Involuntary leg movements affect interstitial nutrient gradients and blood flow in insulin stimulated rat skeletal muscle

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Running title: Interstitial glucose, lactate and insulin concentrations in muscle.
ABSTRACT

To evaluate the effect of passive muscle shortening and lengthening (PSL) on the transcapillary exchange of glucose, lactate, and insulin in the insulin stimulated state, microdialysis was performed in rat quadriceps muscle. Electrical pulsative stimulation (0.1 msec, 0.3-0.6 V, 1 Hz) was performed on the sciatic nerve in one leg to induce passive tension on the quadriceps during a hyperinsulinemic euglycemic clamp (10 mU/kg/min). In the non-insulin-stimulated state (basal state) the muscle arterial-interstitial (A-I) concentration difference of glucose was 1.6±0.3 mM (p<0.01). During insulin infusion, it remained unaltered in resting muscle (1.3±0.3 mM), but diminished during PSL. In the basal state, no I-A difference of lactate was present whereas in the insulin infusion state it increased significantly and was significantly larger in moving (2.8±0.5 mM, p<0.01) relative to resting muscle (0.7±0.4 mM). The A-I concentration difference of insulin was equal in resting (86±7 µU/mL) and moving muscle (100±8 µU/mL). Muscle blood flow estimated by using radiolabelled microspheres was 17±4 ml/100g/min and increased to 34±6 ml/100g/min (p<0.05) during PSL.

These results confirm that diffusion over the capillary wall is partly rate-limiting for the exchange of insulin and glucose and lactate in resting muscle. PSL, in addition to insulin stimulation, increases blood flow and capillary permeability and, as a result, diminishes the A-I-concentration gradient of glucose but not that of insulin or lactate.

Key words: Blood flow; muscle; microdialysis; glucose; lactate; insulin
INTRODUCTION

The metabolism of glucose and lactate in muscle tissue is of quantitative importance for body glucose homeostasis, and the regulation of this metabolism has been investigated intensively with respect to insulin-resistant conditions such as obesity and non-insulin-dependent diabetes mellitus (NIDDM). Most of these studies have focused on muscle glucose metabolism during resting conditions (15, 36). However, a more complex picture may be obtained by investigating exercising muscle, since contraction of the muscle fibers increases the glucose uptake (1) via a signalling pathway which is different from that elicited by insulin (19). Moreover, glucose metabolism may be affected by the increase in blood flow rate that is produced by muscle exercise (6).

Traditionally, the metabolism of glucose in intact muscle was studied by measuring arterio-venous concentration differences in the extremities (15). To get a further insight of the impact of capillary barriers affecting insulin and glucose delivery measurements should be performed in the interstitial fluid (8, 24). The existence of such barriers implies that the capillary wall affects the rate of muscle glucose uptake and, hence, the glucose uptake rate is dependent not only upon the rate of the cellular consumption of glucose but also upon both the blood flow and the capillary permeability surface area. When blood flow measurements are taken in combination with microdialysis the investigation of transport of substrates and hormones from capillaries to the extracellular space are made possible (13).

In a recent study (22) microdialysis measurements in contracting human skeletal muscle demonstrated that the interstitial concentration of both glucose and lactate was increased. However, in the same study, the relative microdialysis recovery
was increased during contraction but concomitant blood flow measurements were not done and, hence, a full picture of the uptake/release balance was not achieved. In order to eliminate a contraction mediated increase of the water pressure near the microdialysis probe an alternative approach was used in the present study. In this study, to evaluate whether PSL affects tissue blood flow and capillary delivery of insulin and glucose, calibrated microdialysis and blood flow measurements were performed in the anesthetized rat during euglycemic clamp conditions. In addition, passive shortening and lengthing of the quadriceps was induced by means of electrical stimulation of the sciatic nerve and contractions thus activated on the flexor side. The data show that the capillary wall is rate-limiting for glucose uptake and lactate release in resting muscle during an insulin infusion. PSL of muscle causes a concomitant increase of the muscle blood flow, which results in a diminished glucose gradient over the capillary wall, with a corresponding increase in the lactate concentration.
METHODS

