Journal of Physiology (1993), **471**, *pp.* 637–657
With 5 figures
Printed in Great Britain

INTERSTITIAL GLUCOSE AND LACTATE BALANCE IN HUMAN SKELETAL MUSCLE AND ADIPOSE TISSUE STUDIED BY MICRODIALYSIS

BY H. ROSDAHL, U. UNGERSTEDT*, L. JORFELDT† AND J. HENRIKSSON

*From the Departments of Physiology III and *Pharmacology, Karolinska Institutet and the †Department of Clinical Physiology, Thoracic Clinics, Karolinska Sjukhuset, Stockholm, Sweden*

(*Received 23 April 1992*)

SUMMARY

1. Microdialysis was used to gain insight into the substrate exchanges in the interstitial space of skeletal muscle and adipose tissue. Probes were inserted in the quadriceps femoris muscle and para-umbilical subcutaneous adipose tissue of thirteen subjects and microdialysis was performed at different flow rates (1–4 μl min^{-1}) and during changes in tissue blood flow.

2. When ethanol (5 mM) is included in the perfusion solution, the ethanol clearance from the probe is a measure of tissue blood flow. Blood flow changes induced by adenosine or vasopressin perfusion, by exercise or by circulatory occlusion resulted in ethanol clearance values of 69–139 % of the basal level. The ethanol clearance was higher in skeletal muscle than in adipose tissue (32–62 %, $P < 0{\cdot}001$), a difference compatible with a higher blood flow in muscle tissue.

3. The fraction of the interstitial glucose concentration that was recovered with the microdialysis was similar in skeletal muscle (the absolute values being 1·70 $\pm$ 0·14 mM at 1 μl min^{-1} and 0·59 $\pm$ 0·05 mM at 4 μl min^{-1}) and adipose tissue (1·89 $\pm$ 0·20 mM at 1 μl min^{-1}; 0·54 $\pm$ 0·05 mM at 4 μl min^{-1}) and correlated inversely with the tissue ethanol clearance, both in the basal state and during changes in tissue blood flow (muscle: $r = -0{\cdot}56$ to $-0{\cdot}67$; adipose tissue $r = -0{\cdot}72$ to $-0{\cdot}95$). Coefficients of variation were 6–8 % (glucose) and 11–16 % (lactate) and were similar during isometric exercise. The reproducibility of the technique (comparison of two contralateral probes; perfusion flow rate 4 μl min^{-1}) was 5·3–8·3 % (ethanol) and 23·9–20·8 % (glucose) in muscle ($n = 6$) and adipose tissue ($n = 4$) respectively.

4. The skeletal muscle dialysate lactate concentration (1 μl min^{-1}: 1·16 $\pm$ 0·2 mM) was higher than in adipose tissue (0·76 $\pm$ 0·08 mM, $P < 0{\cdot}05$), where the absolute amount of lactate that could be removed from the tissue (at 4 μl min^{-1}) was only half of that in skeletal muscle (0·8 $\pm$ 0·11 *vs.* 1·76 $\pm$ 0·23 nmol min^{-1}, $P < 0{\cdot}05$). The dialysate lactate level was not affected in either tissue by large changes in the interstitial glucose concentration indicating that in neither tissue is blood glucose a significant source of lactate formation.

5. The blood flow effects on the dialysate glucose concentration are the likely consequence of probe glucose drainage artificially shifting the balance between the

supply and consumption of interstitial glucose. The results are compatible with a lower blood flow, glucose uptake and lactate release in para-umbilical adipose tissue than in resting skeletal muscle.

