Interstitial Muscle Insulin and Glucose Levels in Normal and Insulin-Resistant Zucker Rats

Agneta Holmäng, Kazuo Mimura, Per Björntorp, and Peter Lönnroth

To study interstitial insulin and glucose concentrations, microdialysis was performed in the medial femoral muscles in normal SD rats as well as in insulin-resistant obese Zucker rats during a euglycemic insulin clamp. [^{14}C]inulin was given (0.1 mCi/rat) as a constant subcutaneous infusion 24 h before the insulin clamp. Insulin infusion rates were 5–8 mU · kg^{-1} · min^{-1} (low rate) for 140 min and 10–20 mU · kg^{-1} · min^{-1} (high rate) for another 100 min. The relationship between insulin and [^{14}C]inulin dialysate recoveries was evaluated in vivo and in vitro in plasma to calculate interstitial insulin concentration. Relative microdialysis recovery of interstitial insulin in vivo was 3.0 ± 0.3% (mean ± SE, *n* = 68). In normal SD rats, plasma and interstitial insulin concentrations were identical when plasma insulin was ≤250 mU/ml, whereas interstitial insulin was lower when plasma insulin was ≥350 mU/ml. Half-maximal glucose infusion rate was achieved in the presence of plasma and interstitial insulin concentrations of ~140 mU/ml, whereas maximal glucose disposal was seen at interstitial insulin concentrations of ~325 mU/ml, corresponding to ~500 mU/ml in plasma. In electrically stimulated and contracting (1 Hz) normal muscle with markedly increased blood flow, the dialysate insulin concentration was significantly higher at high rates, but not at low rates, of insulin infusion. In insulin-resistant obese Zucker rats, the interstitial insulin concentration was similar to that in plasma, even at pharmacological concentrations. The glucose infusion rate was significantly lower in the obese Zucker rats at both insulin infusion rates than in the lean animals. The glucose content in dialysates from skeletal muscle was equal in both obese and lean rats during the low insulin infusion rate. During the high insulin infusion rate, dialysate glucose concentrations decreased significantly in both groups but were significantly higher in the obese Zucker rats. The data suggest that transport of insulin and glucose diffusion across the capillary wall are rate limiting for insulin as well as for glucose metabolism in muscle in normal rats. This does not appear to be the case in the insulin-resistant obese Zucker rats, where the reduced insulin responsiveness in muscle is due to muscular cellular defects rather than an inhibited transcapillary delivery of insulin. *Diabetes* 46:1799–1804, 1997

From the Department of Heart and Lung Disease (A.H., K.M., P.B.), Wallenberg Laboratory, and the Department of Internal Medicine (P.L.), Lundberg Laboratory for Diabetes Research, Göteborg University, Göteborg, Sweden.

Address correspondence and reprint requests to Dr. Agneta Holmäng, Wallenberg Laboratory, Göteborg University, S-413 45 Göteborg, Sweden.

Received for publication 6 November 1996 and accepted in revised form 25 June 1997.

Insulin-resistant states, such as obesity and type 2 diabetes, are characterized by a major defect in the ability of insulin to stimulate glucose uptake in skeletal muscles (1). Previous investigations in skeletal muscle preparations from insulin-resistant subjects have convincingly shown the existence of numerous insulin receptor and postreceptor defects (1). In addition, intensive interest has recently been focused on insulin-induced vasodilation in skeletal muscle (2). Numerous studies have reported that this vasodilatory effect is hampered in several insulin-resistant conditions and correlates with the rate of glucose consumption (3–5). In support of the view that the microcirculation may be critical for the delivery of insulin and glucose, previous (6) and recent (7) studies in lymph indicate that interstitial insulin concentrations are considerably lower than in plasma. Direct measurements in the interstitial fluid by the microdialysis technique (8,9) have confirmed that subcutaneous interstitial insulin is ~50% of the plasma concentration (10). This, and the fact that the kinetics of interstitial but not plasma insulin fit with the kinetics of total body glucose uptake (7), indicates that the transcapillary transport of insulin might constitute a rate-limiting step for insulin uptake and action.

Direct measurement of insulin in the interstitial fluid in skeletal muscles has not been performed. Glucose measurements in skeletal muscle of normal healthy subjects have, however, shown that interstitial muscle glucose levels are lower than in arterial plasma (11). Thus capillary delivery of glucose seems partly rate limiting for the glucose uptake. Interstitial concentrations of hormones and substrates are dependent not only on transcapillary transport or diffusion but also on tissue blood flow rate and cellular uptake and degradation. Thus a putative decrease in capillary blood flow in insulin-resistant muscle might lower interstitial concentrations of both insulin and glucose, provided that the elimination rate of these substances is not altered.

