vertically with a 20-gauge cannula. The steel mandrin was removed, and the microdialysis catheter was subsequently inserted. The inlets of the catheters were connected to a microinjection pump (CMA 100; CMA) and perfused at a flow rate of 2.5 µl/min. The perfusion fluid consisted of isotonic saline with the addition of 1.7 mmol/l glucose, 0.2 mmol/l lactate, and 3.3 mmol/l urea. For calibration of the microdialysis probes in situ according to the internal reference technique, 5 µCi/ml [^{3}H]glucose (Amersham, Arlington Heights, IL) was added to the perfusate (22). The relative recovery of glucose was determined from the microdialysates sampled every 15 min at basal, every 3 min at ischemia, immediately after release of ischemia, and every 10 min postischemia. The percentage loss of radioactivity over the dialysis membrane was taken as an index of the relative recovery of interstitial glucose (22). The internal reference calibration technique was previously validated for measurements in human skeletal muscle and was found to yield relative recovery figures similar to those obtained with the equilibration calibration procedure (22). The radioactivity in microdialysates and the relative recovery was unchanged throughout the study (Table 2). Interstitial concentrations of lactate were calculated according to the formula: *RR* in vitro = *RR* in vivo, where *RR* is the ratio between the relative recovery of glucose and that of the substance of interest (23).

Study protocol. The investigations started between 8:30 and 9:00 A.M. after an overnight fast. The subjects were resting in a supine position throughout the study. Room temperature was kept at 22°C. Resting pulse and fasting samples were taken before the start of the experiment. The right forearm was heated with electric pads to arterialize the venous blood, and a polyethylene catheter was placed in the right forearm vein for blood sampling. Microdialysis probes were inserted as described below, and an equilibration period of 30 min was allowed. The microdialysis probes were then calibrated for a period of 90 min during basal conditions according to the internal reference technique, followed by an ischemic period of 18 min and a recovery period of 39 min. Microdialysates were collected and analyzed for glucose, lactate, urea, and [^{3}H]glucose. To induce ischemia, the arm was constricted with a blood pressure cuff. A cuff pressure of 20 mmHg above the systolic blood pres-

TABLE 2
Radioactivity in the microdialysate in the muscle and in the subcutaneous tissue

	t (min)							
	15	30	45	60	75	90	137	147
Muscle (DPM)								
Control subjects	1,115 ± 45	1,114 ± 44	1,179 ± 36	1,101 ± 34	1,131 ± 35	1,116 ± 62	1,140 ± 29	1,110 ± 25
Type 2 diabetic subjects	1,054 ± 63	1,127 ± 42	1,137 ± 63	1,175 ± 40	1,111 ± 51	1,172 ± 30	1,197 ± 34	1,136 ± 44
Subcutaneous tissue (DPM)								
Control subjects	1,119 ± 22	1,171 ± 44	1,113 ± 23	1,165 ± 45	1,155 ± 54	1,098 ± 50	1,130 ± 18	1,165 ± 29
Type 2 diabetic subjects	1,048 ± 73	1,085 ± 39	1,168 ± 44	1,197 ± 47	1,173 ± 46	1,165 ± 46	1,179 ± 36	1,179 ± 22

Data are means ± SE. The perfusion solution was 1,418 ± 40 and 1,433 ± 53 DPM in the control and type 2 diabetic groups, respectively. The mean relative recovery of glucose in the muscle was calculated to 20.9 ± 1.2 and 21.1 ± 1.3% in the control and the type 2 diabetic groups, respectively. In the subcutaneous tissue, the mean relative recovery was calculated to 19.6 ± 1.7 and 19.8 ± 1.5% in the 2 groups, respectively. There were no significant differences in counted radioactivity (^{3}[H]glucose) in either group over the duration of the study in the muscle or in the subcutaneous tissue. The specific activity of ^{3}[H]glucose was 5 µCi/ml.