INTRODUCTION

For the *in vivo* study of the metabolism of peripheral tissues, such as skeletal muscle or adipose tissue, experiments based on measurements of flow and arteriovenous differences are commonly used. These investigations may be complemented by *in vitro* models when more controlled conditions are necessary. The latter have serious limitations, however, due to the removal of the tissue from its normal physiological control. Therefore results must be verified by other techniques. Microdialysis is a method that seems promising in this respect, since it makes it possible to monitor as well as to manipulate the extracellular environment with the tissue in its normal location. In microdialysis (Delgado, DeFeudis, Roth, Ryugo & Mitraka, 1972; Ungerstedt & Pycock, 1974), a hollow probe with an inflow and outflow side, is introduced into the tissue and perfused with physiological fluid. While flowing through the probe, the fluid passes by a semipermeable membrane at a flow rate low enough to permit substrate exchange by dialysis over the membrane. The low flow rate furthermore ensures a low hydrostatic pressure and therefore that the fluid does not pass through the dialysis membrane to the tissue but continues to the outflow side of the probe and can be collected at the outlet. This has been verified in a large number of studies in the brain (for a detailed account, see Ungerstedt, 1991), and in adipose tissue and skeletal muscle of anaesthetized animals (Arner, Bolinder, Eliasson, Lundin & Ungerstedt, 1988; Hickner, Rosdahl, Borg, Ungerstedt, Jorfeldt & Henriksson, 1991; Ståhle, 1991) as well as in human adipose tissue (Lönnroth, Jansson & Smith, 1987; Bolinder, Hagström, Ungerstedt & Arner, 1989). Furthermore, by including ethanol (5 mM) in the perfusion solution tissue blood flow may be evaluated based on the clearance of ethanol from the probe into the tissue (Hickner *et al.* 1991). Due to its influence on the composition of the interstitial fluid, blood flow is an important variable in microdialysis studies (Hickner, Rosdahl, Borg, Ungerstedt, Jorfeldt & Henriksson, 1992).

One purpose of the present study was to investigate in healthy humans whether or not this technique would permit as stable monitoring of the interstitial space in resting and contracting skeletal muscle as has previously been reported in adipose tissue. The other main purpose was to use microdialysis to obtain information about the exchange of glucose and lactate in the interstitial space of skeletal muscle and adipose tissue by artificially influencing the balance between the supply and consumption of the interstitial pool of these compounds. To this end, dialysate glucose and lactate concentrations were determined at different microdialysis perfusion flow rates and during changes in tissue blood flow, as monitored by the microdialysis ethanol technique (Hickner *et al.* 1991).

METHODS

Subjects

Thirteen healthy subjects, seven men and six women, participated in the study. Nine subjects were moderately well trained and four were sedentary. The subjects' mean age, height and weight were 29·7 years (range 16–54), 174·1 cm (range 160–193) and 66·9 kg (range 50–82). The subjects

were given a detailed description of the study prior to giving their voluntary consent. The study was approved by the Ethical Committee of the Karolinska Institute.

Procedure for microdialysis probe insertion

In the skeletal muscle experiments, a local anaesthetic (0·5 ml lignocaine, 10 mg ml^{-1}; Astra, Södertälje, Sweden) was given subcutaneously and above the muscle fascia. Microdialysis probes were inserted bilaterally into the quadriceps femoris muscles (vastus lateralis), at 25 % of the distance from the superior margin of the patella to the anterior superior iliac spine. First a guide cannula was inserted (parallel to the fibre direction; 45 deg proximally and laterally) approximately 30 mm into the tissue from the point of penetration of the muscle fascia. Before insertion of the probe, the steel guide cannula was removed, leaving only a teflon guide tubing in the tissue, through which the microdialysis probe was gently inserted. The teflon part of the guide was removed by splicing upon retraction. In the adipose tissue experiments, 0·25 ml of lignocaine was given intradermally and the microdialysis probe was then inserted via the guide. The point of penetration of the skin was 60 mm lateral to the umbilicus; from this site the probe was then introduced 35 mm in a medial direction. To avoid bending of the probe membrane, the probes were retracted 3–5 mm before being fixed in their final position. The abdominal wall was covered during the experiment to prevent cooling. The microdialysis probes used in the experiments are described in detail in Fig. 1.

Microdialysis flow rates

The microdialysis flow rates used in the experiments were chosen to be the lowest possible that gave fluid volumes of enough size that could be sampled frequently. We reasoned that if it proved necessary to use microdialysis flow rates lower than 1 $\mu l\ min^{-1}$, the usefulness of the technique would be impaired. The flow rates used resulted in changes in the ethanol outflow/inflow ratio that were large compared to the precision of the ethanol analysis. The importance of taking this into consideration may be illustrated by the fact that no significant effect of vasopressin was detected on the ethanol outflow/inflow ratio in adipose tissue at 4 $\mu l\ min^{-1}$ (when the ethanol outflow/inflow ratio was close to maximal), whereas at 1 $\mu l\ min^{-1}$, a clear increase was noted.