To further investigate this balance, in the present study we performed microdialysis measurements of insulin and glucose in the quadricep muscles of normal and insulin-resistant Zucker rats during euglycemic hyperinsulinemia.

RESEARCH DESIGN AND METHODS

Animals. In experiments attempting to establish the relationship between plasma and interstitial insulin, male SD rats (250 ± 10 g) were used. Male, obese Zucker rats (363 ± 20 g; *fa/fa*), age 7 weeks, and lean littermates (277 ± 7 g) (Fa/–), obtained from Charles River (Kent, U.K.) were used in comparative experiments. All animals were housed in single cages at 23°C and fed commercial rat chow containing 22% protein, 5% fat, and 51.5% carbohydrate; sufficient minerals and vitamin (Ewos, Södertälje, Sweden); and tap water ad libitum. The dark-light cycle

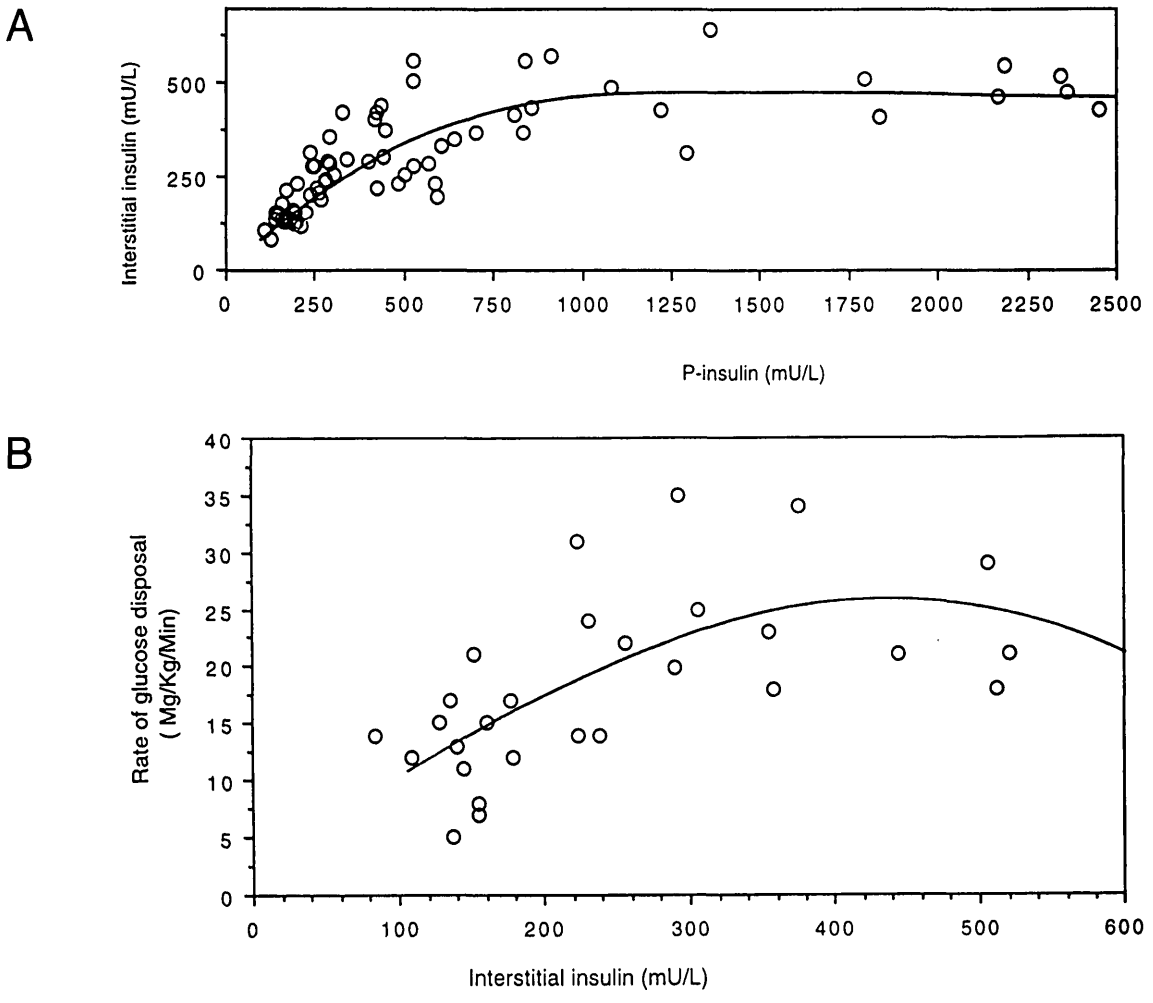

FIG. 1. *A:* **relationship between steady-state plasma and interstitial insulin concentrations in rat femoral muscle during euglycemic hyperinsulinemic clamping conditions.** *B:* **relationship between interstitial insulin concentrations estimated in femoral muscle and total-body glucose uptake. Data are means from two microdialysis catheters in 18–20 rats, each given two insulin infusion rates (8 mU · kg^{-1} · min^{-1}and 12–20 mU · kg^{-1} · min^{-1}).**

was 12:12 h, and the rats were kept under these conditions for at least 1 week before experiments.