Twelve female Sprague-Dawley rats (BK Universal, Sollentuna, Sweden), weighing 267±8 g (mean ± SE), were housed in single cages at 23°C. They were fed rat chow, containing 22% protein, 5% fat, 51.5% carbohydrate and sufficient minerals and vitamins (Ewos, Södertälje, Sweden). The rats also received tap water ad libitum. The dark-light cycle was 12:12 h. Animals were housed under these conditions for at least 1 week before inclusion in the study. The study was approved by the Animal Ethics Committee of the Göteborg University.

Study protocol (Fig 1)

Rats were anesthetized with thiobutabarbitonal sodium (Inactin, RBI, Natick, MA, USA), 12.5 mg/100 g body weight intraperitoneally, and placed on heating pads to maintain adequate temperature (37°C by rectal probe). Catheters were placed in the left carotid artery to draw blood and the right jugular vein to infuse glucose and insulin. 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, while the exposure of the other nerve served as a sham control. The leg was turned outwards in the hip joint and the knee joint was flexed. The bipolar electrode (Grass instrument, Quincy, USA) was connected to a Grass S6 stimulator (Grass instrument, Quincy, USA). After incision of the skin and exposure of quadriceps muscle a microdialysis catheter (o.d. 0.5 mm, length 10 mm, cut-off 50 KD, BAS, IN, USA) was inserted in the quadriceps muscle in both legs of the rat. After closure of the skin and connecting the catheter inlet to a pump (Carnegie, CMA, Stockholm, Sweden), the system was perfused with 1% bovine albumin in isotonic saline with 1mM glucose at a rate of 1 µl/min. Glucose (1mM) was also added to the perfusate in order to prevent the
depletion of glucose from the tissue (20). The catheters were calibrated under steady-state basal conditions in order to estimate the glucose and lactate concentrations in the interstitial fluid (see Microdialysis calibration).

The euglycemic hyperinsulinemic glucose clamp technique that has been described in detail previously (7) was utilized. Catheters were then inserted into the left carotid artery for blood sampling and into the right jugular vein for infusion of glucose and insulin. The body temperature was maintained at 37°C with a heating blanket. After a bolus injection, insulin (100 U/ml, Human Actrapid, Novo, Copenhagen, Denmark) was continuously infused at a rate of 10 mU/kg/min. Briefly, insulin was continuously infused at a rate of 10 mU/kg/min. A 20% solution of glucose in isotonic saline was also infused in order to maintain the blood glucose level at 7.0 mM. The insulin infusion was continued for 100 min, from t = 80 min to 180 min. During steady state clamping conditions, i.e. from t = 120 min to 180 min, samples of the muscle interstitial fluid were collected every 30 min and analyzed for glucose, lactate and insulin concentrations. A total of less than 2 ml blood was used for the determinations and compensated for by the infusion volumes. The data are presented as the mean values obtained during steady state conditions.

Electrical stimulation

The electrical stimulation of peripheral efferent nerves may lead to the activation of sympathetic fibers and vasodilation (2). The goal of the present study was to study the influence of PSL without the interference of a putative sympathetic nervous activation. Therefore, the sciatic nerve was utilized for electrical stimulation and the microdialysis measurements were performed in the non-stimulated passively stretching and contracting quadriceps muscle, not innervated by the sciatic nerve.
During the last 60 min of the clamp, the sciatic nerve of one hind limb was stimulated electrically using square-wave pulses of 0.1 msec duration at a frequency of 1 Hz. The voltage was adjusted (0.3 - 0.6 volt) in order to maintain the same amplitude of dorsal extension of the foot from horizontal position to an angle of ~ 30°.

