Assessment of transcapillary glucose exchange in human skeletal muscle and adipose tissue

Werner Regittnig,1 Martin Ellmerer,3 Günter Fauler,2 Gerald Sendlhofer,3 Zlatko Trajanoski,1 Hans-Jörg Leis,2 Lukas Schaupp,3 Paul Wach,1 and Thomas R. Pieber3

1Department of Biophysics, Institute of Biomedical Engineering, Graz University of Technology; and Departments of 2Biochemical Analysis and Mass Spectrometry and 3Internal Medicine, Diabetes and Metabolism, Karl Franzens University Graz, A-8010 Graz, Austria

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Regittnig, Werner, Martin Ellmerer, Günter Fauler, Gerald Sendlhofer, Zlatko Trajanoski, Hans-Jörg Leis, Lukas Schaupp, Paul Wach, and Thomas R. Pieber. Assessment of transcapillary glucose exchange in human skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab 285: E241–E251, 2003. First published April 8, 2003; 10.1152/ajpendo.00351.2002.—We studied the kinetics of glucose exchange between plasma and interstitial fluid (ISF) in human skeletal muscle and adipose tissue under fasting conditions. Five normal human subjects received an intravenous [6,6-2H2]glucose infusion in a prime-continuous fashion. During the tracer infusion, the open-flow microperfusion technique was employed to frequently sample ISF from quadriceps muscle and subcutaneous adipose tissue. The tracer glucose kinetics observed in muscle and adipose tissue ISF were found to be well described by a capillary-tissue exchange model. As a measure of transcapillary glucose exchange efficiency, the 95% equilibrium time was calculated from the identified model parameters. This time constant was similar for skeletal muscle and adipose tissue (28.6 ± 3.2 vs. 26.8 ± 3.6 min; P = 0.60). Furthermore, we found that the (total) interstitial glucose concentration was significantly lower (P < 0.01) in muscle (3.32 ± 0.46 mmol/l) and adipose tissue (3.51 ± 0.17 mmol/l) compared with arterialized plasma levels (5.56 ± 0.13 mmol/l). Thus the observed gradients and dynamic relationships between plasma and ISF glucose in muscle and adipose tissue provide evidence that transcapillary exchange of glucose is limited in these two tissues under fasting conditions.

open-flow microperfusion; interstitial fluid; capillary-tissue exchange model

STUDIES INVESTIGATING THE MECHANISMS of transcapillary fluid and solute transport suggest that, in most vascular beds, small hydrophilic substances (e.g., glucose) cross the capillary wall primarily by simple diffusion (9, 27). Thus, for small hydrophilic solutes, the capillary wall may be regarded as a porous membrane through which passive solute exchange between the plasma and the interstitial fluid (ISF) occurs (9, 27, 33). The permeability of capillary walls to small hydrophilic solutes has been shown to vary considerably from one microvascular bed to another (9). These variations in capillary permeability are likely to be related to variations in the ultrastructure of the capillary walls in the microvascular beds (e.g., fenestrated vs. continuous capillaries) (46). Because diffusion of solutes into or out of blood also depends on the time that blood spends in the capillary, solute exchange across capillary walls is influenced by blood flow (9, 38). The magnitude of transcapillary exchange of very small solutes with high capillary permeability (e.g., potassium in heart) was shown to depend largely on blood supply (i.e., flow-limited exchange) (9). In the case of solutes with high molecular weight and low capillary permeability (e.g., inulin in heart), solute exchange was found to be practically unaffected by blood flow (i.e., permeability-limited exchange) (9). Therefore, transcapillary exchange of hydrophilic solutes of the size of glucose may be best described as intermediate between the two extreme situations of flow-limited and permeability-limited exchanges (9, 38). Thus, besides the general tissue geometry (e.g., capillary surface area), the main factors determining the transcapillary exchange of a small hydrophilic solute of the size of glucose seem to be the prevailing blood flow and the capillary permeability to the solute.

In recent years, the ISF of peripheral tissues has received considerable attention as an alternative site for the measurement of glucose levels. Various methods, such as microdialysis (4, 18), open-flow microperfusion (40, 51), electrochemical sensors (22), transdermal extraction (23, 49), and hypodermic needles (2, 44), have been proposed for the assessment of glucose in the ISF rather than in blood. The ISF glucose values obtained by such methods may then be used to estimate blood glucose levels. In this way, the ISF-based glucose assessment could potentially replace the more invasive blood glucose measurements (35). Ideally, if transcapillary exchange of glucose were very rapid and unimpeded, the blood and ISF glucose would be in instantaneous equilibrium so that, irrespective of the metabolic rates of cell glucose uptake, the ISF glucose
concentrations would be identical to the blood levels at all times. In this ideal situation, the quality in estimating blood glucose concentrations from ISF glucose values would depend solely on the degree of precision and accuracy of the ISF glucose measurements (1). However, under the circumstances of limited transcapillary exchange of glucose, transient differences between ISF glucose levels and changing blood glucose concentrations may occur, and, if there is an additional glucose consumption by the tissue cells, a blood-to-ISF concentration difference may also exist under steady-state conditions (9, 19, 37, 45). Because of these differences, accurate calculation of blood glucose concentrations from interstitial glucose measurements requires one to postulate a mathematical model of the tissue-specific relationship between blood and ISF glucose levels under steady-state and non-steady-state conditions. Obviously, in the case of limited transcapillary glucose exchange, the quality of the ISF-derived estimate of blood glucose levels will be determined both by the quality of the mathematical model on which the calculation of blood glucose levels from interstitial glucose levels is based and by the accuracy and precision of the interstitial glucose measurements.

