# Measurement by Microdialysis of the Insulin Concentration in Subcutaneous Interstitial Fluid

## Importance of the Endothelial Barrier for Insulin

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**To evaluate the interstitial insulin and inulin concentrations, 20-min microdialysis samples from the abdominal subcutaneous tissue were obtained by using two 45-mm polypropylene dialyzing tubes (o.d. ~0.5 mm, pore size 0.2 μm) during a euglycemic hyperinsulinemic (120 mU · $m^{-2}$ · $min^{-1}$) clamp ($n$ = 9) or during a constant inulin infusion ($n$ = 5). After in situ calibration of the microdialysis catheters during steady-state conditions, interstitial and plasma insulin concentrations were estimated to 654 ± 102 and 1176 ± 66 pM, respectively, i.e., a 44% difference ($P$ < 0.001). A doubling of the insulin infusion rate (240 mU · $m^{-2}$ · $min^{-1}$), leading to supraphysiological plasma insulin levels, raised the interstitial insulin concentrations markedly slower (~20 min) than in plasma. Moreover, at steady state the concentration difference in the two compartments prevailed even during the high insulin infusion rate (55% difference, $P$ < 0.01). In contrast, the interstitial inulin levels were similar to the plasma concentrations in subjects given a constant inulin infusion. Thus, the data suggest the presence of an endothelial barrier for insulin in the subcutaneous tissue. This barrier, in combination with tissue clearance of insulin, leads to lower insulin levels and altered kinetics with a slower rise in the interstitial fluid compared with plasma. *Diabetes* 42:1469–73, 1993**

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BSA, bovine serum albumin; RIA, radioimmunoassay; BMI, body mass index.

Insulin resistance is frequently seen in various conditions linked to a high risk for cardiovascular morbidity such as diabetes, obesity, and hypertension. Therefore, a method that allows proper assessment of insulin sensitivity is needed. Measurements of insulin sensitivity in vivo, such as with the euglycemic clamp technique, are based on the dose-response relationship between the insulin level in the plasma and the systemic insulin effect to promote glucose utilization (1–3). However, several lines of evidence cast doubts on the view that the relationship between plasma insulin and glucose utilization provides a correct index of insulin sensitivity of the tissues. First, the insulin concentration in plasma eliciting half-maximal response on the systemic glucose disposal rate is higher than that required in vitro (4). Second, the kinetics for the increase in glucose uptake during a euglycemic clamp is markedly delayed compared with the change in plasma insulin (5). Third, studies in humans (6) and dogs (7–8) have shown that lymphatic insulin levels are significantly (50–60%) lower than those in plasma. Furthermore, the kinetics for a change in lymphatic insulin is similar to that of the insulin effect but not to that of the plasma insulin level.

These findings have led to the hypothesis (7) that the passage of insulin to the interstitial fluid is restricted and that transendothelial transport of insulin is rate limiting for insulin action in vivo (8). If this is true, then interstitial insulin levels, which are not identical to plasma insulin, may more correctly mirror the insulin signal at the level of the target cells. However, this could be questioned, because collection of lymph fluid may not provide correct information of the interstitial insulin concentration in insulin-sensitive tissues. Thus, it remains to be demonstrated whether interstitial insulin represents the true extracellular insulin signal. To evaluate this, the insulin concentration should be measured directly in the interstitial fluid. Such measurements are now possible with the microdialysis

TABLE 1
Clinical characteristics of study subjects

| | |
|---|---|
| *n* | 14 |
| Age (yr) | 25 ± 1 |
| BMI (kg/m$^2$) | 22.7 ± 0.7 |
| Fasting glucose (mM) | 4.6 ± 0.2 |
| Fasting insulin (mU/L) | 4 ± 1 |

Data are means ± SE.

technique (9). A newly developed method for calibration of the microdialysis device enables accurate estimates of interstitial concentrations of small molecules (9). Furthermore, the kinetics of the concentration changes in the interstitial fluid can also be monitored (10).

The aim of this study was to set up a microdialysis technique to assess, for the first time, the interstitial insulin concentration in vivo and then to test, on the basis of lymph measurements only, the hypothesis that the endothelium represents a substantial barrier to insulin action in vivo. We measured the insulin and inulin concentrations in the subcutaneous interstitial fluid with the microdialysis technique during a constant infusion of these substances. The data show that, under steady-state conditions, the interstitial insulin concentration is only 50–60% of that in plasma, whereas no such concentration difference was seen for inulin. Moreover, the change in the interstitial insulin concentration was markedly delayed (~20 min) compared with plasma.

