Altered Distribution of Insulin Between Intravascular and Interstitial Compartments in STZ-Induced Diabetic Rats

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These studies were undertaken to determine the distribution of insulin between the intravascular and interstitial compartments in 19 nondiabetic and 16 high-dose (60 mg/kg) STZ-induced diabetic rats. Anesthetized male rats underwent cannulation of the thoracic lymph duct with continuous collection of lymph by passive drainage; 90-min hyperinsulinemic (0, 14, 28, or 42 pmol · kg^{-1} · min^{-1}) glucose clamps were performed with serum glucose held at the preinfusion level. Integrated lymph samples were collected, with serum collection at the midpoint. Before insulin infusion, insulin levels in lymph as a percentage of levels in serum were higher in the diabetic rats (lymph = 103 ± 8% of 52 matched serum values, means ± SE) compared with nondiabetic rats (lymph = 83 ± 3% of serum; n = 84, $P < 0.008$). Under steady-state conditions during insulin infusion (66–90 min), lymph insulin as a percentage of serum insulin decreased significantly in nondiabetic animals (range 53–62% in all insulin-infused groups, vs. 94% in the absence of insulin infusion, $P < 0.0001$). However, in the diabetic animals, the proportion was similar in the presence or absence of infused insulin (range 57–63% in insulin-infused groups vs. 70% in the absence of infused insulin, P = NS). Lymph flow rate was significantly higher in diabetic than nondiabetic rats (37.7 ± 7.2 vs. 17.9 ± 1.5 μl/min [means ± SE], $P < 0.02$). Distribution of [^{14}C]inulin was uniform between the two compartments in all animals. In nondiabetic rats, correlation of lymph and serum levels of insulin with hepatic glucose output was equal (r = −0.86 and −0.85 for serum and lymph insulin, respectively) and as was the case for peripheral glucose disposal, r = 0.75 for serum, and r = 0.72 for lymph. Estimated clearance of insulin from serum to lymph was 100% higher in the diabetic rats (22.9 vs. 12.7 μl/min). The mechanism is unclear but may represent increased transcapillary transport of insulin, adaptive to insulin deficiency. *Diabetes* 42:1528–35, 1993

Transcapillary transport may be rate limiting for insulin action (1). In studies conducted on nondiabetic dogs, Rasio et al. (2) and Yang et al. (1) showed that a gradient for insulin exists between the vascular and lymph compartments and that a highly significant correlation exists between lymph insulin levels and the onset of insulin action under nonsteady-state conditions and lymph insulin levels and insulin action at steady state (1). This was true for both suppression of HGO and peripheral glucose disposal. Furthermore, this gradient is maintained at all insulin infusion rates studied. In addition, thoracic duct lymph insulin levels are closely correlated with hindlimb lymph insulin levels (3,4), suggesting that insulin levels in thoracic duct lymph are also representative of those found in lymph draining insulin-sensitive tissues such as the muscle bed of the hindlimb. Insulin concentrations in lymph-draining peripheral tissues of the dog hindlimb were more closely correlated with the onset of insulin action than insulin levels in plasma (4). Comparison of insulin-stimulated glucose oxidation rates between arterially perfused rat adipose tissue and isolated adipocytes also suggests that transcapillary transfer of insulin may regulate insulin action (5). This study was designed to ascertain *1*) whether the approach of Rasio et al. and Yang et al. can be applied to the rat, a small animal model frequently used in the study of aspects of glucose metabolism; and *2*) whether the relationship between serum and lymph insulin is altered under conditions of prolonged insulin deficiency such as high-dose STZ-induced diabetes.

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STZ, streptozocin; HGO, hepatic glucose output; R_d, peripheral glucose uptake; RIA, radioimmunoassay; NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus.

RESEARCH DESIGN AND METHODS

D-[3-^{3}H]Glucose and [^{14}C]inulin were obtained from NEN-Du Pont (Boston, MA). Porcine insulin and STZ were obtained from Sigma (St Louis, MO). Male Sprague-Dawley rats weighing 220–250 g were obtained from the University of British Columbia Animal Care Center (Vancouver, BC). Studies were approved by the University Animal Care and Use Committee. Some of the rats were given an intravenous injection of 60 mg/kg STZ via the tail vein, under brief restraint while awake. STZ-administered animals manifested 2% glycosuria within 48 h. Animals were then housed for an additional 3 wk in climate-controlled conditions with 12-h alternating light-dark cycles and fed on standard rat-chow (49% carbohydrate, 5% fat, 23% protein, and 23% fiber by weight) ad libitum.