There have been few attempts to assess the transcapillary glucose exchange properties of the intact peripheral tissues of the rat (42), dog (35, 36, 48), and sheep (13). In these animal studies, glucose or tracer glucose kinetics simultaneously observed in blood and ISF have been mathematically treated to yield quantitative information on the tissue-specific ability to exchange glucose across the capillary walls. To the best of our knowledge, similar evaluations of transcapillary glucose exchange in peripheral tissues in humans have not been carried out. Therefore, the purpose of the present study was to quantify the kinetics of the exchange of glucose between the plasma and the ISF of human skeletal muscle and adipose tissue under fasting conditions. To achieve this, we simultaneously sampled plasma and ISF of muscle and adipose tissue during intravenous infusions of D-[6,6-2H2]glucose in fasted normal subjects. ISF sampling from the two tissues was accomplished by employing the open-flow microperfusion technique (40, 51). The observed tracer glucose kinetics in plasma and ISF were then analyzed by a capillary-tissue exchange model to yield kinetic parameters describing transcapillary glucose exchange in muscle and adipose tissue.

METHODS

Subjects. Five male volunteers [age 30.2 ± 2.6 yr, range 24–37 yr; body mass index (BMI) 23.4 ± 0.8 kg/m², range 21.7–25.7 kg/m²; means ± SE] participated in this study. They were all healthy as judged by medical history, physical exam, and routine laboratory tests and were not taking any medications. Written informed consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The experimental protocol was approved by the ethics committee of the University of Graz.

Open-flow microperfusion and mannitol calibration technique. The open-flow microperfusion technique has been previously described in detail (10, 37, 40). Briefly, as described in these studies, open-flow microperfusion was based on a double-lumen catheter (18 gauge) with macroscopic perforations (80 holes, each 0.5 mm in diameter). This catheter was placed in the tissue of interest and then perfused with artificial extracellular fluid by means of a peristaltic pump (Minipuls 3; Gilson, Villiers-le-Bel, France). The perfusion fluid entered the probe via the inlet tubing, moved through the inner cannula to the tip of the probe, and streamed back in the space between inner cannula and the perforated outer cannula. The volume of the space between inner cannula and the perforated part of the outer cannula was 4.8 μl. As the perfusate passed by the perforations of the outer cannula, exchange occurred between the perfusate and the surrounding ISF. The medium (now called effluent) then flowed through an 800-mm piece of 250-μm-ID outlet tubing, at the end of which samples were collected for the subsequent analysis of glucose and other substances. The total volume from the middle of the perforated region of the outer cannula to the end of the outlet tubing was 43.5 μl. To achieve an acceptable time resolution, the microperfusion probes are usually perfused at relatively high flow rates (e.g., 2 μl/min). However, at these high flow rates, glucose in the perfusate and the ISF is not complete. In the present study, we applied the mannitol calibration technique to determine the extent of the mixing between perfusate and ISF. In this calibration technique, mannitol is intravenously infused at a constant rate, and the mannitol concentration is then measured in the plasma and probe effluents. Mannitol is a sugar with a composition and molecular weight similar to those of glucose but, unlike glucose, it is not taken up by muscle and fat cells. Thus steady-state mannitol concentrations are identical in plasma and ISF of muscle and fat tissues. Therefore, the extent of the mixing between perfusate and ISF (also called recovery) can be calculated from the mannitol concentrations in plasma and probe effluents. The macroscopic perforations of the microperfusion catheter permit unrestricted exchange of solutes between the perfusion medium and the surrounding ISF. Because of this property, the microperfusion system may be qualified to follow rapid solute concentration changes in the ISF (37, 40).