## RESEARCH DESIGN AND METHODS

We recruited 14 nonobese volunteers. Clinical characteristics are shown in Table 1. All subjects were healthy and took no regular medication. They refrained from tobacco and caffeine for 24 h before the investigation. The subjects participated in the study after giving informed consent, and the study was approved by the Ethical Committee of the University of Göteborg.

[$^{125}$I]insulin and unlabeled insulin were from Novo Nordisk, Denmark. [$^3$H]inulin (specific activity 1–5 Ci/mmol, 5200 $M_r$) was purchased from Amersham (Amersham, UK), and BSA and unlabeled inulin (~5000 $M_r$) were from Sigma (St. Louis, MO).

**Study protocol.** After fasting overnight, the subjects arrived at the laboratory at 0730. The microdialysis catheters were inserted in the subcutaneous tissue on both sides of the umbilicus, and an antecubital vein in the left forearm was cannulated while the subject was resting in the supine position. Insulin or inulin infusions were started at 0800. When steady-state plasma levels were achieved (after 90 and 240 min for insulin and inulin, respectively) samples were collected from the microdialysis catheters and plasma and stored at −20°C.

**Euglycemic clamp.** The clamps were performed essentially according to DeFronzo et al. (11). Because of the low diffusion rate of insulin through the ultrafiltrating membrane, the basal plasma concentration of insulin could not be measured (data not shown). Therefore, to achieve hyperinsulinemia an insulin infusion was started with a primed infusion for 10 min followed by a constant infusion of 120 mU · m$^{-2}$ · min$^{-1}$ for 260 min. The microdialysis measurements started after steady-state plasma insulin concentrations had been achieved (within 60 min in every subject as measured by plasma insulin). Blood glucose was measured every 5 min. The rate of glucose infusion was adjusted to maintain the glucose concentration at euglycemia (~5 mM). Potassium chloride (0.1 M) was infused at a rate of 10 mmol/h during the clamp to prevent hypokalemia.

In five subjects, the clamp was extended to include an increased insulin infusion rate after 260 min to 240 mU · m$^{-2}$ · min$^{-1}$. This rate was maintained for 140 min when the insulin infusion was stopped.

**Inulin infusion.** In a separate study, a bolus injection of 0.2 ml/kg inulin was given followed by a constant intravenous inulin infusion at 24 ml/h in 5 subjects. With this protocol, steady-state plasma inulin levels were achieved within 4 h (12) and maintained as ascertained by 60-min plasma samples throughout each study (Table 2). Then, calibration of the microdialysis catheters and interstitial fluid measurements continued for another 4 h.

**Microdialysis.** The microdialysis technique has been described previously (9–10). A single dialyzing tubing was used as catheter. The catheter device is soft, essentially nontraumatic to subcutaneous adipose tissue, and permits accurate recordings in the interstitial fluid for at least 8 h (9–10). In this study, the dialysis area of the catheter was made of a polypropylene membrane with an average pore size of ~0.2 μm, o.d. of ~0.4 mm, and length of ~45 mm. The dialyzing tubing was glued between two 0.6-mm (o.d.) nylon tubes constituting the outlet and the inlet, respectively (9). One microdialysis catheter was inserted on each side of the umbilicus with the help of an ordinary cannula. The cannula is inserted without anesthetics at a 45° angle and a 5-mm depth through the dermis into the subcutaneous tissue. The subcutaneous tissue is tunneled at a 5-mm depth in parallel to the skin surface. At 55 mm from the insertion point, the cannula again perforates the skin layer. The dialyzing catheter is placed in the cannula, which is then withdrawn.

TABLE 2
Inulin measurements

| | Plasma inulin (mg/100 ml) | | | | | Interstitial inulin (mg/100 ml) | Relative microdialysis recovery (%) |
|---|---|---|---|---|---|---|---|
| | 20 ± 1 | 21 ± 2 | 22 ± 2 | 21 ± 2 | 22 ± 2 | 29 ± 2 | 18 ± 2 |
| Time (min) | 240 | 300 | 360 | 420 | 480 | 240–480 | 240–480 |

Data are means ± SE; *n* = 5. Relative microdialysis recovery is microdialysate concentration divided by interstitial concentration.