Lymph sampling and glucose clamp procedure. After weighing, animals were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital and placed on a warming pad. They then underwent tracheostomy with supplemental oxygen administered via a T-piece. Internal jugular vein and common carotid artery were cannulated with fine bore polyethylene tubing (PE 50) and periodically flushed with 50 U/ml of heparin. Under a Kyowa STZ-TR stereo dissecting microscope (Tokyo, Japan), the thoracic lymph duct was identified and cannulated (6) with very fine bore tubing (PE 10), and lymph was collected by continuous passive drainage. Arterial blood was collected at the midpoint of each lymph collection period. During a 15-min baseline period, lymph and serum were collected at 3-min intervals for insulin measurement, and glucose was also measured in serum samples. Lymph volume was also measured. Thereafter, an adaptation of the glucose clamp technique described in humans (7) was used for rats, as described by Burnol et al. (8). After a ×100 loading dose for 1 min, D-[3-^{3}H]glucose was infused at 0.1 μCi/min throughout the remainder of the study for isotopic measurement of glucose turnover, together with [^{14}C]inulin at 0.02 μCi/min via the jugular vein. An infusion of porcine regular insulin was simultaneously commenced at either 0, 14, 28, or 42 pmol · kg^{-1} · min^{-1}. Sufficient 20% D-glucose was then infused as needed at a rate sufficient to maintain serum glucose at the basal level. Thereafter, lymph and blood (60 μl) were sampled at 3-min intervals (0–30 min) and 6-min intervals (31–90 min) for measurement of glucose, insulin, and lymph volume. At 60 and 90 min, additional blood (75 μl) was obtained for determination of [^{14}C]inulin and D-[3-^{3}H]glucose distribution. Total volume of saline in which insulin, [^{14}C]inulin, and D-[3-^{3}H]glucose were infused was 1.38 ml. This was approximately equal to the total volume of blood sampled (1.1–1.2 ml). Urinary glucose losses were measured by emptying the bladder at the start of the steady-state period and collecting urine at the end of the steady-state period. Urine volume and glucose concentration were determined and included in the glucose turnover calculations. At 90 min, animals were killed by intravenous injection of 250 mg/kg of pentobarbital.

Analytical methods. Serum glucose was determined by glucose oxidase technique in a YSI 23A glucose analyzer (Yellow Springs, OH). Serum and lymph insulin were measured by double-antibody RIA kit (ICN, Costa Mesa, CA). Lymph triglyceride levels were determined colorimetrically by enzymatic production of hydrogen peroxide (9). For determination of D-[3-^{3}H]glucose and [^{14}C]inulin concentrations, serum was diluted 1:4 with water and then added to an equal volume of perchloric acid, final concentration 2.5%. Proteins were precipitated by centrifugation at 2000 *g* for 10 min. Aliquots of supernatant were then dehydrated for 6 h at 55°C and counted in a β-scintillation counter. Glucose disposal was calculated according to Steele's equations for isotope dilution under steady-state conditions (10). HGO was calculated by subtraction of the exogenous glucose infusion rate from the isotopically derived estimate of total unlabeled glucose production. Insulin clearance from serum to lymph (defined as the volume of serum completely cleared of insulin by passage into lymph per unit time) was calculated as

$$\text{Insulin clearance}_{(Se \rightarrow Ly)} = \frac{\text{lymph flow (μl/min)} \times \text{lymph [insulin] (pM)}}{\text{serum [insulin] (pM)}}$$

Statistical methods. Two-sample Student's *t* test was used for continuous variables. Where appropriate, data was analyzed by ANOVA using the CSS: Statistica program (Statsoft, Tulsa, OK).

RESULTS

Characteristics of the animals studied and glucose clamp data are shown in Tables 1 and 2. All groups of animals were weight-matched, although a small but statistically significant difference in weight existed between the diabetic rats who received insulin at 42 pmol · kg^{-1} · min^{-1} compared with the other three diabetic groups. No difference occurred in basal versus steady-state serum glucose concentrations among the animals receiving insulin at the various infusion rates. Because animals were clamped at basal serum glucose levels, profound differences occurred in both basal and steady-state serum glucose levels between the diabetic rats and their nondiabetic counterparts. In the diabetic animals who received 0 pmol · kg^{-1} · min^{-1} infused insulin, R_d is slightly but not significantly higher than HGO because in 1 animal small quantities of exogenous glucose were infused to maintain serum glucose at basal levels in the latter part of the clamp study. The lower plasma glucose during the clamp period in this animal probably represented an expected fluctuation from the mean, because in the remaining 3 animals in this group plasma glucose at steady state was insignificantly different from basal in 2 animals (i.e., within 3%) and 11% higher in the third. Values for HGO are negative in the nondiabetic animals who received insulin infusions at 28 and 42 pmol · kg^{-1} · min^{-1} because cold glucose infusate was not spiked with D-[3-^{3}H]glucose in these studies. Despite the much higher serum glucose levels in the diabetic rats, R_d during insulin infusion was not appreciably greater in the diabetic than the nondiabetic animals, and HGO was not significantly suppressed by insulin in the diabetic animals, confirming insulin resistance. Although serum insulin concentrations are similar