Intravenous infusions and blood sampling. All subjects were studied in the supine position after a 12- to 14-h overnight fast. In the morning at ~8 AM, an 18-gauge catheter was placed into a forearm vein for the infusion of mannitol and tracer glucose. A second catheter was inserted into a dorsal hand vein on the opposite arm to allow blood withdrawal during the experiment. The hand with the sampling catheter was placed in a thermoregulated box and maintained at 55°C to ensure the arterialization of the venous samples. The sampling cannula was kept patent by the slow infusion of 0.9% NaCl (Fresenius Kabi Austria, Graz, Austria). A primed (49.5 mg/kg) continuous (0.45 mg·kg⁻¹·min⁻¹) infusion of D-mannitol (Fresenius Kabi Austria) was then started at ~120 min and continued for the duration of the study. After a mannitol equilibration period of 100 min, a basal blood sample (4 ml) was collected at ~10 min. At time 0, D-[6,6-2H2]glucose (Cambridge Isotope Laboratories, Woburn, MA) was given as a primed (12 mg/kg) continuous (0.1 mg·kg⁻¹·min⁻¹) infusion for 110 min. During the tracer infusion, 4-ml blood samples were taken at 2.5, 4, 5.5, 7.5, 9.5, 12.5, 17.5, 22.5, 27.5, 35, 45, 60, and 90 min after the start of the dorsal hand catheter, one microperfusion probe was placed in the perilumbilical subcutaneous adipose tissue and another in the tissue of the rectus femoris muscle (10). Starting at ~120 min, and continuing for the duration of the study, microperfusion probes were perfused with Ringer solution (Fresenius AJP-Endocrinol Metab • VOL 285 • AUGUST 2003 • www.ajpendo.org
and pyridine to convert glucose and mannitol to their acetate derivatives (6,7). A Trace GC-MS (Thermo Finnigan) equipped with an AS 2000 autosampler (Thermo Quest) and a DB-5MS Capillary GC column (length 15 m, ID 0.25 mm, film thickness 0.25 μm; J&W Scientific) was used to analyze the glucose and mannitol derivatives. The GC injector temperature was set at 280°C, and the transfer line between GC and MS was held at 280°C. The carrier gas was helium. The column temperature was 80°C for 1 min and was increased by 30°C/min to 310°C. Under chemical ionization (CI), ions with mass-to-charge ratios (m/z) of 331, 333, and 337 were monitored for glucose, and ions with m/z ratios of 375 and 376 were monitored for mannitol (6). The CI gas used was methane. The peak areas of the monitored ions were used to calculate the isotope ratios. The variance in the determination of the isotope ratios was assessed by analyzing replicates of plasma and ISF samples enriched with known amounts of [6,6-2H2]glucose and [1-13C]mannitol (Isotec, Miamisburg, OH) in known amounts. To this sample, zinc sulfate and a standard solution of [6,6-2H2]glucose were used to prepare a series of five samples ranging in tracer-to-tracer ratio (TTR) from 0 to 12%. Each sample was aliquoted in five equal volumes. The aliquots were then purified, derivatized, and analyzed. Over this range of TTR values, the measurement of R333/331 had a coefficient of variation (CV) equal to 3.2%. A similar CV in the measurement of R333/331 was obtained for ISF samples. Furthermore, the R337/331 and R376/375 of the replicate plasma and ISF samples enriched with known amounts of [U-13C]glucose and [1-13C]mannitol were determined with a CV equal to 1.5%. Uncertainties associated with the pipetting are included in this CV value.

Calculation of glucose and mannitol concentrations in plasma and effluent samples. Because of the natural occurrence of heavier isotopes in both tracer and tracee, the isotope ratio cannot generally be taken as a direct measure of the TTR in a sample. It can be shown (34,53) that the general form of equation defining the relationship between a measured isotope ratio $R_{K}$ and the number of fragment ions arising from a trace (I) and tracer (I*) in a sample is

$$R_{K} = \frac{(I_{qK} + I_{qL})(I_{qK} + I_{rL})}{I_{qL}}$$

where $q_K$ and $r_K$ are the respective probabilities of occurrence of trace and tracer fragment ions with an m/z of K, and where $q_L$ and $r_L$ are the respective probabilities of occurrence of trace and tracer fragment ions with an m/z of L. Because all tracers used in our study were 99% enriched, the contribution of tracer fragment ions at an m/z of K is negligible (i.e., $r_K \approx 0$). Under this condition, the following relationship may be deduced from Eq. 1

$$TTR = I_{qL}L = \frac{(q_{qL}r_{qL})R_{qL} - (q_{rL}r_{rL})}{(q_{rL}r_{rL})R_{rL} - (q_{qL}r_{qL})}$$

Thus, in this case, TTR (or $I_{qL}$) is linearly related to $R_{qL}$. Numerical values of the slopes (i.e., $q_{qL}r_{qL}$) and intercepts (i.e., $q_{rL}r_{rL}$) were obtained by performing linear regression using data from the GC-MS analysis of the replicate plasma and ISF samples with known enrichments. There was no deviation from linearity over the range of TTR values investigated ($r > 0.997$). The measured TTR values and the determined linear regression lines (Eq. 2) were then used to derive the TTR values for the plasma and effluent samples with unknown enrichments. From the TTR values so obtained, contents of unlabeled glucose (G) and mannitol (M) in the samples were calculated according to the following equations.
where TTRM is the ratio of [1-13C]mannitol tracer to mannitol tracee, TTRG is the ratio of [U-13C]glucose tracer to glucose tracee, and M* and G* are the known quantities of added [1-13C]mannitol and [U-13C]glucose tracers, respectively. By carrying out error propagation analysis (5), it can be shown that the following relationships between the CVs of the derived tracee concentrations and those of the measured RL/Κ values (CVRL/Κ) exist

\[
CV_M = \frac{CV_{R_{\text{M}}}}{1 - \left(\frac{q_{375}r_{257}}{q_{257}r_{375}R_{375257}}\right)}
\]

\[
CV_G = \frac{CV_{R_{\text{G}}}}{1 - \left(\frac{q_{331}r_{237}}{q_{237}r_{331}R_{331237}}\right)}
\]

where \(CV_M\) and \(CV_G\) are the CVs for the determination of mannitol and glucose concentrations, respectively. The average CV for the mannitol data was 2.0% (range 1.7–3.5%; lower when \(R_{375257}\) was higher, see Eq. 5). Because the contribution of glucose tracee fragment ions at \(m/z\) of 337 was negligible (i.e., \(q_{337} = 0\)), the regression line for TTRG exhibited an intercept at the origin (i.e., \(q_{337}r_{237} = 0\)). As a result (see Eq. 6), the CV of the glucose tracee data was close to the experimentally determined CV for \(R_{337231}\) (i.e., 1.5%). From the derived glucose tracee concentrations (\(G\)) and the ratios of [6,6-\(^2\text{H}_2\)]glucose tracer to glucose tracee (TTRG), the content of [6,6-\(^2\text{H}_2\)]glucose (\(G^*\)) in each plasma or effluent sample was calculated according to the following equation

\[
G^* = \text{TTR}_G \cdot G
\]