Experimental

The subjects were told to eat a regular breakfast between 6 and 6.30 a.m. and did not ingest anything thereafter. The experiments were started at 8.00 a.m. and lasted 6–8 h. With the exception of the exercise experiments (see below), the subjects were investigated while sitting reclined, with their hips slightly bent, in a dentist's chair (room temperature 22 °C). The different types of experiment were performed as described below (protocols A–D).

Protocol A. Skeletal muscle: effect of different microdialysis flow rates and adenosine perfusion (7 probes in 5 subjects). After the microdialysis probe had been inserted, an equilibration period of 5–10 min elapsed before the dialysate was collected. The perfusion medium was Krebs–Henseleit buffer containing 5 mM ethanol (KHB–ethanol). The perfusion flow rates were 2 $\mu l\ min^{-1}$ (0–60 min), 1 $\mu l\ min^{-1}$ (60–120 min) and 4 $\mu l\ min^{-1}$ (120–180 min). In five of the probes (from 3 subjects), the perfusion medium was then replaced (at 4 $\mu l\ min^{-1}$) by a KHB–ethanol (5 mM) solution containing 515 μM adenosine. After 60 min of microdialysis perfusion with adenosine, the perfusion medium was switched back to the initial solution for 60 min. Microdialysis samples were collected in 10 min fractions during the basal and recovery periods and in 5 min fractions during the adenosine treatment period. Blood samples were obtained after 2·5 and 5 h of the experiment.

Protocol B. Skeletal muscle: effect of exercise and circulatory occlusion (microdialysis perfusion at 4 $\mu l\ min^{-1}$; 5 probes in 3 subjects). The subjects were investigated while sitting on a modified car seat and held securely to the seat by straps fastened around the waist. The knee angle of the subjects was fixed at 90 deg, with the ankle attached to a strain gauge. In the experimental set-up, the subjects could adjust their power output to 20 or 40 % of their maximal voluntary isometric contraction force. Before the exercise was started, the probe was perfused from 0 to 90 min with Krebs–Henseleit buffer (KHB) without ethanol. During the remainder of the experiment the probes were perfused with KHB containing 5 mM ethanol. From 180 to 240 min of the experiments, the subjects exercised with intermittent contractions (contractions of 5 s duration with 10 s of rest in between) for 30 min at each exercise intensity. Following exercise, the subjects rested for 1 h (240–300 min) while microdialysis samples were collected. Thereafter, a blood pressure tourniquet was attached around the proximal part of the left thigh muscle and