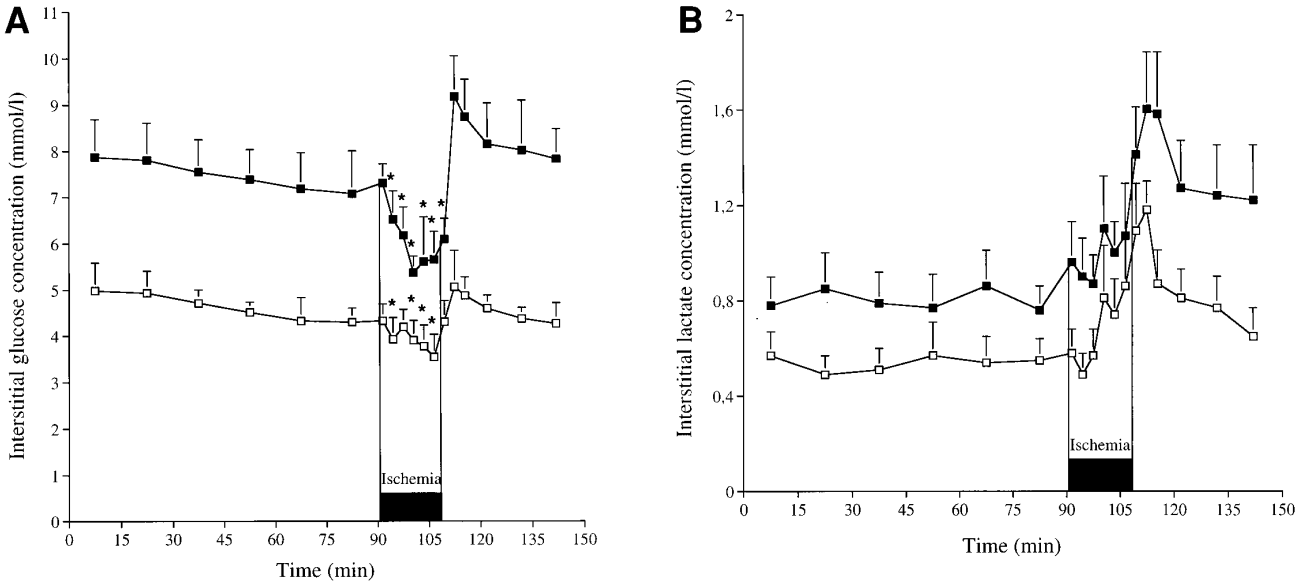

FIG. 1. The interstitial glucose concentration (*A*) and the interstitial lactate concentration (*B*) in brachioradialis muscle measured by the microdialysis technique. Interstitial concentrations were recorded during basal (0–90 min), ischemia (90–108 min), and postischemia (108–147 min) in healthy control subjects (□; *n* = 9) and type 2 diabetic patients (■; *n* = 9). Because 1 type 2 diabetic patient tolerated ischemia for only 15 min, there were 8 patients in the type 2 diabetic group at the last measuring point during the ischemic period. The interstitial glucose concentration decreased significantly ($P < 0.01$ in the type 2 diabetic group vs. $P < 0.05$ in the control group), whereas the interstitial lactate concentration increased significantly ($P < 0.05$) in both the type 2 diabetic and the control groups during ischemia. Data are presented as means ± SE. *$P < 0.05$ for the change in the interstitial glucose concentration in type 2 diabetic subjects vs. the control group.

sure was applied for 18 min. All participants, save 1 type 2 diabetic patient in whom ischemia was interrupted after 15 min, tolerated the ischemic period of 18 min. Microdialysates were analyzed for glucose, lactate, and urea. Arterialized venous blood samples were taken from the right forearm before, during, and after the ischemic period and were analyzed for glucose, lactate, free fatty acid (FFA) urea, and insulin.

Analytical methods. Glucose, lactate, and urea concentrations in plasma and in the dialysate fractions were determined enzymatically in a biochemical analyzer (CMA 600, CMA). Radioactivity ([^{3}H]glucose) was counted in a liquid scintillation counter by using a quenched corrected (external standards) double-isotope program (1900 CA Tri-Carb; Packard Instruments). Insulin was determined by a double radioimmunoassay (Pharmacia, Uppsala, Sweden). FFA levels were determined with an enzymatic colorimetric method (NEFA-C; Wako Chemicals, Neuss, Germany).