The study was approved by the Animal Ethics Committee of Göteborg University.

Study protocol. The rats were anesthetized with diethyl ether, and an osmotic minipump was implanted subcutaneously (Alzet 2001D; Alza, Palo Alto, CA) containing ^{14}C-inulin (Amersham, U.K.) given as a constant infusion (0.1 mCi/rat) 24 h before the clamp study was initiated.

The euglycemic clamp technique (12,13) was used as described previously (14). In brief, rats were anesthetized with thiobutabarbital sodium salt (Inactin; RBI, Natick, MA) intraperitoneally, and catheters were inserted into the right jugular vein for infusions and into the left carotid artery for blood sampling. Body temperature was maintained at 37°C with a heating blanket and a rectal probe. While euglycemia was maintained at ~7 mmol/l plasma glucose concentrations, insulin (Actrapid Human; Novo Nordisk, Copenhagen, Denmark) was infused at submaximal insulin concentrations (5 or 8 mU · kg^{-1} · min^{-1}) for 140 min, followed by a higher insulin concentration (10–20 mU · kg^{-1} · min^{-1}) or 20 mU · kg^{-1} · min^{-1} (comparative study) for another 100 min. Euglycemia was maintained by simultaneously infusing a 20% glucose in saline solution at a rate guided by repeated plasma (30 ml) sampling. A total of <2 ml blood was used for these determinations, compensated for by the infusion volumes.

When indicated, the soleus and extensor digitorum longus muscles and the retroperitoneal adipose tissues were dissected out and weighed.

Electrical stimulation of the sciatic nerve. A subgroup of SD rats (n = 6) undergoing a two-step insulin clamp according to the above protocol were subjected to electrical stimulation of the sciatic nerve on one side. The sciatic nerve of each hind limb was exposed through a lateral incision in the thigh. A bipolar electrode was placed adjacent to the sciatic nerve in one limb. The exposure of the other nerve served as a sham control. The bipolar electrode (Grass Instruments, Quincy, MA) was connected to a Grass S6 stimulator (Grass Instruments). The euglycemic hyperinsulinemic glucose clamp technique was used as described above. During the last 50 min (steady state) of the clamp, samples of the muscle interstitial fluid from both legs were collected, and the medial femoral muscle of one hind limb was stimulated electrically, using square-wave pulses of 0.1-ms duration, at a frequency of 1 Hz. The voltage was adjusted (0.3–0.6 V) to give a standardized intensity of contraction.

Measurement of blood flow. After completion of electrical stimulation in the subgroup of rats described above, blood flow in the individual muscles was estimated using the radioactive microsphere technique (15), with ^{57}Co microspheres (specific activity 11.5 mCi/g in 10% dextran with 0.01% Tween 80; New England Nuclear, Boston, MA). The suspension of microspheres was injected into the left heart ventricle and immediately followed by a flush with 0.4 ml saline. The muscles of the hind limb (tibialis anterior, extensor digitorum longus, gastrocnemius white and red, soleus, and plantaris), heart, kidney, and spleen were excised immediately thereafter. Regional blood flow (Q) to the lower leg was calculated as the average radioactivity of all the muscles and expressed as ml · 100 g tissue^{-1} · min^{-1}. Injections giving more than 10% difference in blood flow between the kidneys, taken as evidence of inadequate distribution of microspheres, were excluded (15,16).

Microdialysis. The microdialysis technique has been described previously (9,10). Briefly, two 12-mm microdialysis catheters (BAS, 50 kD molecular weight cut-off; BAS, Indianapolis, IN) were inserted into the medial femoral muscle, one in each leg. After connecting the catheter inlet to a precision pump (Carnegie, Stockholm, Sweden), the system was perfused with 1% bovine albumin in isotonic saline at a rate of 1 µl/min. Starting 40 min after insertion of the two catheters, samples of the interstitial fluid were collected every 50 min.