Again, using a propagation of errors analysis, it can be shown that the CV for the determination of \(G^*\) (\(CV_{G^*}\)) can be calculated as follows

\[
CV_{G^*} = \sqrt{(CV_G)^2 + (CV_{\text{TTRG}})^2}
\]

where

\[
CV_{\text{TTRG}} = \frac{CV_{R_{\text{M}}}}{1 - \left(\frac{q_{331}r_{237}}{q_{237}r_{331}R_{331237}}\right)}
\]

The average \(CV_{G^*}\) values for the determination of [6,6-\(^2\text{H}_2\)]glucose concentrations in plasma and effluent samples were 5.2% (range 3.9–11.7%).

**Calculation of the ISF glucose concentrations.** As already mentioned, the substrate recovery in the effluent of the microperfusion probe is not complete at the flow rate used in this study (i.e., the effluent concentration is lower than the interstitial concentration). However, provided that the substrate recovery for an effluent sample is exactly known, the ISF substrate concentrations can be calculated as the substrate concentration in the effluent divided by the substrate recovery (40). In the present study, the substrate recovery for each effluent sample (SR) was estimated as the ratio of the mannitol concentration in the effluent sample (ME) to the steady-state concentration of mannitol in the corresponding arterial plasma sample (MA). Thus, for a certain sampling time point, the interstitial concentrations of glucose tracee and [6,6-\(^2\text{H}_2\)]glucose tracer were calculated as defined in Eqs. 10 and 11

\[
G_I = \frac{G_{\text{SR}}}{G_E \cdot \text{TTR}_{\text{GIE}} \cdot \frac{M_A}{M_E}}
\]

\[
G^*_I = \frac{G^*_{\text{SR}}}{G^*_E \cdot \text{TTR}_{\text{GIE}} \cdot \frac{M_A}{M_E}}
\]

where \(G_I\) and \(G^*_I\) are the interstitial concentrations of [6,6-\(^2\text{H}_2\)]glucose tracer and glucose tracee, respectively. The CVs for the determination of \(G_I\) and \(G^*_I\) were calculated from the following equations

\[
CV_{G_I} = \sqrt{(CV_{G^{*}_I})^2 + (CV_{\text{MIE}})^2 + (CV_{\text{ME}})^2}
\]

\[
CV_{G^*_I} = \sqrt{(CV_{G^{*}_I})^2 + (CV_{\text{TTRGIE}})^2 + (CV_{MIE})^2 + (CV_{\text{ME}})^2}
\]

CVs of the interstitial [6,6-\(^2\text{H}_2\)]glucose tracer data averaged 6.0% (range 4.6–12.7%), and the CVs of the interstitial tracee glucose data averaged 3.2% (range 2.8–5.2%). Because each probe effluent sample was collected over a specified time interval (e.g., the effluent sample in the period from 0 to 5 min), the derived interstitial tracer and tracee glucose values were considered valid at the midpoint of the interval (e.g., at 2.5 min).

In the calculation of the substrate recovery in the microperfusion probe effluents (Eqs. 10 and 11), we implicitly assume that mannitol is not taken up by muscle and fat cells and that, therefore, the steady-state mannitol concentrations are identical in plasma and ISF of muscle and adipose tissue. In preliminary experiments in humans, we infused mannitol intravenously and measured the steady-state mannitol concentration in the ISF 3.2%–5.2% above the arterial plasma concentration. In the present study, we utilized this relationship obtained by Johnson and Wilson (19) to describe the transcapillary flux of tracer glucose from blood into muscle and adipose tissue ISF as a function of arterial and interstitial tracer glucose concentrations. With the additional assumption that there is no back flux of tracer glucose from the cell, the
following differential equation for the quantities of tracer glucose in the interstitial space can be set up (52):

\[
d(G^*_I)/dt = +\rho(G^*_A - G^*_I) - \lambda_0 G^*_I
\]

(15)

where \(G^*_A\) is the tracer glucose concentration in the arterial plasma, \(G^*_I\) is the tracer glucose concentration in the ISF, \(\lambda_0\) is the rate constant for uptake of tracer glucose by the cells, and \(V_I\) is the distribution volume of glucose in the ISF space. Dividing the differential equation by \(V_I\) yields

\[
d(G^*_I)/dt = +\rho G^*_A - p_1 G^*_I
\]

(16)

where \(p_1 = \rho V_I\), and \(p_2 = (\rho V_I + \lambda_0 V_I)\). Both parameters are uniquely identifiable from plasma and ISF tracer data. A graphic visualization of the mathematical model defined with Eq. 15 is given in Fig. 1. To characterize the specific properties of the blood-tissue exchange of glucose in the examined tissues, the steady-state ratio of ISF glucose to plasma glucose \((r)\) and the 95% equilibrium time \((T_{95%})\) were calculated from \(p_1\) and \(p_2\) as follows

\[
r = p_1/p_2
\]

(17a)

\[
T_{95%} = -\ln(0.05)/p_2 = -\ln(0.05)/(\rho V_I + \lambda_0 V_I)
\]

(17b)

The time constant \(T_{95%}\) is defined as the time required for the ISF glucose to attain 95% of the steady-state value after a concentration step change in the plasma compartment. Thus the value of \(T_{95%}\) provides a measure of the ISF glucose equilibration delay, which is determined by the fractional rate of glucose exchange \((\rho V_I)\) and the fractional rate of glucose uptake \((\lambda_0 V_I)\).