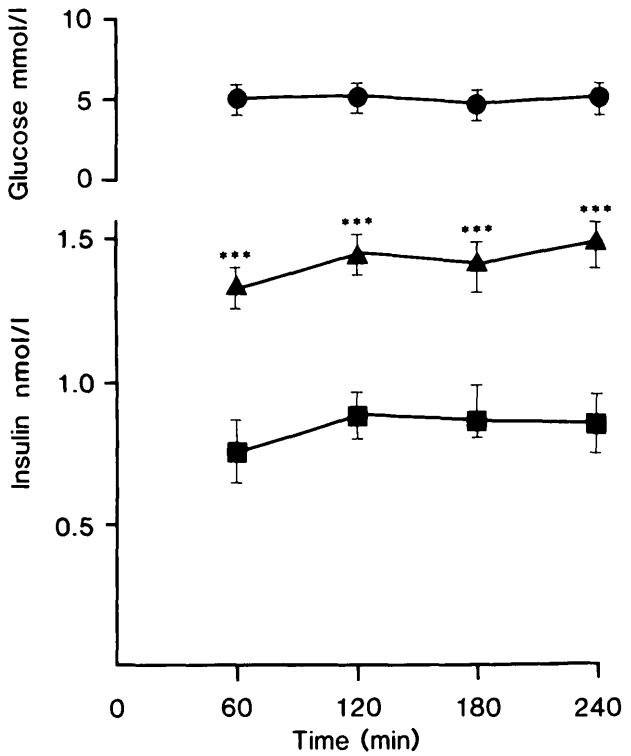


**FIG. 1. Plasma glucose (●) and interstitial (■) and venous plasma (▲) levels of insulin during a euglycemic, hyperinsulinemic clamp. Time 0 = 90 min after initiation of the insulin infusion. Data are mean concentrations ± SE, $n = 9$. ***$P < 0.001$.**

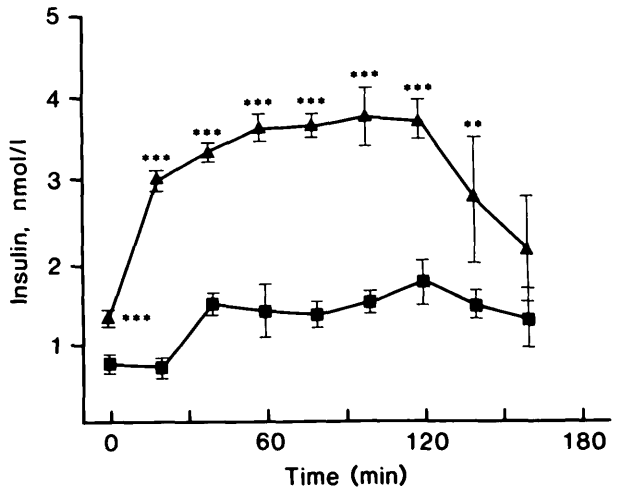


**FIG. 2. Interstitial (■) and venous plasma (▲) insulin levels for 180 min after steady state had been reached (240 min = time 0). The insulin infusion rate was increased from 120 to 240 mU · $m^{-2}$ · $min^{-1}$, and after 140 min, the insulin infusion was stopped. Data are means ± SE, $n = 5$. **$P < 0.01$. ***$P < 0.001$.**

After connecting the catheter inlet to a precision pump (Carnegie, Stockholm, Sweden), isotonic saline containing 10 mg/ml BSA perfused the system at a rate of 2.5 μl/min. Ninety minutes after insertion of the two catheters, samples of the interstitial fluid were collected every 20 min. Because peptides are known to bind to plastic in vivo, data were compared with those obtained from an identical catheter perfused outside the tissue (next to the subject) to evaluate the binding of insulin to the plastic of the tubing and the catheter in each experiment.

Each catheter was calibrated in vivo during insulin or inulin infusion. This calibration technique has been described in detail previously (9). Briefly, when steady-state conditions had been achieved for both insulin or inulin, the microdialysis catheters were perfused with different concentrations of the substance of interest (insulin 0–240 pM and inulin 0–500 mg/L). Because the insulin concentration in the dialysates never exceeded 240 pM, even when plasma insulin was 3500 pM, perfusates were chosen as indicated. The perfusate concentration equilibrating with the interstitial concentration was calculated with regression analysis ($r < -0.9$ in each experiment) and taken as the interstitial concentration (9). The relative recovery was assumed to be the same at low- and high-dose insulin infusions.

The equilibration calibration technique has been used to measure small molecular compounds (9–10). To validate the equilibrium calibration technique for measurements of compounds with an ~5000 $M_r$, a series of in vitro experiments were done in blood where [$^{125}$I]insulin and [$^{3}$H]inulin were measured. In four experiments, two microdialysis catheters were carefully calibrated in an ambient known concentration of [$^{125}$I]insulin and [$^{3}$H]inulin, and a parallel third nondialyzing catheter was perfused for estimation of tubing insulin binding.

