Measurement of interstitial insulin in human muscle

MIKAELA SJÖSTRAND, AGNETA HOLMÄNG, AND PETER LöNNROTH
Lundberg Laboratory for Diabetes Research and the Wallenberg Laboratory,
Department of Internal Medicine and Heart and Lung Diseases, Sahlgrenska
University Hospital, Gothenburg University, S-413 45 Gothenburg, Sweden

Sjöstrand, Mikaela, Agneta Holmäng, and Peter Lönnroth. Measurement of interstitial insulin in human muscle. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E151–E154, 1999.—Previous measurements in lymph and adipose tissue have indicated that interstitial insulin concentrations are ∼40% lower than in plasma. Measurements of insulin in human muscle interstitial fluid have not been performed yet. We developed a new external reference technique for calibration of microdialysis catheters in situ. This technique allows correct assessments of interstitial peptide concentrations and was employed to estimate the insulin concentration in medial quadriceps femoris muscle in 11 individuals (age: 37 ± 3 yr; body mass index: 25.2 ± 1.2 kg/m²) during a two-step euglycemic hyperinsulinemic clamp. At steady-state insulin and glucose infusion, plasma glucose was 5.9 ± 0.2 mmol/l, plasma insulin was 155 ± 17 mU/l, and interstitial muscle insulin was 67 ± 19 mU/l (n = 9, P < 0.01). At a higher insulin infusion rate, the steady-state plasma insulin concentration was 379 ± 58 mU/l, and interstitial insulin concentration was 180 ± 40 mU/l (P < 0.01). The data show for the first time that high physiological and supraphysiological plasma insulin levels give 30–50% lower interstitial concentrations of insulin in the muscle. The importance of capillary delivery as a rate-limiting step for the insulin effect is suggested.

EVALUATION OF THE sensitivity of insulin in muscle tissue involves the relationship between the effect and the concentration of insulin in the ambience of the muscle cells. Recently, investigations on the mechanisms regulating the delivery of insulin to the interstitial fluid have generated a bulk of data suggesting that the concentration of insulin in plasma may differ from that in the interstitial fluid. First, insulin sensitivity in muscle seems to be related to the capacity to increase blood flow and capillary recruitment (4, 10). Second, results from cultured cells in vitro suggest the active transport of insulin to the interstitial fluid by transendothelial transport of the insulin receptor-hormone complex through the capillary endothelium (13). Third, the significance of a saturable pathway for insulin transport has been indicated in different studies of the kinetics of plasma insulin (5, 16). Furthermore, direct measurements of insulin in lymph (1) and in the subcutaneous interstitial fluid (11) suggest that the interstitial concentration of insulin is ∼50% lower than in arterial plasma under steady-state euglycemic insulin clamp conditions. However, direct evidence for the existence of a significant arterial-interstitial concentration difference of insulin in the muscles is not provided.

The present study was performed to further explore the hypothesis that the capillary wall may be rate limiting for insulin delivery to the interstitial fluid in the muscle. Microdialysis measurements of interstitial fluid concentrations of insulin were done in the medial quadriceps femoris muscle (14) under euglycemic hyperinsulinemic conditions in healthy volunteers. The data demonstrate for the first time a significant arterial-interstitial concentration difference of insulin in the muscle.

METHODS

Subjects

Four male and seven female healthy volunteers with normal glucose tolerance and no regular medication were studied. Table 1 lists the clinical characteristics of the subjects. All subjects gave their informed consent, and the study was approved by the Ethics Committee of Gothenburg University.

Study Protocol

The investigations were started at 0800 after an overnight fast. The subjects were studied in the supine position in a room kept at 25°C. A polyethylene catheter was placed in a left forearm vein for blood sampling. The forearm was heated with electric pads (−70°C) to arteriolize the venous blood (12). Each study started with a bolus injection of insulin (Inuject; Kemiflor, Stockholm, Sweden) followed by a constant intravenous infusion (24 mL/h) for 360 min. With this protocol, steady-state plasma insulin levels were achieved within 240 min (18). A euglycemic clamp performed as previously described by DeFronzo et al. (6) started 30 min after the initiation of the insulin infusion. The clamp started with a primed infusion of insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) for 10 min followed by a constant infusion rate of 120 mU·min⁻¹·kg⁻¹ for 120 min. Thereafter, the insulin infusion was increased to 240 mU·min⁻¹·kg⁻¹ and continued for 150 min. Blood samples were drawn every 5 min. The rate of glucose infusion was adjusted to maintain euglycemic plasma glucose concentrations. Potassium chloride (0.1 M) was infused at a rate of 10 mmol/h during the clamp to prevent hypokalemia.