**Microdialysis calibration**

In vivo probe recovery was assessed in situ according to both the equilibrium (20) and the internal reference calibration techniques (21). After reaching steady-state basal conditions (45 min), the equilibrium calibration was carried out as described previously in detail (20). Briefly, known concentrations of glucose and lactate were added to the perfusate in a non-consecutive order and the net increase of both glucose and lactate concentration in the dialysate was measured. The linear relationship between the perfusate glucose/lactate and the increase in dialysate concentration was established, and the point of no net influx (indicating the interstitial glucose/lactate concentration) was estimated by means of linear regression.

The microdialysis catheters were also calibrated in situ according to the internal reference technique (21) in order to attain the interstitial glucose and lactate concentrations. Data obtained by internal reference and equilibration calibration in the same subject have shown similar results (9, 21). For an internal reference calibration, 0.5 mM $^3$H-glucose (0.1 mCi/mL, Amersham, USA) and 0.3 mM $^{14}$C-lactate (0.2 mCi/mL, Amersham, USA) were added to the perfusate and the fractional extraction of radioactivity (relative recovery of the substance of interest) was measured. The
interstitial fluid concentrations of both lactate and insulin were obtained by the use of a reference calibration technique which has recently been validated (31). The recovery (dialysate/interstitial concentrations) of both substances was calculated according to the formulas:

\[
\begin{align*}
\text{recovery of glucose} & = R_1 \\
\text{recovery of glucose} & = R_2 \\
\text{recovery of lactate} & = R_3 \\
\text{recovery of insulin} & = R_4 \\
\text{recovery of insulin} & = R_5
\end{align*}
\]

where the values for \(R_{1-5}\) were calculated from 68 previous consecutive measurements that were made under the same conditions as are used in this study (31). The use of endogenous references where interstitial concentrations of multiple substances can be calculated from knowledge of the dialysate recovery of one of these substances was recently validated (31). The mean calculated recovery of insulin according to the above formula was found to be 0.03±0.01.

During calibration, dialysates were collected for 15 minutes during perfusion with each calibration solution. Calibration using equilibration and internal reference methods were found to yield similar results (Table 1). The mean relative outflux of \(^3\text{H}\)-glucose through the microdialysis catheter in 12 rats (24 hind limbs) did not change over the 200 min period indicating that electrical stimulation and passive muscle movements did not change the relative recovery of interstitial glucose in dialysates (data not shown).
Measurements of blood flow

Immediately before termination of the electrical stimulation (180 min), the blood flow in the individual muscles was estimated by using the radioactive microsphere technique (5). The sphere-suspension with the specific activity of 11.5 mCi/g ([52Co] -microspheres in 0.9% saline with 0.01% Tween-80; New England Nuclear, Boston, MA, USA) were roughly mixed and sonicated to avoid aggregation of the spheres. The mean size of the spheres was 15.5±0.1 μm, which is approximately twice the size of red blood cells and slightly larger than capillaries, which means that the spheres could approximate the distribution of red blood cells until just before impacting in the capillary bed (23). The microspheres (~500 000 microspheres) were immediately injected into the circulation, in the left ventricle via the catheter placed in the left carotid artery, with catheter hip placed inside the ventricle followed by a flush of 0.3 ml saline. A reference blood sample was drawn from the caudal artery with a Minipuls 3 Peristaltic Pump (Gilson France, Villiers-level, France). After the injection the kidneys as well as the quadriceps muscle and the muscles of the lower limb (tibialis anterior, extensor digitorum longus (EDL), gastrocnemius white, gastrocnemius red, soleus and plataris) were immediately excised and their radioactivity measured. Injections which gave more than a 10% difference in blood flow between the kidneys were excluded, as differences this large indicate an inadequate distribution of microspheres (5). By the reference sample technique the blood flow in each individual muscle could be accurately determined, provided that the reference sample contained 200 or more microspheres (12). Regional blood flow (Q) to the lower leg was then calculated as the average radioactivity of all of the muscles and was expressed as mL/100g tissue/min.
Analytical methods

Glucose and lactate concentrations in both the plasma and in the dialysate fractions were determined enzymatically by using 10 µL samples for the simultaneous analyses of glucose and lactate in a YSI 2700 select biochemical analyzer (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, USA). Rat (endogenous) and human (exogenous) insulin were measured with equal efficiency using a double antibody radioimmunoassay (Pharmacia, Uppsala, Sweden). Radioactivity was counted by using a liquid scintillation counter with a quench-corrected (external standards), double-isotope program (1217 Rackbeta, LKB, Uppsala, Sweden). Tissue samples were initially checked for quenching interferences by using internal standards for $^{14}$C and $^3$H.