TABLE 1
Insulin-glucose clamp data in nondiabetic animals

	Insulin infusion rate (pmol · kg^{-1} · min^{-1})			
	0	14	28	42
n	6	4	5	4
Weight (g)	316 ± 12	290 ± 4	319 ± 17	306 ± 15
Basal glucose (mM)	4.9 ± 0.2	5.1 ± 0.1	4.3 ± 0.5	5.3 ± 0.3
Glucose at steady state (mM)	5.3 ± 0.3	4.8 ± 0.1	4.3 ± 0.4	5.0 ± 0.3
R_d (mmol · kg^{-1} · h^{-1})	2.1 ± 0.4	2.1 ± 0.1	3.1 ± 0.1	4.1 ± 0.2*
HGO (mmol · kg^{-1} · h^{-1})	2.1 ± 0.4	0.0 ± 0.2*	−1.1 ± 0.4†	−1.8 ± 0.3†
Serum insulin at steady state (pM)	200 ± 23	512 ± 52	1088 ± 110	1969 ± 168
Lymph insulin at steady state 163 ± 12 (pM)	163 ± 12	294 ± 16	493 ± 39	1023 ± 69

Data are means ± SE.
*$P < 0.005$ vs. zero insulin group.
†$P < 0.001$ vs. zero insulin group.

in the diabetic and nondiabetic rats who did not receive infused insulin (Tables 1 and 2), serum glucose concentration is ~3-fold higher in the diabetic rats, confirming relative insulin deficiency.

Lymph insulin concentration as a percentage of serum insulin concentration under basal conditions before insulin infusion is shown in Table 3. In nondiabetic rats, lymph insulin levels were proportionally lower than serum levels (83 ± 3% in 84 paired determinations, $P < 0.001$), whereas in the diabetic rats, lymph insulin was equal to the corresponding serum value (103 ± 8% in 52 paired determinations, $P > 0.7$). This ratio was significantly different to the ratio in the nondiabetic rats ($P < 0.008$). In several of the diabetic animals, baseline absolute insulin levels were clearly higher in the lymph than the serum.

Insulin levels in lymph and serum over time are shown in Fig. 1 for a diabetic and a nondiabetic animal. The diabetic animal shown was chosen as an example of lymph insulin levels being higher than serum levels during the baseline period. These graphs also illustrate the delay in equilibration of insulin levels in lymph compared with serum, as well as the gradient between the two compartments that persists at steady state. Mean steady-state levels of insulin in serum and lymph are shown in Figs. 2*A* (nondiabetic animals) and 2*B* (diabetic animals) at all infusion rates studied. In both groups of animals under steady-state conditions during insulin infusion, insulin levels in lymph are significantly lower than those in serum, except in the nondiabetic rats who did not receive insulin, in which the serum-to-lymph gradient is similar to that shown during the baseline pre-infusion period (Table 3).

For ease of interpretation, mean steady-state lymph insulin concentration as a percentage of serum insulin concentration is shown in Fig. 3, both for nondiabetic and diabetic animals at the various infusion rates. In the nondiabetic animals, the serum-lymph insulin gradient widens greatly in those animals who received insulin compared with the control group ($P < 0.0001$ for control vs. all insulin-treated animals). However, in the diabetic animals, the serum-to-lymph gradient fails to widen in response to insulin compared with the noninsulin-infused control group, ($P > 0.1$ for control vs. all insulin-treated animals). Any inferences drawn from this data should however take into account that, in the diabetic animals, a significant fall occurs in the lymph-to-serum insulin ratio compared with baseline, even in the absence of infused insulin (Table 2).

The correlations between serum and lymph insulin concentrations and HGO and R_d are illustrated in Figs. 4 and 5. A strong correlation between both lymph and serum insulin and both HGO and R_d exists in nondiabetic animals (Fig. 4). In this study, insulin level in neither compartment was superior in predicting either parameter of insulin action in nondiabetic animals. For R_d, $r = 0.75$ and $r = 0.72$ against serum and lymph insulin, respectively ($P > 0.8$ for a difference between the r values). For HGO, $r = -0.86$ against serum and $r = -0.85$ against

TABLE 2
Insulin-glucose clamp data in diabetic animals

	Insulin infusion rate (pmol · kg^{-1} · min^{-1})			
	0	14	28	42
n	4	4	4	4
Weight (g)	307 ± 22	300 ± 17	311 ± 15	275 ± 7
Basal glucose (mM)	16.8 ± 0.5	15.3 ± 0.8	17.5 ± 1.3	16.5 ± 1.4
Glucose at steady state (mM)	17.3 ± 1.3	15.9 ± 0.8	17.9 ± 0.7	19.1 ± 0.8
R_d (mmol · kg^{-1} · h^{-1})	2.8 ± 0.5	4.6 ± 0.3	4.0 ± 0.4	4.9 ± 1.2
HGO (mmol · kg^{-1} · h^{-1})	2.2 ± 0.4	3.2 ± 0.7	0.9 ± 1.0	1.7 ± 1.0
Serum insulin at steady state (pM)	232 ± 0.4	516 ± 27	924 ± 121	1078 ± 73
Lymph insulin at steady state (pM)	144 ± 18	312 ± 23	492 ± 70	620 ± 14

Data are means ± SE.