Microdialysis

In the present experimental procedures, commercially available custom-made microdialysis catheters (12 × 0.5 mm, 100-kDa molecular mass cutoff; CMA-10; CMA, Stockholm, Sweden) were used. The inlet of the microdialysis catheter was connected to a microinjection pump (CMA 100; CMA) and was perfused with isotonic saline with the addition of 1.5 mmol/l glucose and 1% human albumin at a perfusion speed 1 µl/min. Catheters were inserted without anesthetics in the medial quadriceps femoris muscle ∼10–15 cm above the knee joint by the following procedure. The surface of the disinfected...
Relative microdialysis recovery in vivo for inulin was calculated as dialysate/plasma concentration of [14C]inulin. Recovery of glucose was calculated as the percentage efflux of [3H]glucose according to the internal reference calibration technique previously evaluated (14). In vivo recovery of insulin was calculated according to the exogenous reference technique using the formula $R_1 = R_2$, where $R_1$ is recovery of insulin/recovery of inulin in vitro, and $R_2$ is the corresponding recovery ratio obtained in vivo.

In experiments made in vitro in plasma, microdialysis was performed with the same catheter material and perfusate without labeled glucose (12). Briefly, the microdialysis catheters were placed in a jar containing human plasma with added [14C]inulin (0.025 mCi/ml) and insulin (1,000 mU/l; Actrapid). The jar was placed on a water bath at 37°C (250 strokes/min). Dialysate perfusion speed was 1 µl/min. After 60 min, microdialysate was collected hourly for 4 h and was analyzed for inulin, insulin, and glucose.

The data in Table 2 show that all three substances had ~50% lower recoveries in vivo compared with in vitro. This permitted us to conclude that, first, the previous finding (7) that the relationship between in vitro/in vivo recoveries is similar for any substance also is relevant for insulin measurements, since the insulin concentration in interstitial fluid could be calculated from the knowledge of the in vitro recovery ratio of either reference (i.e., inulin or glucose) and the reference concentration in the interstitial fluid. Second, by the exogenous reference calibration technique, a high-precision estimate of the interstitial insulin concentration was achieved since insulin, if used as a reference in turn, could make accurate calculations of interstitial glucose possible.

Calibration of microdialysis in human muscle. To characterize the different diffusion properties and to correct for differences in binding of the compounds to the catheter, experiments were performed in which inulin and insulin were dialyzed in plasma at 37°C in vitro using the double-cannulated probes for measurements in human muscle. The in vitro experiments were done according to the same protocol described above. Results from nine such experiments demonstrated that the relative recovery of insulin in microdialysates was 47 ± 7%. Relative recovery of insulin in vivo was 21 ± 1, 23 ± 2, 20 ± 2, and 22 ± 1% in the four hourly sampled dialysates.

The mean insulin recovery (dialysate insulin/plasma insulin) in experiments performed in vivo was 11 ± 3%. The in vivo recovery of insulin was then calculated in each subject according to the above formula. The mean calculated in vivo recovery of insulin was 50 ± 0.1%. The in vivo recovery factor was used for recalculating steady-state dialysate insulin content (2 ± 1, 3 ± 1, 10 ± 3, and 9 ± 3 mU/l in the four samples collected at 90-, 120-, 240- and 270-min sampling times, respectively) to interstitial insulin concentrations.

### Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th>n</th>
<th>Age, yr</th>
<th>BMI, kg/m²</th>
<th>Fasting plasma glucose, mM</th>
<th>Fasting plasma insulin, mU/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>36.5 ± 2.8</td>
<td>25.2 ± 1.2</td>
<td>5.0 ± 0.13</td>
<td>4.5 ± 0.9</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of subjects. BMI, body mass index.

Table 2. Relative recovery in vivo of inulin, insulin, and glucose

<table>
<thead>
<tr>
<th></th>
<th>Relative Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>In vivo</td>
</tr>
<tr>
<td>Inulin</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Insulin</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Glucose</td>
<td>62 ± 6</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 9–20 subjects. Relative recovery in vivo of inulin (calculated as dialysate concentration/plasma concentration), insulin (calculated according to the external reference technique), and glucose (calculated according to the internal reference technique). Data were compared with the recoveries obtained in vitro in plasma.
Analyses.