Calculation of interstitial insulin concentrations. The insulin concentration in the interstitial fluid was calculated by means of an external reference with the use of [^{14}C]inulin infused subcutaneously for 24 h (see above). The recoveries of

TABLE 1
Relationship between plasma and dialysate insulin concentrations during hyperinsulinemic-euglycemic clamp in rats (n = 6) undergoing electrical stimulation of the right sciatic nerve

Clamp	Plasma insulin (μU/ml)	Dialysate insulin (μU/ml)	
		Contracting	Noncontracting
5 mU · kg^{-1} · min^{-1}	176 ± 11*	3.36 ± 0.17	3.35 ± 0.33
10 mU · kg^{-1} · min^{-1}	318 ± 15	5.31 ± 0.64	3.14 ± 0.16†

Data are means ± SE. *P < 0.001 when plasma insulin concentrations were compared during 5 and 10 mU · kg^{-1} · min^{-1} euglycemic clamp; †P < 0.01 when contracting and noncontracting legs were compared during 10 mU · kg^{-1} · min^{-1} euglycemic clamp.

both [^{14}C]inulin and insulin in dialysates obtained in vivo were compared, while the interstitial concentration of [^{14}C]inulin was assumed to be the same in plasma. To characterize the different diffusion properties and to correct for differences in binding of the compounds to the catheter, identical experiments were performed where [^{14}C]inulin and insulin were dialyzed in plasma at 37°C in vitro. Results from seven such in vitro experiments demonstrated that the relative recoveries of [^{14}C]inulin and insulin in microdialysates were 0.22 ± 0.01 and 0.03 ± 0.01, respectively. The corresponding [^{14}C]inulin recovery in experiments performed in vivo was 0.22 ± 0.04. The interstitial insulin concentration was then calculated in each animal according to the following formulas:

$$\text{in vivo:} \quad F(1) = \frac{\text{dpm (dialysate)}}{\text{dpm (plasma)} \cdot \text{insulin concentration (dialysate)}}$$

$$\text{in vitro:} \quad F(2) = \frac{\text{dpm (dialysate)} \cdot \text{insulin concentration (plasma)}}{\text{dpm (plasma)} \cdot \text{insulin concentration (dialysate)}}$$

$$F(1) \cdot F(2) = \text{interstitial insulin concentration}$$

Because the in vitro dialysate recovery of [^{14}C]inulin and insulin in plasma was identical to that estimated in vivo, a recovery factor for microdialysis of insulin could be calculated. The in vivo relative recovery of insulin in microdialysates was 3.0 ± 0.3 (mean ± SE; range 1.4–4.2%, n = 68). This is considerably less than previously achieved with a different ultrafiltration dialysis membrane (10). The present technique, however, is more convenient because ultrafiltration leakage of perfusate is less abundant.

To further validate the use of this external reference calibration technique in rat muscle, recovery of glucose was estimated using an external reference ([^{14}C]inulin) and the equilibration calibration method (9). Calculated recoveries for glucose were similar between these methods (data not shown).

Analytical methods. Blood was collected in heparinized microtubes and centrifuged immediately in a Beckman microfuge (Beckman, Palo Alto, CA). Plasma and dialysate glucose concentrations were measured enzymatically with a YSI Model 2700 SELECT (Yellow Springs Instruments, Yellow Spring, OH). Radioactivity was determined in a liquid scintillation counter (1217 Rackbeta; LKB, Uppsala, Sweden). Endogenous and exogenous insulin concentration was measured with equal efficiency by means of a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden).

Statistical analyses. Statistical methods used were Student's t test and, when several comparisons were performed, analysis of variance. Fisher's least significant differences test was used for post-hoc analyses.

RESULTS

Figure 1 depicts the relationship between plasma and interstitial insulin in normal SD rats during euglycemic hyperinsulinemia. Nonlinear regression analyses show that the relationship is curvilinear, and that interstitial insulin deviates from plasma insulin levels when the latter are above ~250 mU/ml (Fig. 1*A*). Furthermore, interstitial insulin concentrations did not exceed ~5–600 mU/ml, even in the presence of very high plasma insulin concentrations (Fig. 1*A*). The relationship between interstitial insulin concentrations and the glucose infusion rate is depicted in Fig. 1*B*. The data show that half maximal glucose infusion rate occurred in the presence of an interstitial insulin level of ~140 mU/ml, whereas maximal glucose infusion was achieved at ~325 mU/ml. According to the data depicted in Fig. 1*A*, the corresponding plasma insulin concentrations were ~140 and ~500 mU/ml, respectively.

Tables 1 and 2 show data obtained in rats undergoing unilateral electrical stimulation of the sciatic nerve during a two-step hyperinsulinemic glucose clamp. During the low rate of insulin infusion, the dialysate concentration of insulin was similar in samples obtained from both legs. During the higher rate of insulin infusion, the insulin concentration in dialysates collected from electrically stimulated muscle was significantly higher than that from the contralateral muscle (Table 1). Also, muscle blood flow was markedly increased on the electrically stimulated side (Table 2).

