Measurement of interstitial albumin in human skeletal muscle and adipose tissue by open-flow microperfusion

MARTIN ELLMERER,1,2 LUKAS SCHAUPP,1,2 GERNOT A. BRUNNER,1
GERALD SENDLHOFER,1 ANDREA WUTTE,1 PAUL WACH,2 AND THOMAS R. PIEBER1
1Department of Internal Medicine, Diabetes and Metabolism, Karl Franzens University Graz, Auenbruggerplatz 15, A-8036 Graz; and 2Department of Biophysics, Institute of Electro- and Biomedical Engineering, University of Technology Graz, Inffeldgasse 18, A-8010 Graz, Austria

Ellmerer, Martin, Lukas Schaupp, Gernot A. Brunner, Gerald Sendlhofer, Andrea Wutte, Paul Wach, and Thomas R. Pieber. Measurement of interstitial albumin in human skeletal muscle and adipose tissue by open-flow microperfusion. Am. J. Physiol. Endocrinol. Metab. 278: E352–E356, 2000.—The absolute concentration of albumin was measured in the interstitial fluid of subcutaneous adipose tissue and skeletal muscle in six healthy volunteers by combining the method of open-flow microperfusion and the no-net-flux calibration technique. By use of open-flow microperfusion, four macroscopically perforated double lumen catheters were inserted into the tissue regions of interest and constantly perfused. Across the macroscopic perforations of the catheters interstitial fluid was partially recovered in the perfusion fluid. Catheters were perfused with five solutions, each containing different concentrations of albumin. Absolute interstitial albumin concentrations were calculated by applying linear regression analysis to perfusate vs. sampled albumin concentration (no-net-flux calibration technique). Interstitial albumin concentrations were significantly lower (P < 0.0001) in adipose tissue (7.36 g/l; r = 0.99, P < 0.0003; range: 4.3–10.7 g/l) and in skeletal muscle (13.25 g/l; r = 0.99, P < 0.0012; range: 9.7 to 15.7 g/l) compared with the serum concentration (48.9 ± 0.7 g/l, mean ± SE, n = 6; range: 46.4–50.4 g/l). Furthermore, interstitial albumin concentrations were significantly higher in skeletal muscle compared with adipose tissue (P < 0.01). The study indicates that open-flow microperfusion allows stable sampling of macromolecules from the interstitial space of peripheral tissue compartments. Moreover, the present data report for the first time in healthy humans in vivo the true albumin concentrations of interstitial fluid of adipose tissue and skeletal muscle.

ALBUMIN IS THE MAJOR PLASMA protein synthesized by the liver. The main functions of albumin are 1) to maintain osmotic pressure, 2) to act as a transport mechanism (e.g., free fatty acids), and 3) to serve as a temporary amino acid storage site. Approximately 60% of total body albumin is in the extravascular compartment of muscle and adipose tissue, and serum albumin exchanges with this pool (17, 21). The vascular compartment contains a much higher concentration of albumin than lymph or interstitial fluid, suggesting that there is a greater restriction on transvascular movement for albumin than for water or small solutes (24). However, few reports are available on interstitial fluid protein contents, and the mechanisms responsible for a redistribution of plasma albumin from the extravascular space of adipose tissue and skeletal muscle to the vascular compartment are still not well understood.

Interstitial fluid albumin concentration, total protein content, and colloid osmotic pressure have been measured in excised skeletal muscle tissue (20) in extracellular fluid sampled by the wick technique (1, 26), the blister suction technique (10), and micropuncture (5). Measurements have been made mostly in rat skeletal muscle (2, 11, 14, 18), in rat skin (1, 15), and in pigs (5, 13). Interstitial albumin concentrations were measured in humans by Rothschild et al. (20) in excised skeletal muscle and by Haeverstad et al. (10) in a skinfold of the leg. Interstitial albumin concentrations, as reported in the above-mentioned studies, varied in a wide range between 21 and 63% of the plasma concentration. To the best of our knowledge, measurement of albumin in the interstitial fluid of adipose tissue or skeletal muscle has not been performed before in healthy humans in vivo.