Fisher's least significant difference test was used for post hoc when several comparisons were performed, ANOVA was used. A t-test for paired observations, and, hence, might balance the interstitial fluid concen-
tration of insulin with varying efficiency.

We recently demonstrated the existence of such a saturable transport system in quadriceps muscle in the rat (8). However, measurements in leg lymph did not give any evidence for saturability of the delivery of insulin to leg lymph (19). The present data do not allow conclusions as to the putative existence of a saturable system for transcapillary transport of insulin. Only indirect evidence was provided by the fact that the relative concentration gradient of insulin was identical at both clamp steps despite the significant increase of leg blood flow at clamp step 2 (Table 3). We cannot explain the difference in data obtained in different tissues and species concerning the putative existence of a saturable transport system for insulin. However, it can not be excluded that diffusion of insulin as well as active transport systems, such as the transendoctyosis pathway, may be differently effective in different organs and, hence, might balance the interstitial fluid concentration of insulin with varying efficiency.

Furthermore, a saturable insulin transport system opens up the possibility that an arterial interstitial concentration gradient of insulin may be less extensive or even absent at a low physiological concentration range. Accordingly, data obtained from microdialysis studies in the rat demonstrated a significant insulin concentration difference over the capillary wall only at plasma concentrations beyond those exerting a half-
maximum effect on glucose disposal (8). Due to the poor efficiency of the microdialysis sampling presently used, low physiological insulin concentrations were not detect-
able in the present study. However, it is clear from the present data that a significant concentration gradient over muscle capillary walls could be demonstrated in

![Fig. 1. Plasma (filled bars) and interstitial (open bars) levels of insulin at steady state during a two-step euglycemic hyperinsulinemic clamp (120 and 240 mU·m⁻²·min⁻¹), ***P < 0.001, n = 11 subjects. Data are means ± SE.](http://ajpendo.physiology.org/ by 10.2203.32464 on October 14, 2017)

**Table 3. GIR for plasma and leg blood flow during a two-step hyperinsulinemic euglycemic clamp**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>60–120</th>
<th>210–270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.9 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>GIR, mg·kg⁻¹·min⁻¹</td>
<td>11.7 ± 0.9</td>
<td>15.3 ± 1.0*</td>
</tr>
<tr>
<td>Leg blood flow, ml·100 g⁻¹·min⁻¹</td>
<td>8.5 ± 0.7</td>
<td>10.3 ± 0.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 11 subjects. GIR, glucose infusion rate. *P < 0.05.
humans at submaximal insulin stimulation, indicating that the insulin concentration difference between the interstitial and plasma compartment may be physiologically significant.

The fact that the capillary wall is rate limiting for the uptake of both insulin (the present study) and glucose (14) suggests that the microcirculatory system and its capacity to recruit muscle capillaries is essential to optimize the time kinetics for the distribution of insulin in peripheral tissues. Interestingly, insulin-resistant human subjects not only have a decreased muscle capillary density (2) but also show a delay in peripheral insulin action (16). In experimental studies in the testosterone-treated rat, we have demonstrated reduction of capillary density as well as delayed insulin distribution and insulin resistance in muscle tissue (9, 15). It may be argued that the present insulin data might have been calculated less precisely due to the fact that the dialysis recovery of insulin was low and the multiplication factor based on insulin measurements thus was high. Furthermore, the variance in dialysate insulin was balanced by the same variance in insulin in vivo recovery in each experiment, since all catheters were calibrated in situ to ensure the accuracy of the measurements. We thus conclude that the concentration gradient of insulin over the capillary wall demonstrated here could not be artifactual, dependent on inaccurate calibration techniques.

In summary, microdialysis measurements in the human quadriceps muscle demonstrate for the first time a significant (~50%) concentration difference of insulin over the capillary wall. The pathophysiological significance of this finding obtained during hyperinsulinemic clamping conditions may be demonstrated further in future investigations including insulin-resistant subjects with a reduced muscle capillary density.

The laboratory assistance provided by Lena Strindberg and Britt-Marie Larsson is gratefully acknowledged.

This study was supported by grants from the Swedish Research Council (project nos. 10864, 11330, and 12206), the Swedish Diabetes Association, Nordisk Insulin Fond, and Inga-Britt and Arne Lundberg Foundation.

Address for reprint requests: M. Sjöstrand, Lundberg Laboratory for Diabetes Research, Sahlgrenska Univ. Hospital, S-413 45 Gothenburg, Sweden.

Received 18 February 1998; accepted in final form 29 September 1998.

REFERENCES