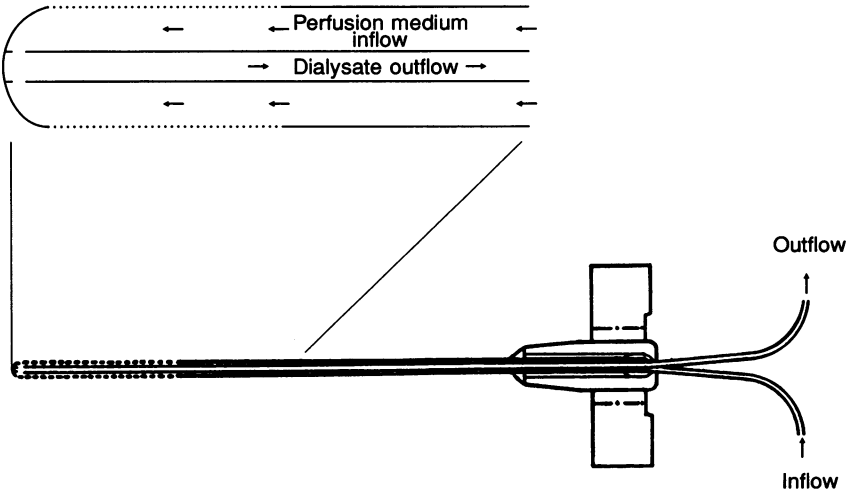

Fig. 1. A schematic drawing of the microdialysis probe. The probes consisted of inner and outer probe tubings as well as a polyamide dialysis membrane. One end of the dialysis membrane was glued to the distal end of the outer probe tubing, while the other end was sealed. The inner probe tubing, located inside the dialysis membrane and the outer probe tubing, was connected at the proximal end to the outlet tubing. The perfusion medium enters the probe through the inlet tubing and flows towards the dialysis membrane in the space between the outer probe tubing and the inner probe tubing. The perfusion medium enters the inner tubing at the distal end of the probe and flows in a retrograde direction to the outlet of the tubing where samples are collected. The dead volume in the tubing from the liquid connecting device to the outlet of the probe is 11·4 μl, and from the probe membrane to the probe outlet, 5 μl. These volumes were taken into account when determining the times for sample collection and changes of perfusion medium. Dimensions: inner probe tubing, length 70 mm, outer diameter 0·40 mm, inner diameter 0·125 mm; outer probe tubing, length 40 mm, outer diameter 0·85 mm, inner diameter 0·625 mm; dialysis membrane (Polyamide), length 20 mm, outer diameter 0·544 mm; inlet tubing, length 150 mm, outer diameter 1·0 mm, inner diameter 0·15 mm; outlet tubing, length 215 mm, outer diameter 1·0 mm, inner diameter 0·15 mm. The microdialysis probe was ethylene sterilized. The distal end of the outlet tubing was equipped with a cap, which fitted tightly on the collection vials; evaporation during sampling was thereby avoided. The inlet tubing is connected, via a connecting device, to a 5 ml sterile syringe (Asik, Denmark) placed in a high-precision syringe pump (CMA/100 microinjection pump, CMA Microdialysis AB, Stockholm, Sweden). In order to avoid air bubbles in the system, the perfusion solutions were kept at room temperature. The microdialysis probes used in the experiments were generously placed at our disposal by CMA Microdialysis-Research AB, Stockholm, Sweden. The performance of the microdialysis system during *in vivo* microdialysis in thigh muscle was checked by weighing sample vials on a high precision balance before and after sample collection. The values given are means $\pm$ S.E.M. of 3–5 probe averages based on six 10 min samples. With the pump set at 1, 2 and 4 μl min^{-1} we found 0·88 $\pm$ 0·03 μl min^{-1} ($n = 3$), 1·86 $\pm$ 0·05 μl min^{-1} ($n = 4$) and 3·74 $\pm$ 0·06 μl min^{-1} ($n = 5$) to be recovered at the probe outlet. With the pump syringes connected to plastic tubing only 1·01 $\pm$ 0·02 μl min^{-1} ($n = 3$); 2·00 $\pm$ 0·03 μl min^{-1} ($n = 4$) and 3·97 $\pm$ 0·04 μl min^{-1} ($n = 5$) were recovered. We do not know if this difference is due to the fact that the pump–syringe system was more loaded with the probes subjected to the tissue pressure *in vivo* or alternatively if a small portion of the inflowing perfusate was lost into the interstitium.

inflated to 200 mmHg, which pressure was maintained for 10 min. After a 5 min rest period the same procedure was carried out on the right thigh muscle of the subjects, followed by a recovery period of 30 min. Microdialysis samples were collected as indicated in the legend to Fig. 2. Blood samples were obtained before the exercise, after 10 and 25 min of exercise at each exercise intensity and 5 min before and 5 min after the circulatory occlusion period.

Protocol C. Adipose tissue: effect of different microdialysis flow rates and adenosine perfusion (8 probes in 5 subjects). The experiments were identical with those described for skeletal muscle in Protocol A. In two probes, the order of microdialysis perfusion was 4, 2 and 1 μl min^{-1}, but this gave results identical with those of the order normally used (2, 1 and 4 μl min^{-1}).

Protocol D. Adipose tissue: effect of vasopressin perfusion (microdialysis perfusion flow rate of 1 μl min^{-1}; 3 probes in 2 subjects). These experiments were performed in two of the subjects of protocol C. Following 180 min of different microdialysis perfusion flow rates (1 μl min^{-1} during the last hour, see above), the probes were perfused during 90 min with a perfusion medium consisting of KHB–ethanol with 4 IU ml^{-1} of vasopressin included. During the recovery period, the perfusion medium was switched back to KHB–ethanol without vasopressin for 60 min. Microdialysis samples were collected in 10 min fractions. Blood samples were obtained after 2·5 and 5 h.

Solutions

The standard perfusion solution was a sterile modified Krebs–Henseleit bicarbonate buffer (KHB) (Krebs & Henseleit, 1932), in which the bicarbonate was replaced by phosphate in order to achieve a stable pH (7·4) at room temperature. The buffer composition was (mM): 140 NaCl, 3·9 KCl, 2·5 $CaCl_2.2H_2O$, 1·2 $MgSO_4.7H_2O$, 0·8 $Na_2HPO_4.2H_2O$, 0·8 KH_2PO_4, adjusted to pH 7·4 using NaOH (Apoteksbolaget, Stockholm, Sweden). In order to prepare KHB–ethanol solutions with an ethanol concentration of 5 mM, 17·2 M sterile ethanol (Apoteksbolaget, Stockholm, Sweden) was used. KHB solutions containing 5 mM ethanol and 515 μM adenosine or 4 IU ml^{-1} vasopressin were prepared from a 19·8 M sterile adenosine solution (Apoteksbolaget, Stockholm, Sweden) or a 20 IU ml^{-1} vasopressin solution (Postacton, Ferring AB, Malmö, Sweden).