Statistics. All statistical calculations were made on absolute values. Plasma glucose and lactate concentrations were unchanged during the study period and are reported as the means from samples achieved at ~0, 100, and 125 min (basal, ischemic, and postischemic period, respectively). Dialysates and the corresponding interstitial concentrations were sampled during the 0–90 min basal period, the 90–108 min ischemic period, and the 108–147 min postischemic period. Dialysate sampling intervals were 15 min at basal, 3 min at ischemia, immediately (108–117 min) after release of ischemia, and 10 min postischemia. The time delay by diffusion and catheter dead space was measured in vitro as <3 min in both catheter types used and withdrawn. The results are expressed by means ± SE. The significance of difference was tested with analysis of variance followed by Fischer's least-significant test for post hoc analyses and, when applicable, with Student's *t* test for paired observations. $P < 0.05$ was considered statistically significant. StatView statistics software for a Macintosh computer was used for all statistical calculations.

RESULTS

Mean insulin resistance estimated by the HOMA index (19,20) was significantly ($P < 0.001$) increased in the type 2 diabetic patients compared with the control subjects (Table 1). Furthermore, the type 2 diabetic patients compared with the control subjects had significantly elevated fasting plasma glucose ($P < 0.001$) and insulin concentrations ($P < 0.05$), whereas the FFA concentrations were similar in both groups (Table 1). Plasma insulin, glucose, lactate, and FFA concentrations did not change significantly during the ischemic and postischemic period in type 2 diabetic patients and control subjects.

Calibration of the microdialysis probes. The specific activity of [^{3}H]glucose in the muscle and in the subcutaneous tissue is given in Table 2. The perfusate was 1,433 ± 53 and 1,418 ± 40 disintegrations/min (DPM) in the type 2 diabetic and control groups, respectively. The mean relative recovery of glucose in the muscle was 21.1 ± 1.3 and 20.9 ± 1.2% in type 2 diabetic and control subjects, respectively. In the subcutaneous tissue, the mean relative recovery of glucose was 19.8 ± 1.5 and 19.6 ± 1.7% in the 2 groups, respectively. There was no significant change in microdialysate content of [^{3}H]glucose over the course of the study in muscle or in subcutaneous tissue (Table 2). The relative recovery of lactate in the muscle tissue was calculated to 24.4 ± 1.8 and 24.0 ± 2.6% in the type 2 diabetic and control groups, respectively. In the subcutaneous tissue, relative lactate recovery was calculated to 22.4 ± 1.2 and 23.6 ± 1.5%, respectively.

Measurements in skeletal muscle. Figure 1*A* depicts the mean interstitial glucose concentration in both groups during the basal, ischemic, and postischemic periods. Plasma glucose concentrations did not change significantly in the type 2 diabetic subjects or the control group during the study period. The interstitial glucose concentration was significantly ($P <$ 0.01–0.001) higher in the type 2 diabetic patients compared with the control subjects throughout the study, but it was significantly ($P < 0.05$–0.01) lower than plasma in both groups during the basal and ischemic periods. The interstitial glucose concentration did not change significantly during the basal period in either group ($P = 0.145$ and 0.449 in the control and type 2 diabetic groups, respectively). The glucose concentration in interstitial fluid decreased significantly ($P < 0.01$ and $P < 0.05$ in the type 2 diabetic patients and control subjects, respectively) during the ischemic period in both groups

TABLE 3
The mean plasma and interstitial concentrations and the A-I differences of glucose and lactate in the muscle during the basal and ischemic periods

	Basal		Ischemia		Postischemia	
	Type 2 diabetic subjects	Control subjects	Type 2 diabetic subjects	Control subjects	Type 2 diabetic subjects	Control subjects
Plasma glucose (mmol/l)	9.4 ± 0.8*†	5.1 ± 0.1†	8.9 ± 0.8‡§	5.0 ± 0.1§	9.1 ± 0.8‡	4.7 ± 0.2
Interstitial glucose (mmol/l)	7.7 ± 0.6*	4.4 ± 0.3	5.4 ± 0.4‡‖	3.6 ± 0.3¶	8.0 ± 0.8‡#	4.1 ± 0.6
A-I difference	1.7 ± 0.6	0.7 ± 0.2	3.5 ± 0.7‡‖	1.4 ± 0.3¶	1.1 ± 0.8	0.6 ± 0.6
Plasma lactate (mmol/l)	0.9 ± 0.1**	0.6 ± 0.1	0.7 ± 0.1†	0.5 ± 0.1†	0.7 ± 0.1†**	0.5 ± 0.1
Interstitial lactate (mmol/l)	0.8 ± 0.1**	0.5 ± 0.1	1.1 ± 0.1¶	0.9 ± 0.2¶	1.2 ± 0.2‡¶	0.7 ± 0.1
A-I difference	0.1 ± 0.1	0.1 ± 0.1	–0.4 ± 0.1¶	–0.4 ± 0.1¶	–0.5 ± 0.2¶**	–0.2 ± 0.1
Dialysate-urea (mmol/l)	3.2 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.2 ± 0.1	3.3 ± 0.2