The obese Zucker rats had a significantly higher total body weight than the lean animals, and the former's retroperitoneal fat depot weight was significantly increased (Table 3). The respective weights of extensor digitorum longus and soleus muscles were comparable in both groups of animals (data not shown). Samples from the tail vein showed that obese Zucker rats had significantly higher plasma concentrations of glucose, lactate, and insulin than controls (Table 3).

TABLE 2
Blood flow (ml · 100 g^{-1} · min^{-1}) in contracting and noncontracting muscle during 10 mU · kg^{-1} · min^{-1} insulin infusion in rats (n = 6)

	Tibialis anterior	Extensor digitorum longus	Gastrocnemius white	Gastrocnemius red	Soleus	Plantaris
Contracted leg	28.1 ± 4.0*	36.4 ± 6.1*	24.0 ± 5.5*	28.3 ± 7.7*	24.1 ± 5.7*	22.5 ± 3.7*
Noncontracted leg	7.8 ± 1.1	8.9 ± 2.1	6.2 ± 0.7	7.6 ± 1.1	10.3 ± 1.3†	8.3 ± 1.3

Data are means ± SE. *P < 0.01, contracted leg versus noncontracted leg. †P < 0.05, tibialis anterior and white gastrocnemius versus soleus of noncontracted leg.

TABLE 3
Total-body and retroperitoneal adipose tissue weights and plasma glucose, insulin, and lactate in obese and lean Zucker rats

	n	Body weight (g)	Retroperitoneal (g)	Plasma glucose (mmol/l)	Plasma insulin (μU/ml)	Plasma lactate (mmol/l)
Lean	10	277 ± 7	1.27 ± 0.15	5.2 ± 0.2	6 ± 0.3	2.4 ± 0.20
Obese	10	363 ± 20*	5.81 ± 0.43†	8.2 ± 0.5†	40 ± 5.0†	3.6 ± 0.40*

Data are means ± SE. *$P < 0.01$; †$P < 0.001$.

Data from insulin measurements in normal and insulin-resistant Zucker rats are given in Table 4. In normal rats, muscle interstitial and plasma insulin were similar during infusion of the low insulin concentration. At higher plasma concentrations of insulin, interstitial insulin was significantly lower than plasma insulin in normal, but not in obese, rats (Table 4).

In obese Zucker rats, the glucose disposal rate during infusion of the low insulin concentration was significantly lower than that in lean animals (Fig. 2). Moreover, the net increase in glucose disposal rate during the high delivery rate of insulin was significantly lower in obese Zucker rats than in the normal animals. Plasma and interstitial insulin concentrations were similar during infusion of both low and high insulin concentrations in the obese Zucker rats. Consequently, interstitial insulin was significantly higher in skeletal muscle of obese Zucker rats than in normal animals (Table 4).

Plasma glucose at steady state was equal in both groups of animals throughout the insulin clamp (Table 5). The glucose content in dialysates from skeletal muscle was equal in both animal groups during the low insulin concentration. During infusion of the high insulin concentration, dialysate glucose concentrations decreased significantly in both groups of rats (Table 5). However, dialysate glucose concentrations were significantly higher in obese Zucker rats than in normal rats (Table 5).

Control measurements of the percentage recovery of plasma [^{14}C]inulin in dialysates demonstrated the maintenance of steady state microdialysis conditions in both groups of rats (Table 6).

DISCUSSION

Estimation of interstitial insulin concentration. The present study is the first to report on the regulation of insulin in the interstitial fluid of skeletal muscles. Estimation by microdialysis of interstitial fluid during hyperinsulinemia clearly showed that the insulin level differs significantly from that in the plasma. In the present study, muscle microdialysis was also performed in muscles during electrical stimulation of the sciatic nerve. The data show that muscle contraction and the concomitant increase of blood flow increase the dialysate content of insulin only during the high rate of insulin infusion. These data are clear evidence that muscle interstitial insulin concentrations are lower than plasma concentrations only during the infusion of the high insulin concentration. Furthermore, the fact that dialysate concentration of insulin was not affected by electrical stimulation during lower insulin infusion rates rules out any tentative influence of muscle contraction on the microdialysis recovery of ambient insulin. Instead, the influence of higher blood flow to overcome the capillary barrier for insulin delivery to the muscle cells must be emphasized.