**Assays.** Insulin in plasma or samples of the interstitial fluid were measured with RIA (Pharmacia, Uppsala, Sweden). Inulin was measured with the method originally described by Hubbard and Loomis (13). Blood glucose was assayed enzymatically (Boehringer-Mannheim, Mannheim, Germany).

**Statistical analyses.** Regression analyses were performed with the least squares method. The Student's $t$ test was used for comparison of paired data. Data are expressed as means ± SE.

## RESULTS

Figure 1 depicts the insulin concentration in plasma and in interstitial fluid during a euglycemic, hyperinsulinemic clamp. Steady-state insulin levels were obtained in both compartments, and the blood glucose concentration was kept constant. During the last 60 min of the insulin infusion rate of 120 mU · $m^{-2}$ · $kg^{-1}$, the mean glucose disposal rate was 15.6 ± 0.8 mg · $kg^{-1}$ · $min^{-1}$. However, the interstitial insulin level was markedly and significantly lower than that in plasma throughout the study. The interstitial-to-plasma insulin ratio was 0.58 ± 0.07 ($n = 9$ volunteers).

In 5 of the subjects, the study was extended to include a rapid rise in plasma insulin to a new steady-state level during the euglycemic clamp (Fig. 2). A rapid increase of the insulin infusion rate resulted in rapidly increasing plasma insulin levels reaching a new steady state after ~60 min. During the last 60 min of the insulin infusion rate of 240 mU · $m^{-2}$ · $kg^{-1}$, the mean glucose disposal rate was 16.0 ± 0.8 mg · $kg^{-1}$ · $min^{-1}$. Euglycemia was maintained throughout the study. Moreover, the rise in the interstitial insulin was delayed ~20 min (Fig. 2). When the

insulin infusion was stopped (at 140 min), plasma and interstitial insulin levels declined (Fig. 2). Because glucose disposal rate was already maximal at the lower insulin infusion rate, changes in insulin action could not be compared with the insulin kinetics.

During the inulin infusion in 5 subjects, plasma inulin measurements every 60 min showed that steady-state levels were maintained after 240 min. The plasma inulin concentrations during the microdialysis calibration procedure (mean levels during 240 min) are given in Table 2. As shown in Table 2, similar inulin levels were seen in plasma and the subcutaneous interstitial fluid. The recovery for inulin during the in vivo calibration was 18 ± 2%, and the linearity in the regression analysis acceptable ($r < -0.9$). No measurements of the inulin kinetics were performed in vivo.

Data from the calibration studies in vitro show that 20 ± 4% of ambient [$^3$H]inulin was recovered in the dialysate, whereas the ambient [$^{125}$I]insulin concentration was underestimated by a factor of 8.5 ± 0.9 ($n = 4$ catheters). However, the slope of the regression line in the calibration experiments was 0.46 ± 0.8 and 0.45 ± 0.3 in vitro and in vivo, respectively, indicating identical dialysis conditions.

Nondialyzing catheters bound 26 ± 4% of the perfusate insulin in the experiments ($n = 8$ catheters). Thus, the correction factor (8.5) was indicative of loss of insulin in the dialyzing region of the microdialysis catheters caused by the binding of insulin or ultrafiltration of the perfusion liquid. This factor could validly be used to calculate the interstitial insulin concentration in vivo. Taking the correction factor into account, the recovery of interstitial insulin was ~10%.

The microdialysis method allows a change in the insulin concentration to be detected with a finite time delay because of the fact that the sampling technique is time consuming. In vitro experiments showed that 65 ± 8 and 100 ± 0% of a change in the insulin concentration was detected after 20 and 40 min, respectively ($n = 4$ catheters), and the kinetic data were adjusted accordingly.