Data are means ± SE. *P < 0.001 vs. the control group; †P < 0.05 vs. the interstitial concentration; ‡P < 0.01 vs. the control group; §P < 0.01 vs. the interstitial concentration; ‖P < 0.01 vs. the basal period; ¶P < 0.05 vs. the basal period; #P < 0.01 vs. ischemia; **P < 0.05 vs. the control group.

(Table 3). The net decrease in interstitial glucose concentration during ischemia was significantly (P < 0.05) larger in the type 2 diabetic group. Immediately after release of ischemia, the interstitial glucose concentration increased significantly (from 5.4 ± 0.4 to 9.2 ± 0.8 mmol/l, P < 0.001, and from 3.6 ± 0.3 to 5.1 ± 0.8 mmol/l, P < 0.05, in the type 2 diabetic and control groups, respectively) (Fig. 1*A*). The significant arterial-interstitial (A-I) glucose concentration difference recorded during ischemia in both study groups was totally abolished during the initial postischemic period. In the later postischemic phase, the A-I glucose difference was restored to levels similar to basal in both groups.

The mean interstitial lactate concentrations in the type 2 diabetic and the control groups during the study are shown in Fig. 1*B*. Plasma lactate concentrations were stable in both groups before, during, and after ischemia (NS). The basal interstitial lactate concentration was significantly (P < 0.05) higher in type 2 diabetic patients compared with control subjects. Interstitial lactate was significantly raised (P < 0.05) during ischemia compared with basal in both groups (Table 3); the net increase was similar in type 2 diabetic patients and control subjects. Interstitial lactate concentrations were significantly (P < 0.05) increased compared with plasma in both groups at ischemia. Within 6 min after release of ischemia, interstitial lactate concentrations increased significantly (from 1.1 ± 0.1 to 1.6 ± 0.3 mmol/l and from 0.9 ± 0.2 to 1.2 ± 0.1 mmol/l, P < 0.05, in the type 2 diabetic and control groups, respectively) (Fig. 1*B*). They declined to near–plasma concentrations after 40 min postischemia in control subjects, yet they were significantly elevated in type 2 diabetic subjects compared with basal (P < 0.05) and with the control group (P < 0.01). The A-I lactate concentration difference increased significantly (P < 0.05) in both groups during the ischemic period compared with the basal period. Postischemia, the A-I concentration difference of lactate declined to basal values in the control group but not in the type 2 diabetic patients.

Subcutaneous measurements. Mean interstitial glucose concentrations in the subcutaneous tissue are depicted in Fig. 2*A*. Type 2 diabetic patients had significantly (P < 0.05) elevated interstitial glucose concentrations compared with the control group during the basal and postischemic periods (Table 4). The interstitial glucose concentration was significantly (P < 0.01–0.05) lower than the plasma concentration in both groups throughout the study. The interstitial glucose concentration and the A-I glucose concentration difference did not change significantly during and after ischemia in either group. As compared with the ischemic period, immediately after stopping the ischemic period, a peak in the interstitial glucose concentration was recorded (from 6.0 ± 1.2 to 8.9 ± 0.8 mmol/l and from 4.0 ± 0.6 to 5.4 ± 0.5 mmol/l, P < 0.05, in the type 2 diabetic and control groups, respectively) (Fig. 2*A*). At 40 min postischemia, the interstitial glucose concentrations, as well as the A-I glucose difference, returned to levels similar to the basal period in both groups.