Transcapillary delivery of insulin to the interstitial space seems to be saturable, given that interstitial insulin concentrations were not higher than ~5–600 mU/ml, even at very high plasma insulin concentrations (~2000 mU/ml). This is in line with data presented from studies in lymph (6,7) and subcutaneous adipose (10) tissue. This may also concur with the view that transcapillary delivery of insulin is a saturable (17), active, receptor-mediated, and transendocytotic process (18). It must be noted, however, that the interstitial concentration is the net result of the balance between transcapillary delivery and elimination of insulin and, therefore, cannot give conclusive information on whether these processes might be regulated by the ambient insulin concentration. In a recent report where lymph and plasma insulin concentrations were measured in dogs, no such saturable process was detected (19). It is unclear whether the discrepancy in data obtained in that study may be explained by the differences in methods (lymph versus interstitial fluid) or reflect regional dif-

TABLE 4
Plasma and interstitial insulin concentrations (μU/ml) in obese and lean Zucker rats during euglycemic clamp at submaximal insulin concentrations (8 mU · kg^{-1} · min^{-1}) for 140 min followed by a higher insulin concentration (20 mU · kg^{-1} · min^{-1}) for 100 min

		8 mU · kg^{-1} · min^{-1}			20 mU · kg^{-1} · min^{-1}	
	n	40 min	90 min	140 min	190 min	240 min
Plasma insulin(μU/ml)						
Lean	10	192 ± 20	203 ± 17	228 ± 38	524 ± 82	574 ± 188
Obese	10	250 ± 22	314 ± 57	330 ± 34	584 ± 87	530 ± 143
Interstitial insulin (μU/ml)						
Lean	10	—	183 ± 27	177 ± 23	398 ± 68*	360 ± 71*
Obese	10	—	310 ± 53	353 ± 46*	578 ± 126†	592 ± 78†

Data are means ± SE. *$P < 0.05$, interstitial versus plasma insulin concentrations; †$P < 0.05$, obese and lean animals.

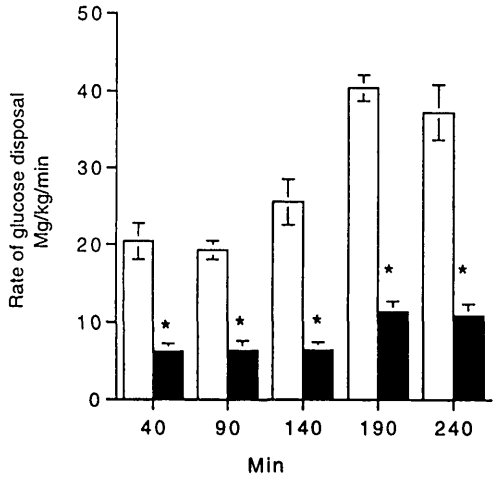

FIG. 2. Total-body glucose uptake during a hyperinsulinemic-euglycemic clamp in obese Zucker rats (■) and their lean litter mates (□). The insulin infusion rate of 8 mU · kg^{-1} · min^{-1} was maintained between 40 and 140 min and then changed to 20 mU · kg^{-1} · min^{-1}. Data are means ± SE, n = 10. *P < 0.001.

ferences in insulin delivery or elimination. Also, in contrast to previous lymph studies (6,7,20), we did not find any difference between plasma and interstitial insulin levels when these were lower than ~250 mU/l. It might be speculated that the microdialysis technique itself partly destroys the capillary barrier, resulting in artifactually high interstitial insulin levels as well as local insulin resistance. This, however, is not a likely explanation for the discrepancy in the data, because the interstitial glucose levels were confirmed (11) to be lower than in plasma.

The finding that maximum glucose consumption occurred at plasma insulin concentrations of ~500 mU/ml and interstitial insulin concentrations of ~325 mU/ml indicates that saturation of the insulin transport system might be of limited physiological importance in normal rats. This was not further evaluated in this study because direct concentration/effect relationships were not obtainable.

It should be noted that the present data refer only to the relationship between plasma and interstitial concentrations of insulin at steady state, and that time kinetics of concentration changes were not recorded. Hence, the delayed kinetics for insulin concentration changes in the interstitial fluid as suggested from data obtained in the lymph (6,7) as well as in the subcutaneous tissue (10) were not emphasized.

Parallel experiments were also performed in normal and insulin-resistant obese Zucker rats. It may be noted that plasma insulin levels in these animals were considerably lower than those reported by others (21,22). The discrepancy in data may reflect differences in analytical techniques for insulin measurements, as well as differences in animal age. The present animals were age 7 weeks, and the insulin level was assayed with rat insulin as the internal standard. Regardless of differences in absolute insulin levels in the literature, it is clear that the obese animals we studied were insulin resistant (Fig. 2).

It is notable that the interstitial insulin concentration in the obese rats was similar to that in plasma, even at high concentrations (Table 3). Thus in this model of insulin resistance, transcapillary delivery of insulin does not seem to be rate limiting for insulin uptake, even at supraphysiological concentrations. In theory, this may be due to either enhanced capillary diffusion of insulin or reduced insulin elimination. The present data suggest that total-body insulin degradation was not altered in the insulin-resistant animals during the insulin clamp.