**Numerical methods.** To perform the kinetic analysis, the observed plasma tracer profiles \((G^*_A)\) were used as an input to Eq. 16, and the parameters \((p_1, p_2)\) were identified by fitting the output \((G^*_I)\) to the observed ISF tracer data. When plasma tracer data were used as an input, the plasma concentrations between sample times were determined by linear interpolation. The numerical values of unknown parameters were obtained by weighted nonlinear least squares by use of a Levenberg-Marquardt algorithm (37) with inverse variance weights. Monte Carlo analysis was performed to determine the precision of the parameter estimates (37). The measurement errors were assumed to be independent, Gaussian of zero mean, and with the experimentally determined variances. The statistical significance of differences was calculated using two-tailed, paired Student’s \(t\)-test analysis. A \(P\) value of <0.05 was considered to indicate statistical significance. Data are reported as means \(\pm SE\). Steady-state values were calculated by averaging data from the final hour of the experiment. MATLAB software packages (The MathWorks, Natick, MA) were used for all of the analyses and statistics (37).

**RESULTS**

**Mannitol concentrations and substrate recovery.** In the five subjects studied, the primed-continuous infusion of mannitol resulted in a constant plasma mannitol concentration of \(1.62 \pm 0.12\) mmol/l (Fig. 2A). In comparison, the mannitol concentrations in the effluents of the muscle and adipose tissue probes attained a steady-state level of \(0.35 \pm 0.07\) and \(0.27 \pm 0.05\) mmol/l, respectively (Fig. 2A). By taking these mannitol values, the average substrate recovery in the muscle and adipose tissue probe effluents was calculated to be \(21.1 \pm 2.9\) and \(17.4 \pm 3.4\%\), respectively. There was no statistical difference in substrate recovery between the two probe effluents \((P > 0.32)\). The relatively constant mannitol concentrations in plasma and effluent samples indicate that steady-state microperfusion conditions were maintained during the experiments.

**Total glucose concentrations.** During the final hour of the study, the average total glucose concentrations (i.e., tracer + tracee concentrations) in the muscle and adipose tissue probe effluents were \(0.670 \pm 0.065\) and \(0.609 \pm 0.121\) mmol/l, respectively (Fig. 2B). By taking the observed effluent glucose and substrate recovery values (Eqs. 10 and 11), the mean total glucose concentrations in muscle and adipose tissue ISF can be calculated to be \(3.32 \pm 0.46\) and \(3.51 \pm 0.17\) mmol/l, respectively. There was no statistical difference in concentrations between muscle and adipose tissue ISF. However, the total glucose levels estimated for ISF of muscle and adipose tissue were significantly lower than those measured in arterialized plasma \((5.56 \pm 0.13\) mmol/l, \(P < 0.01\)). Thus, in these subjects, a significant glucose gradient between plasma and ISF of muscle and adipose tissue was observed in the fasting state (Fig. 2B).
Glucose TTR. Figures 3A and 4A show the time courses of the glucose TTR in plasma and ISF of muscle and adipose tissue. As can be seen, the glucose TTR measured in plasma increased to a peak of 0.118 ± 0.021 at 2.5 min after start of infusion, fell for ~45 min to a level of 0.055 ± 0.004, and remained at this level until the end of the experiment. In the muscle ISF, the glucose TTR increased gradually during the first ~20 min of infusion and then reached a steady-state level comparable to that seen in plasma (Fig. 3A). A similar pattern of change in the glucose TTR was observed in the ISF of adipose tissue (Fig. 4A). The marked dynamic differences between the glucose TTR in plasma and those in the ISF of the two tissues suggest that, at ~20 min after the start of the tracer glucose infusion, the plasma and the interstitial tracer glucose pools attained their exchange equilibrium.

Tracer glucose concentrations and kinetic analysis. As can be seen in Figs. 3B and 4B, the tracer glucose concentration in plasma rose to a peak of 0.67 ± 0.13 mmol/l by 2.5 min after start of infusion, declined for ~45 min, and then remained at a steady-state level of 0.29 ± 0.03 mmol/l. In comparison, interstitial tracer glucose concentrations in both muscle and adipose tissue gradually increased during the first ~20 min of tracer infusion. After this period, tracer glucose concentrations in muscle and adipose tissue ISF paralleled those seen in plasma. During the final hour of the study, the tracer glucose concentrations in muscle and adipose tissue ISF averaged 0.19 ± 0.04 and 0.20 ± 0.02 mmol/l, respectively. In each subject, tracer glucose profiles in plasma and ISF were used to identify the parameters of the capillary-tissue exchange model (Fig. 1). Tables 1 and 2 list the individual parameters derived from the observed exchange kinetics in muscle and adipose tissue, respectively. Parameter CVs as determined by Monte Carlo analysis ranged from 9 to 16%. Thus parameters of the proposed model were estimated with a relatively high degree of precision. Average model fits for tracer glucose kinetics observed in muscle and adipose tissue ISF are shown in Figs. 3B and 4B, respectively. As can be seen, interstitial tracer glucose kinetics in both tissues were well described by the capillary-tissue exchange model. The value of the model parameter $p_2$ derived from adipose tissue data was similar to the value of $p_2$ derived from muscle data (0.120 ± 0.015 vs. 0.110 ± 0.012 min$^{-1}$, $P > 0.57$). When the values of $p_2$ are used, the 95% equilibrium
times ($T_{95\%}$) for skeletal muscle and adipose tissue were calculated to be 28.6 ± 3.2 and 26.8 ± 3.6 min, respectively. As was the case with parameter $p_2$, the value of $p_1$ derived from muscle data did not differ from the value identified from adipose tissue data (0.063 ± 0.007 vs. 0.081 ± 0.012 min$^{-1}$; $P > 0.31$). By taking the values of $p_1$ and $p_2$, the steady-state ratios of ISF to plasma glucose can be calculated to be 0.58 ± 0.06 and 0.66 ± 0.03 for skeletal muscle and adipose tissue, respectively. There was no significant difference in the ratios between the two tissue beds ($P > 0.38$).