Originally, the method of open-flow microperfusion was developed to gain access to the interstitial fluid compartment for monitoring subcutaneous glucose concentration as an alternative to currently available home blood glucose meters (23, 25). In open-flow microperfusion a double lumen catheter with a macroscopically perforated outer tubing is set into the tissue region of interest and constantly perfused. The concentration of a substance in the perfusate sampled by using open-flow microperfusion is not a direct measure...
of interstitial concentration. The degree of equilibration (recovery rate) between the interstitial fluid and the perfusate is primarily dependent on the perfusion flow and the exchange area of the catheter and must be considered to obtain absolute concentrations. The no-net-flux method is a well-established calibration technique applied by Lönnroth et al. (16) for the microdialysis technique and by Kramer et al. (15) for the wick technique (wicks loaded with diluted serum) to calculate the absolute concentration of a substance in the interstitial fluid. Using open-flow microperfusion we applied this calibration technique in recent studies to estimate the absolute concentrations of glucose (22) and lactate (7) in the interstitial fluid of subcutaneous adipose tissue.

The aim of the present study was 1) to demonstrate that open-flow microperfusion allows sampling of macromolecules from the interstitial fluid of peripheral tissues, and 2) to measure the absolute albumin concentrations of the interstitial fluid of adipose tissue and skeletal muscle in healthy humans in vivo.

METHODS

Subjects. Six healthy male subjects (26.7 ± 4.5 yr; mean ± SD) participated in the study after giving their informed consent. They were all of normal weight (24.2 ± 2.0 kg/m²; mean ± SD) and none were taking any medication at the time of the study. The study was approved by the local ethics committee of the University of Graz. The subjects were investigated at 0730 after an overnight fast while supine at a room temperature of 21°C.

Open-flow microperfusion. The open-flow microperfusion technique has been described in detail previously (7, 22). The system consists of a perfusate reservoir (10 ml perfusate bag, Technoflex, Bidart, France), a conventional intravenous cannula (18 gauge; 50 × 1.2 mm diam; Angiocath, Becton-Dickinson Sandy, UT), a peristaltic pump (Minipuls 3, Gilson, Villier-le-Bel, France), and vials collecting the perfusate (PCR tube-fluor 0.2 ml, Biorheology, Oldendorf, Germany). The intravenous cannula was perforated with 80 holes (0.5 mm diam) using an Excimer Laser (LPX 205i, Lambda Physik, Gottingen, Germany) and was placed in the tissue of interest using a steel mandrel. After catheter insertion the steel mandrel was replaced with Teflon (OD, 0.76 mm; ID, 0.3 mm; PTFE tubing, Cole-Parmer Instrument, Vernon Hills, IL) and Tygon tubing (OD, 2.01 mm; ID, 0.25 mm; Tygon Autoanalysis Tubing, Cole-Parmer Instrument), which were connected to the peristaltic pump. The perfusate bag, the perforated catheter, and the tubing system were sterilized by gas sterilization (formaldehyde) at 60°C. Figure 1 represents a schematic view of the double lumen catheter. The exchange of substances between the interstitial fluid and the perfusate takes place via the macroscopic perforations of the outer tubing of the double lumen catheter.

In the present study, four double lumen catheters were placed in each subject. After local skin anesthesia, two catheters were placed in the adipose tissue of the abdominal region, and two catheters were placed in the tissue of the rectus femoris muscle. In each tissue region, catheters were placed ≈30 mm apart. The correct position of the catheter in the skeletal muscle was controlled in an experiment by using magnetic resonance spectroscopy (data not shown). All catheters were perfused at a constant flow of 0.5 µl/min, and samples were obtained in 30-min intervals. Perfusion of the catheter was started immediately after catheter insertion, but sampling started ≥1 h later to avoid contamination of the samples due to local tissue trauma (7). During the experiments, the amount of fluid collected was determined by weighing the sample vials before and after collection.