The finding of higher insulin levels in insulin-resistant muscles agrees with previous findings that lymph insulin in the leg was high in insulin-resistant dogs (23) and normal (not reduced) in obese human subjects (20). Altogether, the data from various models of insulin resistance suggest that the defect in insulin responsiveness resides in the muscle cells.

Glucose uptake in insulin-resistant muscles. Calibration of the microdialysis recovery of glucose was not carried out in the present study. However, previous investigations in rat (24) and human skeletal muscle (11) have demonstrated that the interstitial glucose concentration is lower than in venous plasma. The present data show that the dialysate concentration, as a semiquantitative measure of interstitial glucose, was rapidly decreasing when plasma insulin levels (Table 4) and glucose disposal rates (Fig. 2) were increased. Control experiments in which the equilibrium calibration technique (9) was used have shown that, under identical conditions, the relative recovery of muscle glucose in dialysates is 31 ± 2% (mean ± SE, n = 24). Thus the interstitial glucose levels in normal rats during low and high insulin infusion rates could be estimated to be 3.0 ± 0.4 and 1.5 ± 0.2

TABLE 5
Plasma and dialysate glucose concentrations (mmol/l) in obese and lean Zucker rats during euglycemic clamp at submaximal insulin concentrations (8 mU · kg^{-1} · min^{-1}) for 140 min followed by a higher insulin concentration (20 mU · kg^{-1} · min^{-1}) for 100 min

		8 mU · kg^{-1} · min^{-1}			20 mU · kg^{-1} · min^{-1}	
	n	40 min	90 min	140 min	190 min	240 min
Plasma glucose (mmol/l)						
Lean	10	7.1 ± 0.29	6.9 ± 0.13	6.5 ± 0.23	7.0 ± 0.15	6.6 ± 0.4
Obese	10	7.6 ± 0.35	7.5 ± 0.27	7.1 ± 0.20	7.0 ± 0.18	6.9 ± 0.1
Dialysate glucose (mmol/l)						
Lean	10	—	0.95 ± 0.11	0.93 ± 0.13	0.58 ± 0.07†	0.46 ± 0.07†
Obese	10	—	1.1 ± 0.09	1.0 ± 0.05	0.89 ± 0.4*	0.74 ± 0.1*†

Data are means ± SE. *P < 0.001 obese versus lean animals; †P < 0.001 dialysate glucose concentrations during infusion of submaximal versus maximal insulin concentrations.

TABLE 6
Control measurements of the percentage recovery of plasma [^{14}C]inulin in dialysate in obese and lean Zucker rats

		8 mU · kg^{-1} · min^{-1}		20 mU · kg^{-1} · min^{-1}	
	n	90 min (%)	140 min (%)	190 min (%)	240 min (%)
Lean	10	21.1 ± 2.0	22.8 ± 5.9	18.1 ± 3.1	20.1 ± 3.1
Obese	10	28.5 ± 2.5	20.4 ± 2.6	24.6 ± 3.9	24.4 ± 5.7

Data are means ± SE.

mmol/l, respectively. It may then be concluded that, in normal skeletal muscles, the capillary diffusion rate of glucose is partly rate limiting for insulin-stimulated glucose uptake. In the insulin-resistant Zucker rats, however, the glucose concentration was higher in interstitial fluid because of the reduced rate of glucose uptake. Yet the interstitial glucose level decreased to a small extent following the high insulin infusion rate in insulin-resistant animals; hence capillary delivery of glucose was, to a lesser extent, rate limiting for glucose uptake. Preliminary data obtained in our laboratory suggest that the ability to decrease interstitial glucose levels in muscles in response to insulin is further diminished in diabetic animals.

The present data may give us further insight into the consequences of diminished vasodilation demonstrated in insulin-resistant muscle (3–5). We found higher-than-normal interstitial insulin and glucose concentrations in the insulin-resistant muscles despite the possibility that capillarization might be reduced (21). This strongly suggests that the main mechanism responsible for insulin resistance resides in the muscle cells, and that the vasodilatory effect of insulin and glucose then becomes less important.

In summary, microdialysis measurements of insulin and glucose in muscle interstitial fluid provide evidence that transcapillary delivery of these substances is rate limiting for the uptake and metabolism of both substances in normal insulin-sensitive rats, but less so in insulin-resistant Zucker rats. The insulin resistance in the latter animals seems to be related to a cellular defect in glucose uptake, rather than to insufficient transcapillary delivery of glucose or insulin.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Medical Research Council (K95-19P-11330-01A, project no 10864), the Swedish Society of Medicine, the Swedish Diabetes Association, Nordisk Insulinfond, and the Inga-Britt and Arne Lundberg Foundation.