TABLE 3
Basal insulin compartmentalization and inulin distribution, lymph flow, and lymph triglyceride in rats during glucose clamps

	Nondiabetic rats	Diabetic rats
Preclamp insulin (lymph/serum × 100)	83 ± 3	103 ± 8*
Lymph flow rate (μl/min)	17.9 ± 1.5	37.7 ± 7.2†
Lymph triglyceride (mM)	8.0 ± 1.1	11.3 ± 2.3
Counts per minute in [^{14}C]inulin (lymph/serum × 100)	106 ± 4	104 ± 5

Data are means ± SE.
*$P < 0.008$ vs. nondiabetic animals.
†$P < 0.001$ vs. nondiabetic animals.

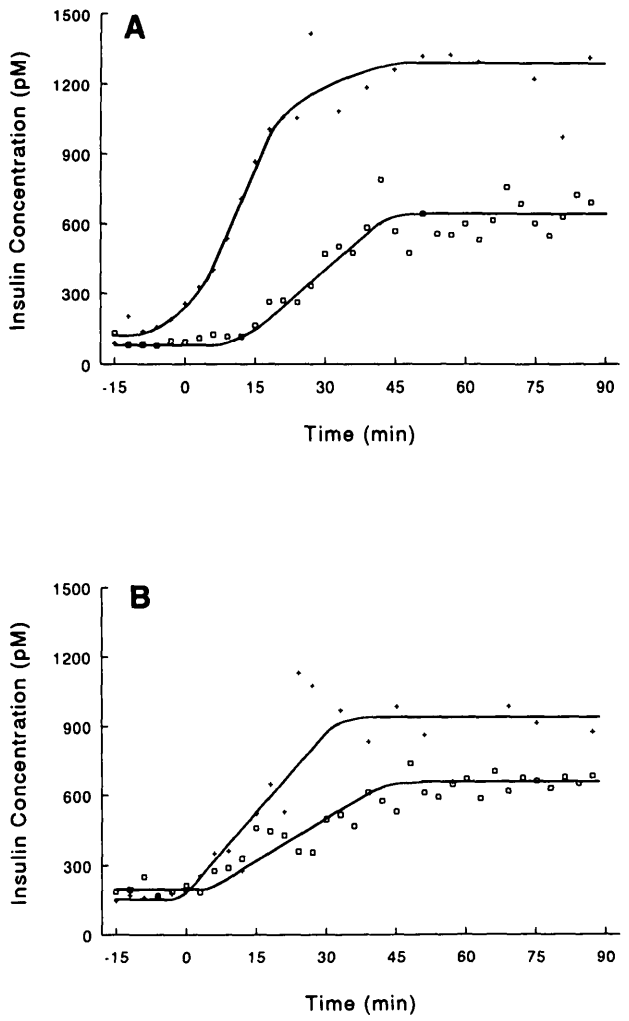
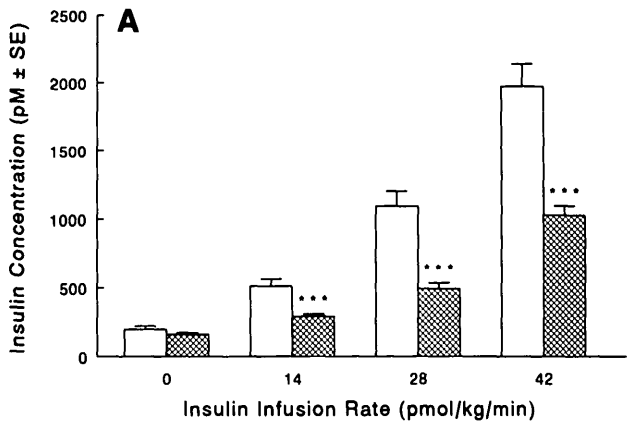


FIG. 1. Insulin concentration in lymph (□) and serum (+) in a nondiabetic (*A*) and a diabetic (*B*) rat. Lymph was collected by passive drainage over 3 min, with serum sampled at midpoint. Time shown is minutes of insulin infusion at 42 pmol · kg^{-1} · min^{-1}; −15–0 min is baseline sampling period before insulin infusion.