Statistics

Results are presented as the mean ± SE. Linear regression analyses were performed according to the least square method. The significance of differences was tested with Student's t-test for paired or non-paired observations. Non-parametric tests were done by means of the Wilcoxon signed ranked test for paired comparisons of blood flow, glucose uptake and lactate production. A two-tailed p-value of < 0.05 was considered statistically significant. When multiple comparisons were performed, analysis of variance (ANOVA) was used. The StatView Version 4.0 program was used in the Macintosh system for all analyses.
RESULTS

Resting basal non-insulin stimulated state

The arterial plasma glucose concentration in the basal state (7.7±0.2 mM) was significantly greater than the estimated interstitial glucose level in muscle (6.1±0.2 mM, p<0.001). However, the mean plasma lactate concentration in the basal state did not differ from the estimated interstitial concentration of lactate (Table 1). The estimated interstitial concentrations of glucose and lactate were determined by using both the equilibrium (20) and internal reference calibration techniques (21), and were found to be similar (Table 1). The mean basal plasma insulin concentration was determined to be 19.8±1.5 µU/mL. The microdialysate levels of insulin were below the detectable limit (2 µU/mL of dialysate insulin or ~10 µU/mL of interstitial insulin concentration) of the analytical methods that were used.

Effects of insulin infusion and electrical stimulation

During the steady state (90 min) hyperinsulinemic clamping plasma conditions, insulin concentrations were kept at 247±21 µU/mL. The mean glucose infusion rate during the last 60 min of the insulin infusion was found to be 0.7±0.1 mmol/kg/min, while the plasma glucose concentration during the clamp was 7.1±0.1 mM. Although the interstitial glucose concentration during PSL (6.7±0.3 mM) did not differ from the average plasma concentration, the interstitial glucose concentration of the resting leg (5.8±0.2 mM) was found to be significantly lower than that in the plasma (p<0.05) (Figure 2a).

In the basal state, the arterial-interstitial (A-I) concentration difference of glucose was 1.6±0.3 mM (p<0.01). During insulin infusion, the A-I difference of
glucose decreased and became statistically non-significant in PSL muscles (0.4±0.6 mM), but was unaltered in resting muscles (1.3±0.3 mM, p<0.05) (Fig. 2a).

The interstitial lactate concentrations of the PSL (p<0.001) and resting (p<0.05) muscles were significantly greater than the average plasma lactate concentration (Fig. 2b) during insulin infusion. Moreover, the interstitial lactate concentration in PSL muscle (4.6±0.39 mM) was roughly two times greater than that in the resting muscle (2.5±0.5 mM, p<0.05).

In the basal state, no significant I-A concentration differences of lactate were found. However, during insulin infusion, the I-A differences of lactate increased significantly in both legs, the amplitude of the change being higher (2.8±0.5 mM, p<0.01) in PSL than in resting leg (0.7±0.4 mM, p<0.05) (Fig. 2b).

The interstitial insulin concentrations were found to be roughly equal in both the PSL and resting muscles (161±22 vs. 148±14 µU/mL, n.s.) (Fig. 2c). The A-I concentration difference of insulin during the insulin infusion did not differ between PSL (86±7 µU/ml)) and resting muscles (100±8 µU/ml) (Fig. 2c). Relative microdialysis recovery monitored according to the internal standard technique was unchanged throughout the study (not shown).

**Blood flow**

Blood flow was found to be significantly increased in all of the moving muscles including the PSL quadriceps muscle that were studied during the hyperinsulinemic euglycemic clamp relative to that of resting muscles. The mean blood
flow (Q) in quadriceps muscle was found to be 17±4 and 34±6 ml/100g/min, (p<0.05) in the control and electrically stimulated legs, respectively (Table 2).