No-net-flux calibration protocol. The no-net-flux technique is based on the principle that perfusates containing different concentrations of the substance of interest above and below the expected interstitial fluid concentration are exposed to the exchange mechanism between interstitial fluid and perfusate. Perfusates loaded with lower concentrations than interstitial fluid concentration become concentrated, whereas perfusates loaded with higher concentrations become diluted. When linear regression analysis is applied to perfusate vs. sampled minus-perfusate concentration, an equilibrium point can be estimated where no-net-flux, (i.e., neither concentration nor dilution between the interstitial fluid and the perfusate) occurs. This equilibrium point represents the absolute interstitial concentration of the substance of interest. The slope of the regression line represents the recovery rate of the extracellular fluid in the perfusate (16).

Study protocol. Subjects arrived at the laboratory at 0730 and were investigated during fasting conditions in supine position. An intravenous cannula was inserted into a cubital vein to obtain venous blood samples. Two double lumen catheters were inserted into the adipose tissue, and two catheters were inserted into the skeletal muscle (see Open-flow microperfusion). Before starting the study, 10 ml of blood were withdrawn, coagulated, and centrifuged. The serum of this 10-ml sample from each subject was used to add endogenous albumin to the perfusate. Each catheter was perfused with Krebs-Ringer solution with 2 mmol/l glucose (without albumin), and afterward with two different dilutions of the subject’s serum with Krebs-Ringer solution. Each tissue region (two catheters) was perfused with five different dilutions of serum with Krebs-Ringer solution (1:3, 1:4, 1:6, 1:12, and Krebs-Ringer undiluted). Different levels of concentration were perfused in randomized order in each experiment. Each level of concentration was sampled for a period of 150 min at intervals of 30 min (five samples of 15 µl). The albumin concentrations of samples 1 and 5 of a concentration step were not considered for analysis of the no-net-flux data. The albumin concentrations of samples 2, 3, and 4 were averaged for further analysis. The entire experiment lasted 8.5 h (60-min equilibration period, 3 × 150-min concentration levels). Serum, perfusate, and sampled interstitial fluid albumin concentrations were determined immediately after the experiments (see next section).

Absolute interstitial albumin concentrations were calculated in three different ways. First, to get an indication of the comparability of two catheters placed in the same tissue...
Table 1. Albumin concentration and recovery rate for albumin in the interstitial fluid of adipose tissue and skeletal muscle calculated by linear regression analysis of the individual no-net-flux data.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Adipose Tissue</th>
<th>Skeletal Muscle</th>
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<tbody>
<tr>
<td></td>
<td>Alb*</td>
<td>r</td>
</tr>
<tr>
<td>1</td>
<td>9.5</td>
<td>0.99</td>
</tr>
<tr>
<td>2</td>
<td>6.6</td>
<td>0.95</td>
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<td>3</td>
<td>4.3</td>
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<td>4</td>
<td>5.3</td>
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<tr>
<td>5</td>
<td>10.7</td>
<td>0.95</td>
</tr>
<tr>
<td>6</td>
<td>8.1</td>
<td>0.90</td>
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Recovery rate is calculated as the slope of the regression line of the individual experiments.

*Alb* = Albumin concentration (g/l); †recovery rate for albumin (%).

region, for each catheter both the recovery rate (i.e., slope of the linear regression line) and the single catheter albumin concentration were calculated by applying linear regression analysis. Second, the individual albumin concentration of each tissue region (Table 1) was calculated by applying linear regression analysis to the no-net-flux data obtained from one tissue region (two catheters of the same tissue were regarded as one no-net-flux data set, i.e., one regression plot, Table 1). Third, the mean interstitial albumin concentration was calculated by applying linear regression analysis to the mean values of the five concentration levels (Fig. 2). Corresponding to the interstitial fluid samples, three serum samples were withdrawn during each level of concentration.

Probe handling and analysis. To prevent evaporation of the sampled interstitial fluid, the sample vials were covered and cooled on ice. To determine the exact sample volume and to control the average flow of the perfusate solution, the sample vials were weighed before and after sampling. The samples were stored at −70°C and analyzed after the experiment. Serum, perfusate, and interstitial albumin concentrations were determined photometrically, using a Cobas Integra 700 Laboratory Analyzer (Basel, Switzerland). The analyzer was adapted for the used interstitial sample vials. Calculation of absolute concentrations from the no-net-flux data was performed by applying linear regression analysis. Student’s paired t-test was used to compare recovery rates and interstitial albumin concentrations within an investigated tissue region and to compare serum, adipose tissue, and skeletal muscle albumin concentrations. All diagrams and statistical analyses were performed using Micro Cal Origin (technical graphics and data analysis, Microcal Software, Northampton, MA).