Blood samples

Fingertip blood samples were obtained from a prewarmed hand. After lancing of the fingertip, 3×50 μl of blood were collected and deproteinized by mixing with an equal volume of 3 M perchloric acid, left at room temperature for 15 min and centrifuged at 3000 r.p.m. for 10 min, after which the supernatant was stored at 4 °C for subsequent analysis.

Analyses

All dialysate samples were collected in 300 μl polyethylene vials (Milian, Geneva, Switzerland), capped immediately and stored at + 4 °C until analysed. The glucose and lactate analyses were made according to standard enzymatic fluorometric procedures (Lowry & Passonneau, 1972) within 2 days of the experiment. Analyses of ethanol were made within 1 day of the experiment. Ethanol analyses were performed using a modification of the procedure described by Bernt & Gutmann (1974). Briefly, 2 or 4 μl of sample was added to 1 ml of reagent mixture consisting of glycine–hydrazine buffer at pH 8·9 (74·6 mM sodium pyrophosphate, 22·0 mM glycine, 50·0 mM hydrazine) and 0·54 mM NAD^+. Samples were read fluorometrically against a standard curve before and after the addition of 50 μl of enzyme (1·7 mg alcohol dehydrogenase (195 units mg^{-1} solid) in 22 mM ammonium sulphate buffer). In a storage test performed before the study, we found the ethanol concentration to be correct if the vials (10 μl sample) were kept at + 4 °C for 1 day until analysed (before, 5·67 $\pm$ 0·03 mM; after 1 day, 5·65 $\pm$ 0·12 mM, $n = 8$). In contrast, we found many samples to have decreased ethanol concentrations following 1 week of storage at + 4 °C or when samples were stored at −20 or −80 °C overnight or for a week. Unless stated otherwise, chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA) or from Boehringer Mannheim GmbH Biochemica (Mannheim, FRG).

Statistics and calculations

All results are given as means $\pm$ S.E.M. The ethanol results are given as the outflow/inflow ratio: $[\text{ethanol}]_{\text{collected dialysate}}/[\text{ethanol}]_{\text{infused perfusion medium}}$. Statistical analyses were made using the Mann–Whitney rank-sum test for non-paired data (differences between muscle and adipose tissue) or by one-way analysis of variance (effects of blood flow changes). When required, the analysis of variance was followed by the Newman–Keuls *post hoc* test. Regression analysis was performed according to standard procedures.

RESULTS

Effects of different perfusion flow rates (Table 1)

In the basal period the ethanol outflow/inflow ratio was significantly higher in adipose tissue than in skeletal muscle at all perfusion flow rates ($P < 0.001$). In skeletal muscle the ethanol outflow/inflow ratio increased linearly with increasing microdialysis flow rate, but in adipose tissue the increase was clearly lower between 2–4 μl min^{-1} than between 1–2 μl min^{-1}. On the other hand, the dialysate glucose concentrations did not differ in the two tissues. The highest values, at 1 μl min^{-1}, were 36–38 % of the simultaneously determined blood glucose values, which were 4.95 ± 0.13 mM in the skeletal muscle experiments and 5.02 ± 0.06 mM in the adipose tissue experiments (n.s.). Generally, there was a negative correlation between the dialysate glucose concentration and the ethanol outflow/inflow ratio both in muscle and adipose tissue (Fig. 2). In these correlations, each value is the mean for one probe (based on four samples), i.e. the same values as in Table 1, complemented with three additional experiments for 4 μl min^{-1}. In both tissues,