DISCUSSION

The data that are herein presented demonstrate for the first time that reciprocal changes are present in lactate and glucose concentration gradients over the capillary wall during PSL. Furthermore, the measurement of insulin in dialysates showed no evidence for mediated changes in insulin delivery to the interstitial fluid during these movements.

The microdialysis technique used in this study was calibrated using two different techniques: the equilibrium calibration method (20) and the internal calibration method (21). Values which were obtained by using both of these methods were similar and regression analyses indicated that a strong correlation was present between data generated by the two techniques (Table 1). Furthermore, the fractional outflux of an internal standard (not shown) and the recovery data (Table 1) indicated that the microdialysis technique did not affect either the interstitial concentration or the blood flow in muscle tissue during the study. Thus, it may be concluded that the concentration gradients that were studied during insulin infusion and electrical stimulation were not affected by any factors other than the rate of cellular uptake/release, the capillary diffusion capacity and the blood flow.

Data from previous studies involving subcutaneous adipose tissue have shown that microdialysis calibration with an internal reference may lead to an overestimation of the interstitial concentration of glucose due to an accumulation of reference compound near the catheter (21). However, the present finding that microdialysis
calibration with an internal reference gives the same results as those from equilibration calibration indicates that microcirculation near the catheter was sufficient to prevent reference accumulation, thus justifying the use of the internal reference calibration technique in rat muscle tissue.

**Resting state**

The data obtained from resting muscle confirm that the interstitial muscle glucose concentration is lower than that in arterial plasma (8, 24) (Fig. 2a). The capillary wall thus constitutes a functional diffusion barrier for glucose uptake in muscle, both during fasting and after oral glucose (24) and also during insulin infusion (the present study). The insulin stimulated increase of blood flow in the skeletal muscle could be brought about by either an increase in the flow rate without a concomitant change in muscle blood volume, and/or by the recruitment of capillaries (25). In a previous microdialysis study (24) we have shown that, the glucose utilization in muscle is enhanced and the muscle blood flow is increased following an oral glucose load. Despite this increase in blood flow, the arterial interstitial glucose concentration difference is increased. This implies that the increased glucose consumption in the muscle cells after oral glucose leads to an increased glucose gradient over the capillary wall as a result of the enhanced cellular uptake and by the metabolism of glucose.

The presence of an arterial-interstitial concentration difference allows for the calculation of the rate of glucose uptake according to Fick’s principle (13, 24), by the equation: GU=(I-A) x (1-e^{-PS/Q})x BF where GU = glucose uptake rate, A = arterial blood glucose concentration, I = interstitial glucose, PS = permeability surface area product (a marker for the number of recruited capillaries), Q= plasma flow rate and BF
blood flow. PS is assumed to be 5 mL/100g/min for glucose in the resting state (4) and is approximately 10 mL/100g/min in the contracting muscle (27). Application of the above formula then gives an estimated glucose uptake rate of ~10 µmol/100g/min in the femoral muscles during insulin infusion in agreement with earlier data obtained in AV-difference and perfusion studies in the leg (29, 30).

In the resting state, the muscular, interstitial lactate concentration in the femoral muscle was not different from the arterial lactate concentration (Fig. 2b). In contrast to the present data, the interstitial lactate concentration has been demonstrated to be higher than the arterial lactate concentration in resting gastrocnemius muscle (3). However, results presented by Okuda (26) from the medial thigh muscles indicated that interstitial lactate and arterial lactate were equal in the basal state. It should be noted that data from different muscle groups may differ in this respect because of regional differences in muscle fiber composition and blood flow as well as in capillary diffusion capacity. In the present work, the lactate production could not be estimated using the above equation because the capillary diffusion was not rate-limiting for lactate release from the muscle in the basal state (24).