Mean interstitial lactate concentrations in the subcutaneous tissue are illustrated in Fig. 2*B*. The interstitial lactate concentration was significantly (P < 0.001–0.05) elevated compared with the plasma concentration over the course of the study in both groups. Interstitial lactate concentrations increased significantly (P < 0.05) during the ischemic period compared with the basal period in both groups (Table 4). The increase in the interstitial lactate concentration was similar in the type 2 diabetic patients and the control group. In the initial postischemic phase, a significant peak in the interstitial lactate concentration (from 2.0 ± 0.2 to 3.0 ± 0.3 mmol/l, P < 0.001, and from 1.8 ± 0.3 to 2.3 ± 0.2 mmol/l, P < 0.05, in the type 2 diabetic and the control groups, respectively) was registered in both groups. At 40 min after ischemia, the interstitial lactate concentration declined in both groups but was still significantly (P < 0.05) higher than basal concentrations. The A-I lactate concentration difference was significantly (P < 0.05) increased during ischemia compared with the basal level in both groups, and it did not change significantly during the postischemic period.

Control measurements of urea. Plasma urea concentration did not change significantly during the study period in either the type 2 diabetic group or the control group (data not shown), and there was no significant difference in plasma urea concentration between type 2 diabetic and control subjects. Dialysate urea concentrations in muscle and in the subcutaneous tissue were stable during the basal, ischemic, and postischemic periods and did not change significantly (Tables 3 and 4). Furthermore, there were no significant group differences in dialysate urea concentrations in muscle and the subcutaneous tissue during the study period.

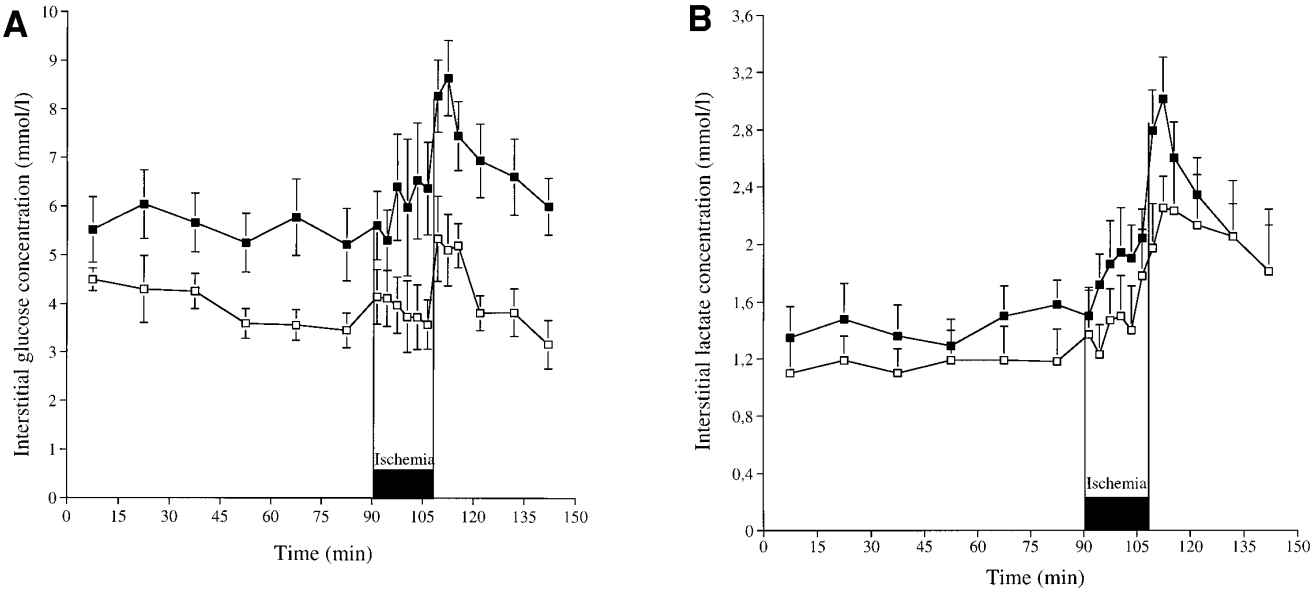

FIG. 2. The interstitial glucose concentration (*A*) and the interstitial lactate concentration (*B*) in the subcutaneous adipose tissue during basal (0–90 min), ischemia (90–108 min), and postischemia (108–147 min) in healthy control subjects (□; *n* = 9) and type 2 diabetic patients (■; *n* = 9). No significant change could be registered either in plasma or in the interstitial glucose concentration in the type 2 diabetic or the control group during the study periods. The interstitial lactate concentration was significantly (*P* < 0.05) elevated in both groups during the ischemic period. Data are presented as means ± SE.