The authors thank Lena Halvordsson for excellent technical assistance and Gudrun Jonson and Raija Saikkonen for skillful secretarial help.

REFERENCES

1. DeFronzo RA: Lilly Lecture: the triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 37:667–687, 1988
2. Christensen NJ: Acute effects of insulin on cardiovascular function and noradrenaline uptake and release. *Diabetologia* 25:377–381, 1983
3. James DE, Burleigh KM, Storlien LH, Bennett SP, Kraegen EW: Heterogeneity of insulin action in muscle: influence of blood flow. *Am J Physiol* 251:E422–E430, 1986
4. Laakso M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. *J Clin Invest* 85:1844–1852, 1990
5. Baron AD, Brechtel G, Johnson A, Fineberg N, Henry DP, Steinberg HO: Interactions between insulin and norepinephrine on blood pressure and insulin sensitivity. *J Clin Invest* 93:2453–2462, 1994
6. Rasio E, Mach E, Egdahl R, Herrera M: Passage of insulin across vascular membranes in the dog. *Diabetes* 17:668–672, 1968
7. Yang Y, Hope J, Ader M, Bergman R: Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest* 84:1620–1628, 1989
8. Delgado JMR, DeFendis FV, Roth RH, Ryngo DK, Mitruka BM: Dialytrode for long-term intracerebral perfusion in awake monkeys. *Arch Int Pharmacodyn Ther* 198:9–21, 1972
9. Lönnroth P, Jansson P-A, Smith U: A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol* 253:E228–E231, 1987
10. Jansson P- A, Fowelin J, von Schenck H, Smith U, Lönnroth P: Measurement by microdialysis of the insulin concentration in subcutaneous interstitial fluid. *Diabetes* 42:1469–1473, 1993
11. Müller M, Schmid R, Nieszpaur-Los M, Fassolt A, Lönnroth P, Fasching P, Eichler HG: Key metabolite kinetics in human skeletel muscle during ischaemia and reperfusion: measurement by microdialysis. *Eur J Clin Invest* 25:601–607, 1995
12. Kraegen E, James D, Bennett S, Chisholm D: In vivo insulin sensitivity in the rat determined by euglycemic clamp. *Am J Physiol* 245:E1–E7, 1983
13. Terretaz J, Jeanrenaud B: In vivo hepatic and peripheral insulin resistance in genetically obese (fa/fa) rats. *Endocrinology* 112:1346–1353, 1983
14. Holmäng A, Svedberg J, Jennische E, Björntorp P: Effects of testosterone on muscle insulin sensitivity and morphology in female rats. *Am J Physiol* 259:E555–E560, 1990
15. Flaim S, Zelis R: Effects of diltiazem on total cardiac output distribution in conscious rats. *J Pharmacol Exp Ther* 222:359–366, 1982
16. Elander A, Idström J, Scherstén T, Bylund-Fellenius A: Metabolic adaptation to reduced muscle blood flow. I. Enzyme and metabolite alterations. *Am J Physiol* 249:E63–E69, 1985
17. Prigeon RL, Roder ME, Porte D, Kahn SE: The effect of insulin dose on the measurement of insulin sensitivity by the minimal model technique: evidence for saturable insulin transport in humans. *J Clin Invest* 97:501–507, 1996
18. King GL, Johnson SM: Receptor-mediated transport of insulin across endothelial cells. *Science* 227:1583–1585, 1985
19. Steil GM, Kerstin R, Moore DM: Receptor independent transport of insulin across capillary endothelial cells in vivo. *J Clin Invest* 97:1497–1503, 1996
20. Castillo C, Bogardus C, Bergman R, Thuillez P, Lillioja S: Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. *J Clin Invest* 93:10–16, 1994
21. Lash JM, Sherman WM, Hamlin RL: Capillary basement membrane thickness and capillary density in sedentary and trained obese Zucker rats. *Diabetes* 38:854–860, 1989
22. Pénicaud L, Ferré P, Terretaz J, Framekinebanyan M, Leturque A, Doré E, Girard J, Jeanrenaud B, Ricon L: Development of obesity in Zucker rats: early insulin resistance in muscles but normal sensitivity in white adipose tissue. *Diabetes* 36:626–631, 1987
23. Ader M: Diabetes alters transendothelial insulin transport kinetics. *Diabetes* 43 (Suppl. 1):41A, 1994
24. Hickner RC, Ungerstedt U, Henriksson I: Regulation of skeletal muscle blood flow during acute insulin-induced hypoglycemia in the rat. *Diabetes* 43:1340–1344, 1994