TABLE 1. Effect of different microdialysis perfusion flow rates

	Flow 1 μl min^{-1}		Flow 2 μl min^{-1}		Flow 4 μl min^{-1}	
	Skeletal muscle	Adipose tissue	Skeletal muscle	Adipose tissue	Skeletal muscle	Adipose tissue
Ethanol (o/i ratio)	0·13±0·016	0·34±0·030	0·33±0·037	0·57±0·029	0·58±0·033	0·74±0·017
Glucose (mM)	1·70±0·143	1·89±0·196	0·99±0·128	1·04±0·158	0·59±0·053	0·54±0·050
Lactate (mM)	1·16±0·201	0·76±0·082	0·63±0·100	0·51±0·059	0·44±0·057	0·20±0·027

Influence of different microdialysis flow rates on the ethanol outflow/inflow ratio, and on dialysate glucose and lactate concentrations in skeletal muscle and adipose tissue. Probes were perfused during 60 min at each flow rate and samples collected every 10 min. In order to avoid impure samples in connection with the change in dialysis flow rate, the first and the last 10 min sample from each flow rate was excluded when calculating the means of each probe. Values given are means ± S.E.M. for 7 probes in skeletal muscle and 8 probes in adipose tissue. Whole blood glucose and lactate values, simultaneously determined, were 4.95 ± 0.13 mM and 1.02 ± 0.046 mM in the muscle experiments and 5.02 ± 0.06 mM and 1.09 ± 0.012 mM in the adipose tissue experiments.

the slopes and Y-intercept decreased with increasing microdialysis perfusion flow rate. In neither tissue was there a significant correlation between the dialysate lactate concentration and the ethanol outflow/inflow ratio, or between the dialysate lactate and glucose concentrations.

At the lowest perfusion flow rate (1 μl min^{-1}) in skeletal muscle, the dialysate lactate concentration was higher than the blood lactate concentration in four out of six experiments, although average values were not significantly different (1.16 ± 0.20 *vs.* 1.02 ± 0.046 mM, $n = 6$, n.s.). On the contrary, the adipose tissue dialysate lactate concentration at 1 μl min^{-1} was significantly lower than the blood glucose

concentration (0·759 ± 0·086 *vs.* 1·09 ± 0·012 mM, $n = 8$, $P < 0·05$). In four additional experiments at a perfusion rate of 0·5 μl min^{-1} and with probe membrane length of 30 mm (on two separate subjects) the muscle dialysate lactate concentration was 1·78 ± 0·13 mM (blood lactate concentration 0·67 ± 0·01 mM). In these experiments

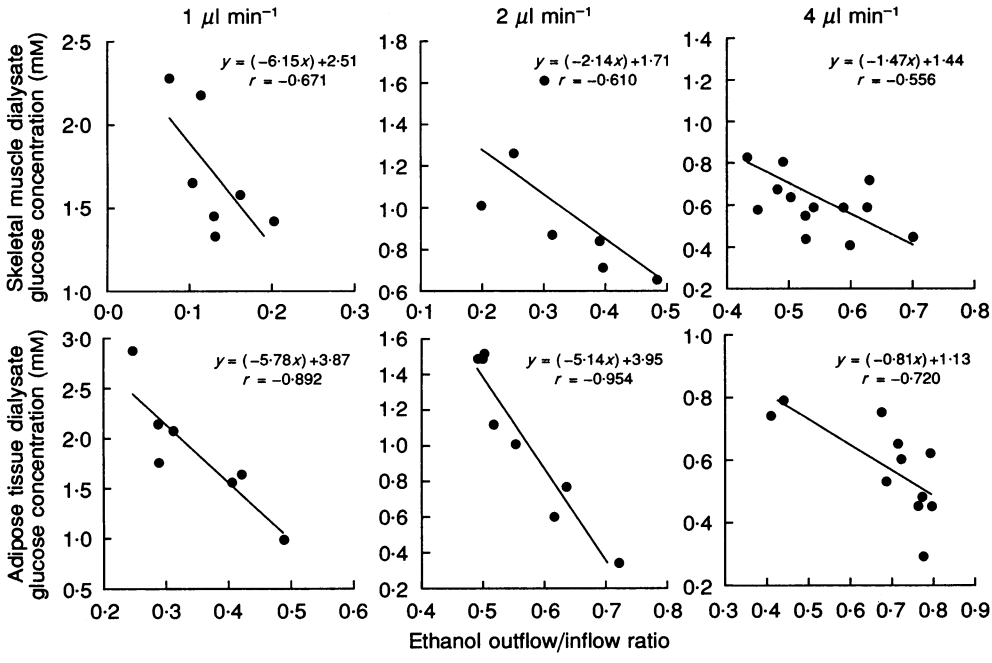

Fig. 2. Correlation between the dialysate glucose concentration and the ethanol outflow/inflow ratio (the inverse marker of blood flow). Probes were inserted in the thigh muscle and the para-umbilical subcutaneous adipose tissue of 8 subjects and perfused at 1, 2 or 4 μl min^{-1} with Krebs–Henseleit buffer containing 5 mM ethanol. Each dot is the mean for 1 probe (based on four 10 min samples).