RESULTS

Serum albumin concentrations were constant during the whole study period. Mean concentration was 48.9 ± 0.7 g/l (mean ± SE; range: 46.4–50.4 g/l). Mean interstitial albumin concentrations were 7.36 g/l (r = 0.99, P < 0.0003) and 13.25 g/l (r = 0.99, P < 0.0012) for adipose tissue and skeletal muscle, respectively (Fig. 3). The mean interstitial fluid-to-serum ratio was 0.15 for adipose tissue and 0.27 for skeletal muscle, respectively. The individual interstitial albumin concentrations and the corresponding recovery rates are depicted in Table 1. Interstitial albumin concentrations were not significantly different when mean single catheter albumin concentrations were compared within group (adipose: 7.42 ± 1.01 g/l vs. 7.65 ± 0.93 g/l, P = 0.38; muscle: 13.03 ± 0.95 g/l vs. 13.49 ± 1.13 g/l, P = 0.49; mean ± SE, n = 6). Interstitial albumin concentrations were significantly higher in skeletal muscle compared with adipose tissue (P < 0.01). Mean recovery rates were 21.4 ± 3.4 and 32.3 ± 2.8% (mean ± SE; n = 6) for adipose tissue and skeletal muscle, respectively (P < 0.01). Within the two catheters of a single investigated tissue region the recovery rates were not significantly different (adipose: 19.7 ± 4.1 vs. 20.5 ± 3.4%, P = 0.85 and muscle: 32.1 ± 4.0 vs. 33.3 ± 5.1%, P = 0.72; mean ± SE, n = 6). The duration for the albumin concentration in the probe effluent and in the perfusate is given in Fig. 2 and is presented separately for each level of perfusate concentration.

DISCUSSION

In the present study it was demonstrated that open-flow microperfusion allows stable sampling of macromol-
molecules from the interstitial fluid of peripheral tissue compartments. Combining open-flow microperfusion and the no-net-flux calibration technique, we estimated for the first time in humans in vivo the absolute albumin concentration in the interstitial fluid of adipose tissue and skeletal muscle. The results suggest that the interstitial albumin concentration is significantly lower both in both adipose tissue (15% of serum) and skeletal muscle (27% of serum) compared with the serum concentration.

The method of open-flow microperfusion is giving direct access to the extracellular space of peripheral tissue compartments. The macroscopic perforations of the double lumen catheter responsible for the exchange mechanism between the perfusate fluid and the interstitial fluid surrounding the catheter do not restrict the passage of macromolecules and therefore allow the sampling of large molecules (e.g., proteins) from the interstitial fluid compartment (6). Due to the fact that the inner tubing of the double lumen catheter is slightly shorter than the perforated outer tubing and due to the continuous and equable fluid flow through the catheter, the cells of the investigated tissue region are not directly drained by the perfusate fluid. A recent study investigated the ion status, the lactic dehydrogenase concentration, and the contamination of the sampled perfusate with blood (8). The results of this study suggest that the catheter as constructed for the open-flow microperfusion technique causes only minor and transient damage to the tissue surrounding the catheter, and that using open-flow microperfusion enables the true interstitial fluid compartment to be investigated.

To estimate the absolute albumin concentration in the interstitial fluid of subcutaneous adipose tissue and skeletal muscle, we decided to combine the method of open-flow microperfusion and the no-net-flux calibration technique using a modified protocol compared with previous studies (7, 22). First, to obtain an expedient indicator for the stability of the sampling technique, each perfusate concentration was applied for an extended period of 150 min and sampled at intervals of 30 min (Fig. 2). Second, to consider the risk of drainage of albumin from the investigated tissue region, as was observed in a previous study, (6) the catheters were perfused at a lower perfusion flow (0.5 µl/min). Preliminary experiments (data not shown) indicated stable sampling of interstitial albumin during steady-state conditions at a perfusate flow of 0.5 µl/min and for a period of ≥6 h. Third, to attain a higher physiological correspondence between the interstitial fluid and the perfusate, and to avoid any possible risk of infections, we applied the approach of Kramer et al. (15), who used diluted serum to obtain different protein concentrations.