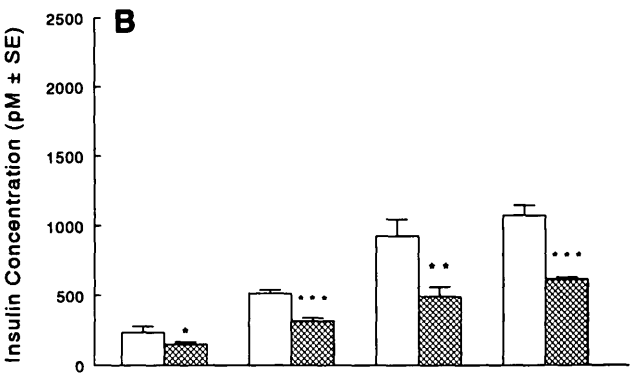


FIG. 2. Mean steady-state insulin concentrations in serum (□) and lymph (▩) during clamp studies in nondiabetic (*A*) and diabetic (*B*) rats. In nondiabetic animals, serum concentrations were 200 ± 23, 512 ± 52, 1088 ± 110, and 1969 ± 168 pM (means ± SE) at insulin infusion rates of 0, 14, 28, and 42 pmol · kg^{-1} · min^{-1}, respectively. Corresponding lymph insulin levels were 163 ± 12, 294 ± 16, 493 ± 39, and 1023 ± 69 pM. In diabetic animals, similarly expressed, serum levels were 232 ± 44, 516 ± 27, 924 ± 121, and 1078 ± 73 pM. Lymph levels were 144 ± 18, 312 ± 23, 492 ± 70, and 620 ± 14 pM. Number of animals is shown in Tables 1 and 2. *$P < 0.05$, **$P < 0.005$, and ***$P < 0.001$ vs. matched serum level.

lymph insulin concentration ($P > 0.8$ for a difference between the r values). In the diabetic group, neither lymph nor serum insulin concentration exhibited a strong correlation with suppression of HGO ($r = -0.26$ for serum insulin, P = NS; $r = -0.10$ for lymph insulin, P = NS), presumably a reflection of hepatic insulin resistance (Fig. 5*A*). Similarly, no correlation existed between either serum or lymph insulin concentration and R_d ($r = 0.18$ for lymph insulin, $P > 0.5$; $r = 0.17$ for serum insulin, $P > 0.5$) (Fig. 5*B*). Again, correlation was not significantly greater between R_d and insulin concentration in either compartment in the diabetic animals.

The correlation between serum and lymph insulin concentrations is shown in Fig. 6. In both nondiabetic (Fig. 6*A*) and diabetic (Fig. 6*B*) animals, correlation between serum and lymph insulin concentrations was very strong ($r = 0.98$, $P < 0.001$ for nondiabetic and $r = 0.93$, $P < 0.001$ for diabetic animals). The slope of the regression line was similar for both groups of animals, although absolute values were lower for diabetic rats, presumably reflecting loss of endogenous insulin production and possibly accelerated insulin clearance.

Lymph flow rate was twice as high in diabetic animals

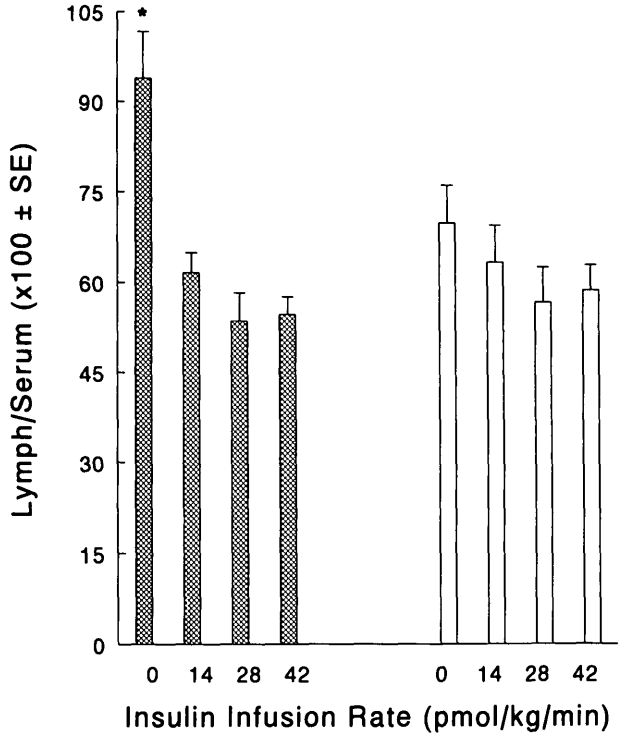


FIG. 3. Mean steady-state lymph insulin concentration expressed as a percentage of serum concentration during glucose clamps in nondiabetic (▩) and diabetic (□) rats. Data (left to right) are 94 ± 8, 62 ± 3, 53 ± 5, and 55 ± 3% (means ± SE) for nondiabetic animals and 70 ± 6, 63 ± 6, 57 ± 6, and 59 ± 4% for diabetic animals. Number of animals is shown in Tables 1 and 2. *$P < 0.0001$ vs. all insulin-infused nondiabetic animals.