Stimulation by insulin was found to increase the lactate production, which resulted in the interstitial lactate concentration increasing significantly compared to the plasma concentration, which is in accordance with previous data (32). In this insulin-stimulated state, then, capillary diffusion should be regarded as partly rate-limiting for the lactate release.
Passive muscle shortening and lengthening (PSL)

It should be noted that this study was designed to investigate the effects of passive shortening and lengthing in the quadriceps muscle through stimulation of the sciatic nerve that induces active contractions of the flexor groups. By using this approach, the observed changes in glucose metabolism should be regarded as resulting from the activation of the non-insulin dependent signaling pathway as a means for stimulating glucose transport and metabolism (19) without the presence of direct interference from the electrical stimulation, nor from increasing interstitial water pressure gradients induced by active contractions.

The interstitial muscle glucose concentration was found to be higher in the PSL quadriceps muscle than in the quadriceps muscle of the control leg. This finding suggests that in moving muscles, capillary recruitment, blood flow and/or the permeability surface (27) was increased to a magnitude which was high enough to compensate for the increase in cellular glucose uptake and high enough to allow glucose to equilibrate over the capillary wall. In this situation, the capillary wall is no longer rate-limiting for muscle glucose uptake. Instead, a maximum concentration gradient of glucose was applied over the plasma membrane in order to drive glucose transport through facilitated diffusion.

Earlier studies (17, 30) have demonstrated that increasing plasma glucose concentrations may enhance the blood flow, while the A-V concentration difference of glucose remains constant, which may indicate that the increase in blood flow could explain the total increase in muscle glucose consumption in these subjects (17). In the present study, PSL produced a substantial increase in muscle blood flow, which was enough to compensate for the increased tissue glucose uptake, as well as to diminish the
A-I concentration difference. In contrast, the interstitial lactate concentration in the PSL muscle was significantly higher than either the concentration that was measured during the insulin-infusion in the control resting leg or the concentration of lactate in the plasma (Fig. 2b). It is thus clear that the increase in blood flow, which blunts the glucose gradient over the capillary wall during electrical stimulation and PSL movements, is not enough to prevent a concomitant increase in the lactate I-A concentration gradient. This indicates that the PSL of muscle partly switches the glucose metabolism to enhance glycolysis. Furthermore, this change in glucose metabolism is compatible with the theory that the increase in blood flow may be mediated by the accumulation of lactate or other vasoactive glucose metabolites.

According to this theory, the increase in blood flow is regulated such that reciprocal changes in glucose and lactate concentration gradients over the capillary wall allow for autoregulation of glucose A-I concentration differences (9, 10).

Previous investigations including measurements in lymph (35) and in interstitial fluid (8, 31, 32) have shown that the interstitial concentration of insulin is 40-60% lower than in plasma. Thus, the capillary wall is rate limiting for the delivery of insulin to the muscle cells. The delivery rate per se is dependent on the blood flow rate and on the permeability of the capillaries. The capillary permeability is a function of the number of open capillary beds as well as the permeability of each capillary. The existence of a transendocytotic and regulated pathway for insulin has been demonstrated (14) but its physiological significance for the transcapillary insulin delivery is yet unclear (33). The interstitial concentration of insulin in muscle was not affected by the PSL (Fig. 2c). This is consistent with previous data which demonstrated that muscle contraction and the concomitant increase of blood flow only provide for an increase in the interstitial concentration of insulin in the presence of non-
physiologically high plasma insulin concentrations and when the transcapillary concentration gradient of insulin is high (8). It should be noted that insulin delivery over the capillary wall is not readily increased by changes in blood flow due to the poor capillary permeability (4, 27, 31).

It is important to consider that the design of the present investigation does not permit us to fully differentiate the effect of PSL movements from that of insulin or other humoral factors. For obvious technical reasons an insulin free experiment could not be performed and, furthermore, we wanted to study the transcapillary delivery of insulin during PSL. Therefore, passive muscle movements were induced in the insulin stimulated state and the effect exerted should be considered as additional to insulin and other hormones.