**DISCUSSION**

The objective of this investigation was to assess transcapillary glucose exchange efficiency in human skeletal muscle and adipose tissue under basal conditions. For this reason, we frequently sampled plasma as well as muscle and adipose tissue ISF during intravenous [6,6-3H$_2$]glucose infusion in fasting, nonobese humans. The ISF sampling from the two tissues was accomplished by applying the open-flow microperfusion technique (40). To achieve a relatively high time resolution with this technique, a perfusion flow rate of 2 μl/min was employed. At this high flow rate, the mixing between the perfusate and the ISF surrounding the probe is not complete. Therefore, to determine the extent of perfusate-ISF mixing in the probe effluents (i.e., recovery), a constant intravenous infusion of the extracellular marker mannitol was initiated 120 min before the beginning of tracer infusion and was continued throughout the experiment. In this way, a constant plasma mannitol level was established during the plasma and ISF sampling period (Fig. 2A). Because steady-state concentrations of mannitol are similar in plasma and ISF of muscle and adipose tissue (see METHODS), the observed effluent/plasma ratios of mannitol were taken as estimates of substrate recovery in the collected probe effluents. From the substrate recovery and glucose values in the probe effluents, we were then able to determine the interstitial glucose concentrations in muscle and adipose tissue. We found that, in the fasting state, the glucose levels in the ISF of both adipose tissue and skeletal muscle are ~60% of arterIALIZED plasma levels (Fig. 2B). This result suggests that a significant glucose concentration gradient between plasma and ISF of muscle and adipose tissue exists in the fasting state.

In a recent microperfusion study, by employing different calibration techniques, we found similar glucose concentrations in adipose tissue ISF in normal subjects under fasting conditions (40). Furthermore, some other investigators have measured interstitial glucose levels in human adipose tissue using microdialysis (26, 29, 32, 41), ultrafiltration (41), and/or an equilibration technique (41) and have also reported glucose levels in the ISF to be significantly lower than those in plasma, with levels ranging from 50 to 75% of arterialized plasma values. However, contrary to these results, a number of studies using microdialysis in normal subjects have estimated the basal interstitial glucose concentrations in adipose tissue to be 85–100% of arterialized plasma levels (4, 18, 24, 28, 39, 47, 50). Concerning the human skeletal muscle, previous studies have employed the microdialysis technique to measure glucose levels in the muscle ISF and have found fasting interstitial glucose concentrations to be equal to (25, 26, 30, 31) or higher (85–100% of arterIALIZED plasma values (7, 15, 28, 32, 39)) than those measured in the present microperfusion study. Although spatial heterogeneity in the interstitial glucose levels within a studied tissue bed (e.g., due to local variations in blood flow) cannot be excluded, it is reasonable to suspect that technical and procedural details can account for the different findings. For example, Hamrin et al. (14) have reported that the hydrostatic pressure applied to the perfusate of the microdialysis catheter influences the

### Table 1. Model parameters obtained by analysis of tracer glucose kinetics in skeletal muscle ISF

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>$\text{p}_1$, min$^{-1}$</th>
<th>$\text{p}_2$, min$^{-1}$</th>
<th>$r$</th>
<th>$T_{95%}$, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.087(11)</td>
<td>0.127(16)</td>
<td>0.68(6)</td>
<td>23.6(16)</td>
</tr>
<tr>
<td>2</td>
<td>0.070(12)</td>
<td>0.146(14)</td>
<td>0.48(5)</td>
<td>20.6(14)</td>
</tr>
<tr>
<td>3</td>
<td>0.060(9)</td>
<td>0.081(13)</td>
<td>0.74(5)</td>
<td>36.9(13)</td>
</tr>
<tr>
<td>4</td>
<td>0.052(9)</td>
<td>0.084(12)</td>
<td>0.61(5)</td>
<td>35.5(12)</td>
</tr>
<tr>
<td>5</td>
<td>0.045(9)</td>
<td>0.114(13)</td>
<td>0.40(6)</td>
<td>26.3(13)</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.063(10) ± 0.007(1)</td>
<td>0.110(14) ± 0.012(1)</td>
<td>0.58 (5) ± 0.06(1)</td>
<td>28.6 (14) ± 3.2 (1)</td>
</tr>
</tbody>
</table>

Nos. in parentheses represent the precision of parameter estimate expressed as percent coefficient of variation. ISF, interstitial fluid. For definitions of $p_1$, $p_2$, and $r$, see METHODS.