## DISCUSSION

This study shows for the first time the application of the microdialysis technique to assess the subcutaneous interstitial insulin concentration in humans. To our knowledge, no other technique has been used to measure insulin directly in the extracellular space in vivo. The microdialysis technique may be particularly useful in such studies because previous data have shown that the interstitial water space, and not plasma, is measured (10) and that calibration of microdialysis catheters in vivo results in correct measurements of interstitial concentrations in the subcutaneous tissue (9). In this study, each catheter was calibrated in vivo and in vitro with a good linearity in each experiment ($r < -0.9$), and the interstitial/medium concentration was calculated. We assumed that [$^{125}$I]insulin had the same dialyzing properties as cold insulin and thus that the recovery measured in whole blood in vitro and in adipose tissue in vivo was not different. Furthermore, the similarities of the insulin kinetics in plasma and the interstitial fluid in vivo support the validity of our method. Moreover, bioassays and precipitability data have shown that insulin in the dialysates both in vitro and in vivo was not altered by hormonal degradation or change in the hormonal activity (data not shown). The limitation of the method is dependent on the fact that dialysis membranes with larger pores are mainly ultrafiltrating the perfusion liquid leading to low recovery rates of interstitial insulin. Therefore, physiological concentrations of insulin under basal conditions could not be investigated in this study, and hyperinsulinemia had to be induced.

Taking these limitations into consideration, the data show that the insulin concentration in the subcutaneous interstitial fluid is 40–50% lower than in plasma even under steady-state conditions. Furthermore, a rapid increase in plasma insulin leads to a much slower (~20 min) rise in the interstitial compartment. However, no concentration difference was seen between the plasma and interstitial inulin concentrations. Hence, the data are in agreement with previous lymph studies (6–8) and support the existence of an endothelial barrier for insulin in the subcutaneous tissue. The magnitude of the difference in insulin concentrations and kinetics seems to be similar between the subcutaneous interstitial fluid and leg lymph of the dog (7–8), suggesting that such a barrier may be present in both species. In a preliminary study, Lillioja et al. (14) reported similar findings in human leg lymph from lean subjects.

Despite similar molecular size, insulin and inulin exhibit different kinetics in plasma and lymph (6,8). Previous studies have shown that a large fraction of the circulating insulin rapidly binds to various organs (15). The binding of insulin to specific insulin receptors on the endothelial cells (16) has been proposed as an important mechanism for the passage of insulin to the interstitial fluid (17). Furthermore, endocytosis and retroendocytosis of the insulin hormone-receptor complex may constitute such a route for insulin transport through the endothelial wall (17). In vivo evidence for the importance of this passage was shown recently with the perfused rat heart (18).

This finding that the difference between plasma and interstitial insulin levels became more pronounced when the insulin infusion rate was increased (Fig. 2) suggests that the transporting mechanism for insulin into the interstitial fluid is saturable. Thus, the data in this and previous lymph studies are compatible with the existence of a receptor-mediated passage of insulin into the interstitial space. However, the data do not rule out the possibility that different physicochemical properties for insulin and inulin may explain the difference in diffusion capacity and equilibration time. Moreover, these and other data support the importance of an interstitial or remote compartment (5) for the transport of insulin into an organ.

The finding that the subcutaneous interstitial insulin concentrations were low during the euglycemic, hyperinsulinemic clamp suggests that previous $EC_{50}$ values calculated from plasma insulin levels may be erroneous. It is well-known that isolated rat (4) and human (19) subcutaneous adipose cells are highly sensitive to insulin and that half-maximal antilipolytic effect may be seen at ~30 pM. The low basal insulin levels in the interstitial fluid

and the slower rise compared with plasma insulin indicate that a high cellular sensitivity in combination with a rapid insulin secretion is necessary for the marked inhibition of lipolysis after oral glucose (20). In this study, no correlation could be made between interstitial insulin and the glucose disposal rate, because the rate of disappearance for glucose was maximal already at the lower rate of insulin infusion (Fig. 1). However, note that a 20-min time difference between the rise in plasma and interstitial insulin is in perfect harmony with the time difference in plasma and lymph insulin; the latter following the same time kinetics as rate of disappearance for glucose (7–8).

The existence of an endothelial barrier for insulin also implies that the transport of insulin to the interstitial fluid may be a rate-limiting step for insulin action. The impairment of the insulin passage may be an underlying factor for insulin resistance in various tissues (22). Furthermore, a slower interstitial diffusion in muscles from obese individuals has also been proposed (22). Thus, studies of the interstitial insulin levels with the microdialysis technique may give additional information about potential mechanisms for insulin resistance in obesity and diabetes. However, the methodology needs to be improved in future studies enabling sampling of physiological interstitial insulin concentrations.

In summary, microdialysis of subcutaneous tissue shows that the insulin levels in the interstitial fluid under steady-state conditions are 40–50% lower than in plasma. Furthermore, the kinetics for a change in interstitial insulin is delayed ~20 min. Because the inulin concentration was similar in plasma and interstitial fluid after a constant infusion, the data suggest that the transcapillary route might be a rate-limiting step for insulin action in human adipose tissue.

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