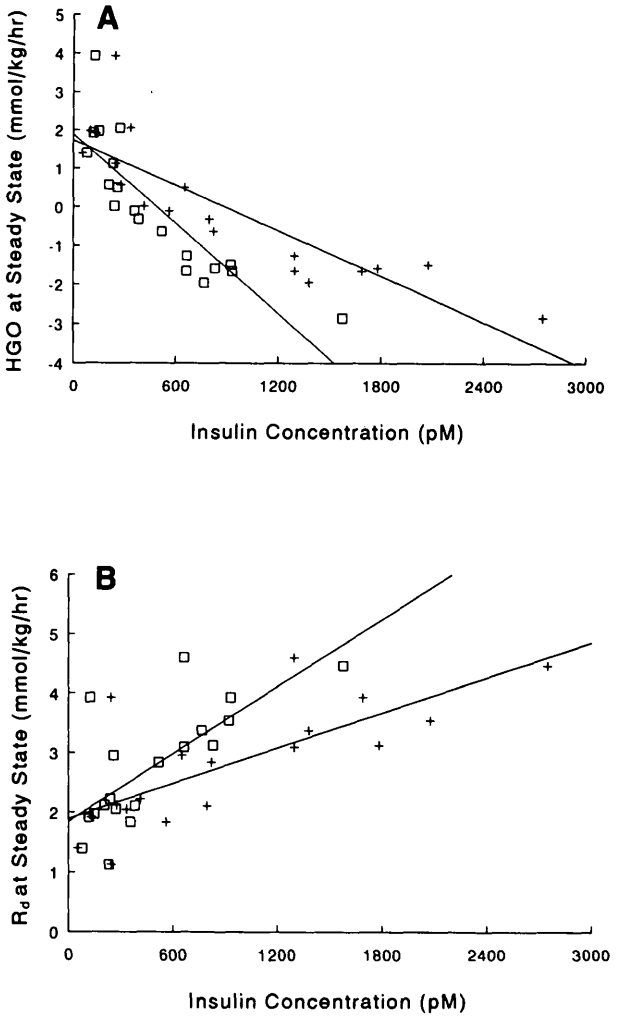


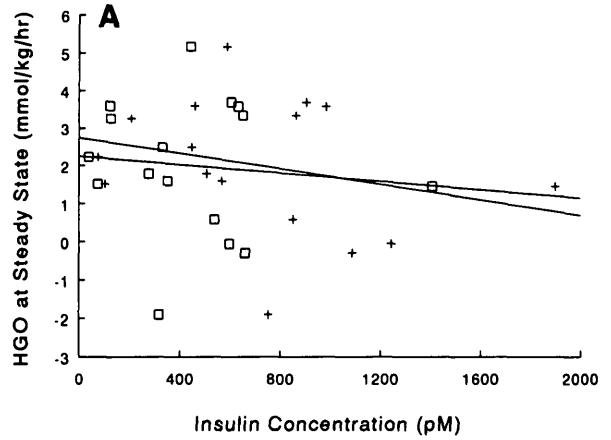
FIG. 4. Correlation between steady-state serum (+) and lymph (□) insulin concentrations during glucose clamps in nondiabetic animals and HGO (*A*) and R_d (*B*). For HGO vs. serum insulin level, $r = -0.86$ ($P < 0.0001$), and for HGO vs. lymph insulin level, $r = -0.85$ ($P < 0.0001$). For R_d vs. serum insulin, $r = 0.75$ ($P < 0.001$), and for R_d vs. lymph insulin, $r = 0.72$ ($P < 0.001$).

as nondiabetic animals, as shown in Table 3. Lymph lipid content was also measured, because diabetic lymph was milkier in appearance than nondiabetic lymph and a marked increase in lipid might result in artificially low measured concentrations of water-soluble substances. Although measured lymph triglyceride was higher in diabetic animals, this difference was not significant, and in both groups estimated total lymph lipid concentration was <2%. Table 3 also shows the distribution of [^{14}C]inulin, a carbohydrate molecule similar in size and M_r to insulin, between serum and lymph. No difference in inulin distribution between the two compartments existed in either diabetic or nondiabetic animals.

DISCUSSION

Many defects at the target-tissue level have already been described that in part provide explanations of the insulin resistance that is characteristic of NIDDM and may also occur in IDDM. These include abnormalities in insulin receptor number (11), insulin receptor tyrosine kinase activity (12,13), and abnormalities of glucose transporters (14). However, the effect of either condition on the ability of insulin in plasma to gain access to the interstitial fluid, in which the concentration of insulin determines insulin receptor occupancy, is largely unknown. Capillaries perfusing skeletal muscle have tight endothelial junctions and are nonfenestrated (15). In addition, capillary endothelial cells have been shown to both possess insulin receptors (16) and to take up labeled insulin against a concentration gradient, favoring transfer from the capillary luminal surface to the basement membrane surface (17). In contrast, capillaries perfusing liver parenchyma are widely fenestrated and presumably represent no barrier to the passage of insulin. In addition, the current interest in the association between insulin resistance and hypertension (18,19) suggests that there may be a defect common to both disorders. If this is the case, the pathogenic mechanism responsible for abnormal vascular tone in hypertension might also result in altered passage of insulin from plasma to interstitium.