DISCUSSION

The present study was undertaken to examine the effects of acute ischemia on glucose uptake in muscle and in the subcutaneous tissue in type 2 diabetic patients. The type 2 diabetic subjects were characterized by a significantly higher HOMA index and elevated fasting plasma glucose and insulin concentrations, indicating a marked insulin resistance. The data presented in this study show that acute ischemia caused a significant decrease in the interstitial glucose concentration in muscle, but not in adipose tissue, of both type 2 diabetic patients and control subjects. The net decrease in the interstitial glucose concentration in muscle during the ischemic period was more pronounced ($P < 0.05$) in the hyperglycemic type 2 diabetic group, thus suggesting that the elimination rate of glucose from the muscle interstitial fluid in vivo in hyperglycemic type 2 diabetic patients is at least as high as that in normoglycemic control subjects during ischemia. Furthermore, the data demonstrate that ischemia-induced lactate production in muscle and adipose tissue is equally efficient in type 2 diabetic and healthy control subjects during ischemia. The data suggest the importance of the putative signaling pathway, which is different from that induced by insulin to stimulate muscle glucose uptake in hyperglycemic type 2 diabetes (2–4).

Microdialysis measurements allow for correct estimations of the rate of glucose and lactate production in situ under basal conditions. Because the microdialysis technique records the metabolic events through sampling from the interstitial fluid (21), an approximate estimation of the extraction fraction of nutrients and hormones could be calculated by means of Fick's principle (24). Since basal tissue blood flow was not measured in the present study, we were only able to speculate about the glucose uptake rate during

TABLE 4
The mean plasma and interstitial concentrations and the A-I differences of glucose and lactate in the subcutaneous tissue during the basal and ischemic periods

	Basal		Ischemia		Postischemia	
	Type 2 diabetic subjects	Control subjects	Type 2 diabetic subjects	Control subjects	Type 2 diabetic subjects	Control subjects
Plasma glucose (mmol/l)	9.4 ± 0.8*†	5.1 ± 0.1†	8.9 ± 0.8†‡	5.0 ± 0.1§	9.1 ± 0.8‡§	4.7 ± 0.2§
Interstitial glucose (mmol/l)	5.9 ± 0.6‖	3.9 ± 0.3	6.0 ± 1.2	4.0 ± 0.6	6.7 ± 0.9‖	3.8 ± 0.4
A-I difference	3.5 ± 0.8	1.2 ± 0.2	2.9 ± 1.0	1.0 ± 0.3	2.4 ± 0.9	0.9 ± 0.4
Plasma lactate (mmol/l)	0.9 ± 0.1‖§	0.6 ± 0.1§	0.7 ± 0.1¶	0.5 ± 0.1¶	0.7 ± 0.1‖¶	0.5 ± 0.1†
Interstitial lactate (mmol/l)	1.4 ± 0.2	1.1 ± 0.1	2.0 ± 0.2#	1.8 ± 0.3#	1.7 ± 0.2#	1.7 ± 0.5#
A-I difference	–0.5 ± 0.1	–0.5 ± 0.1	–1.3 ± 0.2#	–1.3 ± 0.3#	–1.0 ± 0.2#	–1.2 ± 0.5#
Dialysate urea (mmol/l)	3.2 ± 0.3	3.4 ± 0.2	3.3 ± 0.3	3.4 ± 0.3	3.2 ± 0.2	3.2 ± 0.2

Data are means ± SE. *$P < 0.001$ vs. the control group; †$P < 0.01$ vs. the interstitial concentration; ‡$P < 0.01$ vs. the control group; §$P < 0.05$ vs. the interstitial concentration; ‖$P < 0.05$ vs. the control group; ¶$P < 0.001$ vs. the interstitial concentration; #$P < 0.05$ vs. the basal period.