In summary, the present data demonstrate that the capillary wall is rate-limiting for glucose uptake in resting muscle during euglycemic clamp conditions, but is not rate-limiting for lactate release under these conditions. Passive muscle shortening and lengthening results in elevated blood flow and glucose consumption with a shift in rate-limiting steps and an increased lactate production. These data suggest that in PSL, the capillary wall is rate-limiting for delivery of lactate to the plasma but not for taking up glucose into the muscle cell.
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FIGURE LEGENDS

Fig 1. Study protocol. Calibration of microdialysis catheters was carried out for 80 min. (basal state).

During steady state clamping conditions, 10 mU/kg/min (from 120 min to 180 min) samples of the muscle interstitial fluid were collected every 30 min. During the last 60 min of the clamp, the medial femoral muscle of one hind limb was stimulated electrically passive muscle shortening and lengthening (PSL). At the end of electrical stimulation, blood flow measurements (microsphere study) were performed.

Fig. 2 a Glucose concentration in plasma and interstitial fluid in medial femoral muscles during basal and insulin infusion conditions (euglycemic hyperinsulinemic clamp, 10 mU/kg/min) and during passive muscle shortening and lengthening (PSL).

** p < 0.01,  * p < 0.05, interstitial vs arterial plasma concentrations.

Concentrations are means ± SE,  n = 8-12.

Fig. 2 b Lactate measurements in experiments depicted in Fig. 2 a.

*** p < 0.001,  * p < 0.05 interstitial vs arterial plasma concentrations.

† p < 0.05 concentration differences in legs.

Concentrations are means ± SE,  n = 8-12.

Fig 2 c Insulin concentrations in plasma and interstitial fluid. Symbols as in Fig. 2 a.

*** p < 0.001 interstitial vs arterial plasma concentrations.

Concentrations are means ± SE,  n = 8-12.
Table I
Calibration of microdialysis catheters by means of “equilibration” and “internal reference” techniques.

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equilibration technique</td>
<td>Equilibration technique</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.31±0.02</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td>Estimated interstitial concentrations (mM)</td>
<td>6.1±0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Plasma concentrations (mM)</td>
<td>7.7±0.2***</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td></td>
<td>Internal technique</td>
<td>Internal technique</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.36±0.02</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td>Estimated interstitial concentrations (mM)</td>
<td>6.1±0.3</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Plasma concentrations (mM)</td>
<td>7.7±0.2**</td>
<td>1.3±0.2</td>
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</tbody>
</table>

Numbers of observations are 12 rat (24 legs).

**p<0.01, ***p< 0.001 estimated interstitial glucose vs. plasma glucose values. Means±SE.
Table II

Blood flow (ml/100g/min) in skeletal muscles of contracting and passive shortening and lengthing (PSL) and resting quadriceps muscle control leg during hyperinsulinaemic euglycemic clamp (10 mU/kg/min).

<table>
<thead>
<tr>
<th></th>
<th>Tibialis anterior</th>
<th>EDL</th>
<th>Gastrocnemius white</th>
<th>Gastrocnemius red</th>
<th>Soleus</th>
<th>Plantaris</th>
<th>Quadriceps</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSL</td>
<td>115±22**</td>
<td>127±33**</td>
<td>52±7**</td>
<td>61±11*</td>
<td>68±14*</td>
<td>70±8***</td>
<td>34±6*</td>
</tr>
<tr>
<td>Resting leg</td>
<td>30±7</td>
<td>32±10</td>
<td>19±5</td>
<td>24±7</td>
<td>33±9</td>
<td>23±6</td>
<td>17±4</td>
</tr>
</tbody>
</table>

n=6, Means±SE. EDL, extensor digitorum longus.

* p<0.05, **p<0.01, ***p<0.001, Contracting vs. resting leg.
Fig 1

Calibration

Hyperinsulinemic euglycemic clamp (10 mU/kg/min)

Left leg muscle passively shortening and lengthening (PSL)

Microsphere injection

- Basal state (calibration)
- Insulin infusion state
- Steady-state 120-180 min
- Sampling every 15 min
- Sampling every 30 min

0 40 80 120 160 200 (min)