In this study, the finding that basal insulin levels in lymph are altered and sometimes higher than corresponding serum levels in the diabetic rats is similar to findings by Rasio et al. (20), who reported that insulin levels in thoracic duct lymph in dogs pancreatectomized 40 min to 4 h previously were consistently higher than serum levels. In contrast to the animals reported here, those reported by Rasio et al. were not yet appreciably hyperglycemic, and therefore altered tissue perfusion,



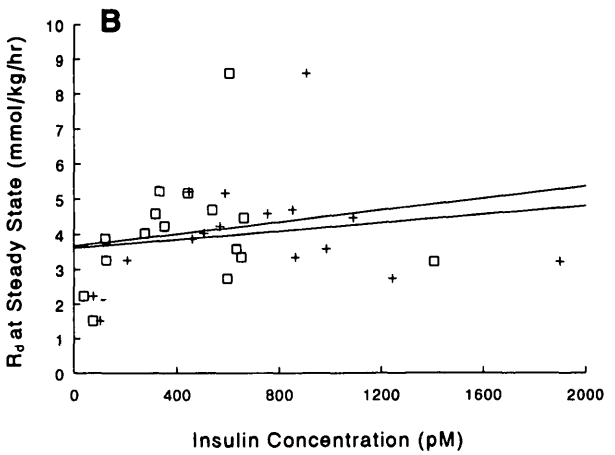
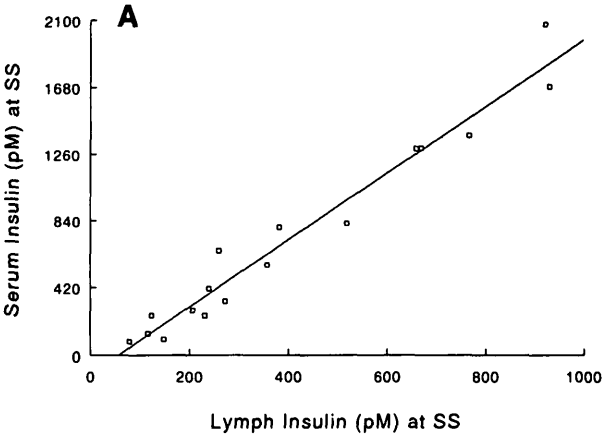


FIG. 5. Correlation between steady-state serum (+) and lymph (□) insulin concentrations during glucose clamps in diabetic animals and HGO (*A*) and R_d (*B*). For HGO vs. serum insulin level, $r = -0.26$ ($P > 0.3$), and for HGO vs. lymph insulin level, $r = -0.10$ ($P > 0.7$). For R_d vs. serum insulin, $r = 0.17$ ($P > 0.5$), and for R_d vs. lymph insulin, $r = 0.18$ ($P > 0.5$).



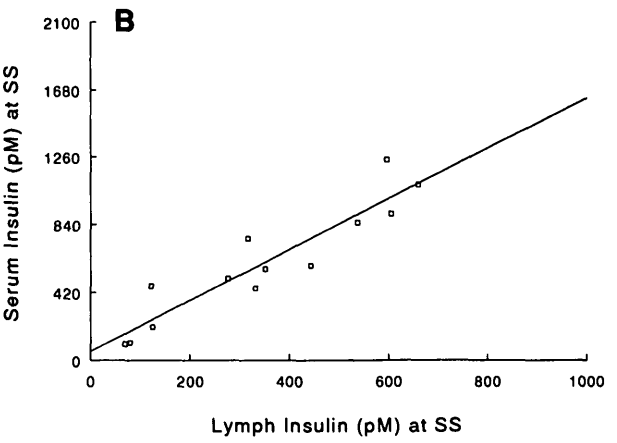


FIG. 6. Correlation between steady-state serum and thoracic duct lymph insulin during glucose clamps in nondiabetic (*A*) and diabetic (*B*) rats. For nondiabetic animals, $r = 0.98$ ($P < 0.0001$), and for diabetic animals, $r = 0.93$ ($P < 0.0001$).

hydration state, abnormalities of lymph formation, or cellular abnormalities do not appear to provide sufficient explanation for this finding. Although the reason for this is unknown, possible explanations might include accelerated insulin clearance or degradation in plasma occurring at very low plasma insulin concentrations, or accelerated transcapillary transport of insulin as an immediate adaptation to tissue insulin deficiency.