the basal study period. The following formula can be used to calculate the extraction fraction:

$$EF = [A\text{-}I] \cdot [1 - e^{-PS/Q}]$$

where EF is the extraction fraction, , and Q is the plasma flow. The basal A-I concentration difference of glucose tended to be higher, albeit not significantly, in type 2 diabetic patients compared with control subjects in the present study. This difference is in perfect harmony with the previously noted enhancement of elevated basal glucose uptake during hyperglycemia (25). The present A-I concentration difference and the assumption that PS for glucose is ~5 ml · 100 g^{-1} · min^{-1} (24) when the forearm blood flow is ~5 ml · 100 g^{-1} · min^{-1} in both type 2 diabetic and control subjects (26) allow us to make an approximation of the muscle glucose uptake rate under basal conditions. The apparent muscle glucose uptake rate could, under the described assumptions, be estimated to be ~3 µmol · 100 g^{-1} · min^{-1} in control subjects and ~6 µmol · 100 g^{-1} · min^{-1} in type 2 diabetic subjects during the basal period. Even if muscle blood flow is somewhat reduced in the type 2 diabetic group (26), the above approximation still suggests that the muscle glucose uptake in hyperglycemic type 2 diabetic subjects is not less than that in normoglycemic control subjects in the postabsorptive state. Fasting hyperglycemia provides a compensatory mechanism to increase the non–insulin–dependent glucose metabolism in the face of insulin deficiency, insulin resistance, or both (27,28). The increase in the rate of glucose uptake in response to high levels of extracellular glucose has partially been attributed to the mass-action effect of glucose. Also, more recent reports have shown that hyperglycemia per se could induce translocation of GLUT4 to the plasma membrane by a mechanism suggested to be mediated by Ca^{2+} and independent of PI 3-kinase, thereby enhancing the rate of glucose uptake (29). Thus, in addition to the increase in glucose uptake due to the mass-action effect, hyperglycemia appears to increase glucose transport by autoregulation of the glucose transport system, which includes a transporter-mediated process (29).

Under the ischemic conditions presently used, blood flow was 0, thus permitting equilibration of the concentration gradient of nutrients and metabolites over the capillary wall. Under such conditions, the capillary wall is no longer rate-limiting for glucose uptake from the blood. Subsequently, the elimination rate of glucose from the interstitial fluid is proportional to the glucose uptake rate (30). During ischemia, the glucose uptake rate in muscle tissue could be estimated by means of the formula for calculating the rate of glucose disappearance:

$$R_d = \ln[C_0/C_t]/t$$

where R_d is the rate of disappearance, C_0 is the basal concentration, and C_t is the concentration at time t (30). During ischemia, the application of the above formula, with the assumption that the extracellular volume is 11 ml · 100 g^{-1} · $muscle^{-1}$ (31), estimated the muscle glucose uptake rate to be ~7 and ~14 µmol · 100 g^{-1} · min^{-1} in control and type 2 diabetic subjects, respectively. In conclusion, on the basis of these assumptions, acute ischemia was estimated to induce at least an ~100% increase in muscle glucose uptake in type 2 diabetic and control subjects.

In this context, however, it should be noted that the effects induced may not be quantitatively comparable in the 2 groups because they had different fasting levels of glucose and insulin. Thus, in the present study, we were not able to draw firm conclusions regarding the existence of an effect of hyperglycemia per se interacting with the effect induced by ischemia.

The decrease in interstitial glucose concentration in muscle under ischemia could not be explained by a consumption of glucose by erythrocytes, because their glucose uptake capacity is low (268 ± 62 µmol · h^{-1} · 10^{-12} cells and $5 \cdot 10^6$ red blood cells/ml blood) and would therefore lower the interstitial glucose concentration by only ~0.5 µmol/l during 18 min of ischemia (32). Also, consumption of glucose by the microdialysis device could not explain the increase in glucose elimination during ischemia. Urea was used as an internal standard to ascertain steady-state conditions during microdialysis. Urea was chosen because, under fasting conditions, it is produced and metabolized minimally in the observed tissues, and, moreover, it is rapidly equilibrated in different tissue compartments (33). The results show that the urea concentration was stable in plasma and in the dialysates from both the subcutaneous tissue and the muscle for the duration of the study in both groups, thereby indicating prevailing steady-state microdialysis conditions.