### Table 2. Model parameters obtained by analysis of tracer glucose kinetics in adipose tissue ISF

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>$\text{p}_1$, min$^{-1}$</th>
<th>$\text{p}_2$, min$^{-1}$</th>
<th>$r$</th>
<th>$T_{95%}$, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.087(10)</td>
<td>0.141(14)</td>
<td>0.61(5)</td>
<td>21.3(14)</td>
</tr>
<tr>
<td>2</td>
<td>0.073(11)</td>
<td>0.109(15)</td>
<td>0.66(5)</td>
<td>27.5(15)</td>
</tr>
<tr>
<td>3</td>
<td>0.066(10)</td>
<td>0.108(12)</td>
<td>0.61(4)</td>
<td>27.8(12)</td>
</tr>
<tr>
<td>4</td>
<td>0.053(9)</td>
<td>0.076(13)</td>
<td>0.69(5)</td>
<td>39.2(13)</td>
</tr>
<tr>
<td>5</td>
<td>0.128(10)</td>
<td>0.164(12)</td>
<td>0.75(4)</td>
<td>18.3(12)</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.081 (10) ± 0.012 (1)</td>
<td>0.120 (13) ± 0.015 (1)</td>
<td>0.66 (5) ± 0.03 (1)</td>
<td>26.8 (13) ± 3.6 (1)</td>
</tr>
</tbody>
</table>

Nos. in parentheses represent the precision of parameter estimate expressed as percent coefficient of variation.

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fluid and substance transport across the microdialysis membrane. Hydrostatic pressure changes induced by varying the vertical position of the orifice of the microdialysis outlet tubing (i.e., below or above the inlet of the microdialysis catheter) resulted in significant changes in the dialysate substrate concentrations, as well as in substantial variations in the degree of perfusate losses (due to net perfusate flux across the membrane into the tissue). Thus the divergent findings of previous microdialysis studies may be partially attributable to different hydrostatic pressures applied to the perfusate of the microdialysis probes. Counteracting the hydrostatic pressure by adding osmotically active substances to the perfusate (e.g., dextran-70) may abolish this source of error (14).

In the present study, the initiation of the intravenous tracer glucose infusion in the primed-continuous format produced a rapid rise in the plasma tracer glucose concentration (Figs. 3B and 4B). After attaining peak values during the first 2.5 min of tracer infusion, plasma tracer concentration fell, first rapidly and then more slowly until, after ~45 min, the plasma level remained constant for the remainder of the experiments. In contrast to the rapid rise and fall with an early, sharp peak seen in the plasma tracer levels, the interstitial tracer concentration in both muscle and adipose tissue rose more slowly to a smooth peak at ~20 min after the tracer infusion was begun. Subsequently, the tracer glucose concentrations in ISF of muscle and adipose tissue paralleled those seen in plasma (Figs. 3B and 4B). Similar dynamic differences in the time course of the TTR between the plasma and ISF of muscle and adipose tissue were observed during the first ~20 min of the experiments (Figs. 3A and 4A). These dynamic differentials seen between the plasma and interstitial tracer levels, as well as the plasma and interstitial TTRs, strongly suggest that, after administration of a primed-continuous tracer glucose infusion, the process of tracer equilibration between plasma and ISF of adipose tissue and skeletal muscle takes ~20 min.

Previous studies in humans have shown that the disappearance of labeled glucose from plasma can be described by the sum of two or more exponential components (8, 11, 16, 17, 37, 43). The different exponential components of the tracer disappearance curve have then usually been interpreted as indicating diffusion from plasma into different anatomic fluid compartments, such as interstitial and intracellular fluids of various organs and tissues (17). For example, Ferrannini et al. (11) and Cobelli et al. (8) have studied in detail the plasma dynamics of injected labeled glucose in normal subjects, both in the fasting state and in the high-insulin euglycemic state. The authors found that, under both experimental conditions, the plasma disappearance curves of labeled glucose were best described by the sum of three exponentials, thus indicating the presence of two separate compartments exchanging glucose with the plasma compartment at two different rates. The initial rapid exponential decline observed during the first ~2 min after tracer injection was attributed to fast tracer diffusion from plasma into extravascular fluid spaces of liver, spleen, and endocrine, whereas the decay of the slower component observed during the first 20–30 min was interpreted as indicating slow diffusion into the extravascular fluid spaces of insulin-sensitive tissues, such as the interstitial space of skeletal muscle and adipose tissue (8, 11). These interpretations given by the authors are supported by the well-known facts that both the capillary permeability and blood supply are considerably lower in skeletal muscle and adipose tissue than in splanchic organs (9). Because of the technical difficulties in obtaining observational access to the ISF compartment of human skeletal muscle and adipose tissue, this physiological meaning assigned to the slower exponential component of the plasma tracer disappearance curve has not been previously verified in vivo. However, in the present study, by combining open-flow microperefusion with the mannitol calibration technique and a sensitive glucose-measuring method (GC-MS), we were able to assess interstitial glucose kinetics in muscle and adipose tissue with an acceptable time resolution. Our observations of marked dynamic differentials between plasma and interstitial tracer glucose in muscle and adipose tissue during the first ~20 min of intravenous tracer infusions (Figs. 3B and 4B) thus provide direct experimental evidence that the interstitial glucose pool of muscle and adipose tissue is indeed part of the postulated compartment that is in slow equilibrium with plasma glucose.