The glucose clamps in the diabetic animals were performed at hyperglycemia rather than at euglycemia for two reasons. First, hyperglycemia results in increased plasma flow and tissue perfusion (provided dehydration does not supervene), and the goal of the study was to examine the effect of the diabetic state on insulin transfer between the intravascular and interstitial compartments. Possibly, tissue perfusion and lymph flow might influence this. Second, at the insulin infusion rates used, it would not have been possible to achieve euglycemia during the course of the clamp studies without either administering a loading dose of insulin or markedly prolonging the clamp period. A loading dose of insulin would have precluded study of the time course to equilibration of insulin in serum and lymph.

As reported previously (1–4,20), kinetics of distribution of insulin between serum and lymph during insulin infusion in the rat are similar to those in the dog, with a slower time course to equilibration in lymph than serum. The small lymph volumes obtained (averaging 54 and 111 μl/sample in the nondiabetic and diabetic animals, respectively) together with the four different insulin infusion rates used did not allow sufficient precision in the insulin assay for determination of the time to steady-state for each animal.

The gradient between serum and lymph during insulin infusion in nondiabetic rats is significantly increased in comparison to animals who did not receive infused insulin (Figs. 2*A* and 3). Yang et al. (1) did not report an increase of the serum-to-lymph insulin ratio during euglycemic clamp studies in conscious dogs; however, insulin infusion rates were appreciably lower (4 pmol · kg^{-1} · min^{-1}) than those used here. Thus, a threshold concentration may exist below which transport of insulin

from plasma to interstitial fluid is unaffected by insulin concentration. Although Camu and Rasio (21) reported that no increase in insulin concentrations in hindlimb lymph occurred when serum insulin concentration was <500 pM, insulin infusion rates were ~1% of those used in this study and 3.3% of those used by Yang et al. (1). In addition, infusions were maintained for only 30 min and a review of their data suggests that lymph insulin concentrations appear to rise toward the end of the infusion. In this study, the fact that the serum-to-lymph insulin ratio is more or less constant (between 1.6 and 1.9:1) at the three infusion rates used suggests that the transcapillary transport process is linear in the physiological range of serum insulin.

In the diabetic rats, no appreciable alteration occurred in the serum-lymph insulin gradient in the insulin-infused animals compared with those who did not receive insulin (Figs. 2*B* and 3). However, an appreciable increase occurs in the gradient between baseline and 90 min in the absence of insulin infusion (lymph/serum × 100 = 103% at baseline and 70% at steady state). Lymph flow rate remained unchanged during the study, making impaired tissue perfusion unlikely. If similar factors are operative in all the diabetic animals, the serum-lymph gradient fails to increase in response to insulin. Despite this, the serum-lymph gradient is similar to that found in nondiabetic animals, and thus the data are subject to limitations in interpretation.

In contrast to previous reports (1,2), lymph insulin concentration did not show greater correlation with HGO or R_d than serum insulin concentration (Figs. 4*A* and *B*). This may be because these comparisons were made only at steady state in this study, rather than during activation, deactivation, and steady states as reported previously, where time courses of lymph insulin, suppression of HGO, and R_d were almost superimposable (1).

Transcapillary transport of insulin appears to be a partially regulated process. This is supported by in vitro studies (17) and the finding that inulin, a carbohydrate molecule of similar size and M_r to insulin, freely distributes between the two compartments. Others have also reported similar findings (1–3). However, transcapillary transport of insulin may be influenced by tissue perfusion and the net passage of fluid from the vascular to the tissue compartments in high-perfusion states such as uncontrolled hyperglycemia. It may also be upregulated in insulin-deficient states by mechanisms that are not well understood. Of interest is that the steady-state serum-lymph gradient is similar in the nondiabetic and diabetic animals during insulin infusions.

The difference in lymph flow rate between the nondiabetic and diabetic animals has implications for clearance of insulin from the vascular to interstitial compartments. Serum insulin concentration, lymph insulin concentration, and lymph flow rate are known. It is reasonable to assume that passage of insulin is principally from the vascular compartment to the tissue compartment (17,22), and any return to the vascular compartment via the thoracic duct has been interrupted under these study conditions. Presumably, net extraction of insulin from the interstitial compartment (e.g., by cellular uptake, degradation, etc.) is not appreciable. As shown in Table 3, insulin clearance from serum to lymph was ~2-fold higher in diabetic animals. However, because inulin concentrations are approximately equal in serum and lymph in both diabetic and nondiabetic rats, inulin clearance likewise increases in direct proportion to lymph flow rate, suggesting that this phenomenon is not specific to insulin.

Thus, in the setting of marked hyperglycemia and insulin deficiency, distribution of insulin between the vascular and tissue compartments and lymph clearance are altered favoring the tissue compartment. During insulin administration, the serum-lymph insulin gradient is similar, although it appears that in the diabetic hyperglycemic state, transcapillary transport of insulin is less responsive to acute regulation. Additional studies are required to determine whether this is also true in disorders where insulin resistance is the primary disturbance.

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