The present findings are in harmony with previous described observations in which glucose transport was investigated in incubated muscle strips from lean, obese, and obese type 2 diabetic subjects after exposure to hypoxia (18). In that study, hypoxia-stimulated glucose transport in insulin-resistant human skeletal muscle was similar to that of lean control subjects. In another report, in situ contraction was shown to stimulate muscle glucose transport in muscles from insulin-resistant obese rats to a magnitude comparable with that obtained in muscles from lean control rats (34). Furthermore, the results from another study demonstrated that an acute bout of treadmill running stimulated muscle glucose transport to the same degree in insulin-resistant rats as that in controls (35). Interestingly, in trained versus untrained subjects, Dela et al. (6) reported a slight (~20%) enhancement of the effect of exercise to stimulate glucose uptake in the leg. This finding may further suggest that insulin sensitivity is related to the activity to stimulate glucose uptake through the insulin-independent pathway. However, a direct comparison may not be made with the present data, because insulin-resistant subjects were not investigated in the study by Dela et al. (6). In contrast, muscle glucose uptake was not normalized by insulin-resistant muscle strips from high-fat–fed rodents, in which both the contraction and the insulin-stimulated glucose transport activity was reduced by ~50% (36). The reduction in glucose transport activity was suggested to result from an impairment in the GLUT4 translocation. In another report on the effect of acute exercise studied in human muscle biopsies, it was shown that GLUT4 translocation in response to contraction was similar in type 2 diabetic and control subjects, although the plasma membrane content of GLUT4 was ~32% lower (not statistically significant) in the type 2 diabetic group (37). These data may imply that the non–insulin-mediated pathway for activation of glucose uptake might interact with the insulin signal and thereby enhance insulin sensitivity. Furthermore, they may also imply that GLUT4 recruited by contraction might have a higher intrinsic activity.

In adipose tissue, basal glucose uptake is predominately non–insulin-dependent and is mediated by GLUT1 proteins residing in the plasma membrane, thus allowing enhanced rates of glucose transport during the prevailing hyperglycemia in type 2 diabetes (25). In the current study, the basal A-I difference of glucose in the adipose tissue was similar in type 2 diabetic and control subjects, which confirmed the findings of a previous report (25). In contrast to the results from skeletal muscle, ischemia did not increase glucose uptake in the subcutaneous adipose tissue. One previous study reported that the membrane content of GLUT1 could be elevated in cultured 3T3-L1 adipocytes by hypoxia, thereby inducing an adaptive response to increase cellular glucose uptake for use by nonoxidative pathways (38). The physiological relevance of such a mechanism was not evident in the present study.

With the release of ischemia, a postischemic hyperemic phase should appear. In the initial postischemic period, a peak increase in the interstitial glucose concentration was observed in muscle and subcutaneous tissue in both groups. As a result of the hyperemia and higher rates of glucose delivery, as well as the concomitant time delay of the insulin effect, the glucose concentration gradient over the capillary wall disappeared. In this context, it is once again worth noting that the interstitial fluid concentration of urea did not change dramatically, indicating that the relative microdialysis recovery of the substances of interest was unaltered. In agreement with the present data, the insulin gradient has been shown to be restored in <10 min (39), and the glucose concentration gradient has been shown to be restored ~30 min after release of ischemia (29).

In the postabsorptive state, the plasma lactate concentration was significantly higher in the type 2 diabetic patients compared with the control subjects, which confirmed previous investigations in which plasma lactate levels were shown to be elevated in insulin-resistant subjects during fasting (40,41). In muscle and fat, the basal A-I lactate concentration difference in type 2 diabetic subjects was similar to that in control subjects, which is in accordance with previous findings (40,42). In agreement with data previously reported by Jansson et al. (41), which showed that subcutaneous adipose tissue is a significant source of whole-body lactate release in the postabsorptive state, the present data show that the interstitial lactate concentration in the subcutaneous tissue was significantly higher than in plasma in both subject groups. As expected (30,43), the interstitial lactate concentration increased rapidly and significantly during ischemia ($P < 0.05$) in both muscle and the subcutaneous adipose tissue. The lactate appearance rate was equally enhanced in both type 2 diabetic and control subjects, indicating a normal nonoxidative glucose metabolism in hyperglycemic type 2 diabetic subjects during ischemic conditions.

In summary, ischemia activates muscle glucose uptake and lactate production in insulin-resistant hyperglycemic type 2 diabetic subjects and normoglycemic control subjects to a similar magnitude. The results from the present study emphasize the importance of the previously suggested non–insulin-dependent pathway for activating glucose uptake in muscle. The data also indicate that this signal is intact in type 2 diabetic subjects.

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