The constancy of the plasma tracer glucose concentration during the experiments (data not shown) and the similarity of the pattern of change in the tracer glucose concentration and TTR (Figs. 3 and 4) indicate that the endogenous glucose system was not perturbed from its steady state by the tracer infusion. This condition allowed us to apply a published capillary-tissue exchange model for the analysis of tracer glucose kinetics (Fig. 1). This model was first proposed by Johnson and Wilson (19) to describe the transcapillary exchange kinetics of nonmetabolizable tracers and was later modified by Watson (52) to include the effects of cell uptake for metabolizable tracers. Mathematical models of more complex form have been proposed for the description of capillary-tissue exchange. However, on the basis of fewer simplifying assumptions, these models contain many more adjustable parameters than can possibly be determined experimentally in humans (see Ref. 3 for review). The capillary-tissue exchange model used in the present study represents the interstitial space in terms of a well-mixed compartment (Fig. 1) and, thus, implicitly assumes that glucose diffusion in the ISF is an instantaneous process. The conception of the ISF of muscle and adipose tissue as single well-mixed compartments is a considerable simplification, but it may be justified by the fact that, in muscle and adipose tissue, the longest intercapillary diffusion distance is 30–50 μm (9), and that glucose diffusion over such a distance is very rapid [within a few seconds (9, 21)]. Also implicit in the model is the assumption that the capillary transit time of an ele-
ment of blood is short compared with time constants of the tracer equilibration between blood and ISF (19, 52). This assumption seems to be appropriate for the mathematical treatment of the transcapillary glucose exchange process in muscle and adipose tissue, because the capillary transit time is on the order of 1–2 s (9), and the observed 95% equilibrium time of the interstitial glucose is on the order of 30 min in these tissues (Tables 1 and 2; Figs. 3 and 4). The model further assumes that tracer is phosphorylated as rapidly as it is translocated into the cell, so that back flux of tracer from the cell is negligible. This latter assumption appears to be justified in our experimental situation, because myocytes and adipocytes may have very little or no free intracellular glucose under basal euglycemic conditions (6, 7, 12, 20).

To perform the kinetic analysis, the interstitial tracer glucose concentrations were fitted with the capillary-tissue exchange model by using the plasma tracer profile as an input. As can be seen in Figs. 3B and 4B, the interstitial tracer glucose kinetics in both muscle and adipose tissue were well described by the model, and, as indicated by the low CV values, the model parameters were estimated with a relatively high degree of precision (Tables 1 and 2). To obtain a measure of the transcapillary glucose exchange efficiency in skeletal muscle and adipose tissue, the 95% equilibrium time (T95%) was calculated from the identified model parameters. We found that the value of T95% is 28.6 ± 3.2 min for skeletal muscle and 26.8 ± 3.6 min for adipose tissue. There was no significant difference in the T95% value between the two tissues (P = 0.60). The mean values of T95% estimated in the present study are lower than those found by Steil et al. (48) and Rebrin and Steil (35), who analyzed the hindlimb lymph kinetics of labeled glucose in the dog under basal and hyperinsulinemic conditions. These authors observed a T95% value of 36.9 ± 7.2 min in the basal state and 40.4 ± 6.9 min in the hyperinsulinemic state (T95% is related to the reported T95% as T95% = –ln(0.05) T63% (35)). Because hindlimb lymph fluid is primarily derived from the ISF of skeletal muscle (48), the T95% values observed in this previous lymph study may therefore largely reflect the transcapillary glucose exchange efficiency of skeletal muscle in dogs. In a more recent study, Rebrin et al. (36) have measured interstitial glucose kinetics by means of a glucose sensor implanted in the subcutaneous tissue of the dog and have observed T95% values (range 10–37 min) that are on the average similar to those derived in our study. Furthermore, in a previous review article, Crone and Levitt (9) provided a summary of permeability-surface area product (PS) values that had been obtained by various investigators using different measurement methods. From the PS values, Crone and Levitt calculated T95% values for skeletal muscle and adipose tissue. These T95% values (range 9–30 min) are on the average lower than those obtained in the present and previous (36, 48) in vivo studies. However, as pointed out by Crone and Levitt, the values of T95% were calculated with the assumption of permeability-limited exchange (i.e., no influence of the blood flow), and thus may not reflect the actual in vivo situation, where both the capillary permeability and the prevailing blood flow determine the transcapillary glucose exchange rates.

In summary, our results obtained using open-flow microperfusion demonstrate that, in the fasting state, the glucose levels in the ISF of human skeletal muscle and adipose tissue are ~60% of arterialized plasma levels. Furthermore, the mathematical analysis of the interstitial tracer glucose kinetics measured in muscle and adipose tissue during intravenous tracer infusions indicates that the 95% equilibrium time for the two tissues is ~28 min. Thus the observed ISF-to-plasma glucose gradients and derived 95% equilibrium times provide strong evidence that transcapillary exchange of glucose is limited in human skeletal muscle and adipose tissue. The interstitial glucose pool of muscle and adipose tissue may therefore be considered as part of a compartment that is in relatively slow equilibrium with plasma glucose.

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DISCLOSURES

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REFERENCES

5. Box GEP, Hunter WG, and Hunter JS. Statistics for Experi-