The comparison of recovery rates resulted in a good correspondence between catheters placed in the same tissue region. This finding is an indicator of high reproducibility of the open-flow microperfusion technique. As the different perfusates of each catheter were applied in randomized order, this result also counts as a reliable indicator of the stability of the sampling technique. On the basis of the finding of the high reproducibility of the open-flow microperfusion technique, it was possible to calculate the albumin concentration and the recovery rate of a single tissue region, regarding the results of two different catheters as a single no-net-flux data set (Table 1). For future studies this approach paves the way for the frequent measurement of absolute substrate concentrations by perfusing at least two catheters in one tissue region with different substrate concentrations in parallel (frequent no-net-flux technique).

Another finding of the present study was the lower recovery rate in adipose tissue compared with skeletal muscle. In a recent study, Rosdahl et al. (19) found a similar effect by applying the microdialysis technique. They reported a negative correlation between skinfold thickness and the recovery rate of glucose and urea in adipose tissue. They also found a markedly lower recovery rate for glucose and urea in adipose tissue compared with that in skeletal muscle. In agreement with Rosdahl et al., we suggest that these phenomena are an effect of the different diffusivity of substances in the tissues due to the different cell size and cell structure of the individual investigated tissue region.

A primary function of proteins is to cause colloid osmotic pressure at the capillary membrane. Albumin is the major plasma protein, contributing to ∼75% of the plasma colloid osmotic pressure content (9). The reason for the osmotic pressure across the capillary membrane is that plasma proteins leak only slightly through the capillary pores into the interstitial spaces. Backtransport of proteins from interstitial fluid to plasma is accomplished mainly by the lymphatic system. On the basis of these principles, which prevent an increase of the interstitial protein content, plasma protein concentration is ∼3.5 times that of the fluid outside the capillaries. Several studies have been done to investigate interstitial albumin concentration. Most of the reported results were higher compared with the results of the present experiments. Measurements of albumin in rat skeletal muscle ranged between 33 and 55% of the plasma concentration. Forty-two percent of plasma albumin was found by Creese et al. (5) in the fluid of guinea pig skeletal muscle collected by micro-puncture using glass capillary tubes.

Only a few results are available on humans, and it is questionable whether the interstitial protein content is similar in animals and humans, or whether the methods used are reliable enough to investigate the true interstitial fluid compartment. Methodological questions concerning the method of open-flow microperfusion have been discussed in detail previously (8). An indication of the reliability of the present findings is the good correspondence between our results from skeletal muscle (27% of plasma albumin) and the results of a very early study of Rothschild et al. (20), who measured the albumin concentration in excised skeletal muscle from humans and found concentrations between 21 and 35% of the albumin concentration in plasma. In addition, the present results report for the first time a clear...
difference between the interstitial albumin concentration of adipose tissue and skeletal muscle (15 and 27%, respectively, of serum albumin). The reason for this difference remains unclear. The literature gives an indication of the importance of skeletal muscle on whole body albumin content. The findings of Auckland and Reed (3), Baumgartner et al. (4), and Heilig and Pette (12) clearly indicate that albumin turnover is dependent on the metabolic activity of skeletal muscle, and that albumin concentration is dependent on skeletal muscle mass. However, this does not necessarily explain the present observation of a significantly higher albumin concentration in skeletal muscle compared with that in adipose tissue. Further investigations are necessary to gain physiological understanding of the present results.

In conclusion, this study indicates that open-flow microperfusion allows a stable and highly reproducible sampling of macromolecules from the interstitial space of peripheral tissue compartments in healthy humans in vivo. This finding opens a new path in clinical research, e.g., frequent measurement of hormones at in vivo. This finding opens a new path in clinical research, e.g., frequent measurement of hormones at.

REFERENCES