also the adipose tissue lactate concentration (1·01 ± 0·06 mM) was higher than in blood, but probably equal to plasma. The amount of glucose or lactate removed from the tissue per unit time increased significantly with higher microdialysis perfusion flow rates in muscle, but not in adipose tissue. Thus, in skeletal muscle, the amount of glucose removed by microdialysis increased from 1·70 nmol min^{-1} at 1 μl min^{-1} to 2·36 nmol min^{-1} at 4 μl min^{-1} ($P < 0·05$). The corresponding values for adipose tissue were 1·89 to 2·16 nmol min^{-1} (n.s.). These modest changes can be compared to the marked effect of the dialysis flow rate on the amount of glucose removed by microdialysis *in vitro* with the probe immersed in a 5 mM glucose solution (1 μl min^{-1}, 3·1 nmol min^{-1}; 3 μl min^{-1}, 7·5 nmol min^{-1}). With respect to lactate, there was a clearly different pattern in adipose tissue than in skeletal muscle. While in muscle 51 % more lactate (1·76 *vs.* 1·16 nmol min^{-1}, $P < 0·01$) was removed from the tissue at 4 than at 1 μl min^{-1}, no corresponding difference was detected in adipose tissue (0·80

vs. 0·76 nmol min^{-1}, n.s.). In contrast to these glucose and lactate values, the absolute amount of ethanol cleared from the probe into the tissue increased in both skeletal muscle and adipose tissue with increasing microdialysis perfusion flow rate (muscle at 1 and 4 μl min^{-1}: 4·35 and 8·4 nmol min^{-1}, $P < 0{\cdot}01$; adipose tissue at 1 and 4 μl min^{-1}: 3·3 and 5·2 nmol min^{-1}, $P < 0{\cdot}01$).

Within-subject variation for skeletal muscle

Six subjects had probes perfused simultaneously (at 4 μl min^{-1}) in the right and the left thigh muscles. The within-subject variation was calculated from the differences between the two legs in the basal periods of adenosine and exercise experiments (4 consecutive 10 min samples). The coefficients of variation of a single value were: ethanol outflow/inflow ratio, 5·3 %; glucose, 23·9 % and lactate, 19·9 %. In addition, the coefficients of variation were calculated for each probe from the repeated sampling during the basal periods before the adenosine (5 consecutive 10 min samples) and exercise experiments (8 consecutive 10 min samples) as well as during the exercise experiments (5 consecutive 5 min samples at each exercise intensity). Average values for the adenosine experiments (5 probes) were: ethanol outflow/inflow ratio, 4·7 ± 0·9 %; glucose, 7·7 ± 1·2 %; lactate, 15·1 ± 3·2 % and, for the exercise experiments (5 probes): ethanol outflow/inflow ratio, 4·5 ± 0·3 %; glucose, 8·3 ± 1·6 %; lactate, 16·5 ± 5·6 %. With the two levels of exercise the coefficients of variation were: ethanol outflow/inflow ratio, 8·2 ± 1·1 and 9·2 ± 2·3 %; glucose, 7·0 ± 0·9 and 6·1 ± 1·2 %; lactate, 10·3 ± 1·5 and 9·2 ± 1·6 %. The constancy of microdialysis results was tested in a pilot study at a perfusion speed of 0·5 μl min^{-1} (30 mm dialysis membrane) with sampling each 15 min. The average glucose value of four probes was 4·22 ± 0·28 mM during the first hour and 3·91 ± 0·14 mM (n.s.) during the fifth hour.

Within-subject variation for adipose tissue

Four subjects had probes perfused simultaneously (at 1, 2 and 4 μl min^{-1}) in the subcutaneous adipose tissue on the left and the right side of the umbilicus. The coefficients of variation of a single value were: ethanol outflow/inflow ratio, 21·6 % (1 μl min^{-1}), 10·6 % (2 μl min^{-1}) and 8·3 % (4 μl min^{-1}); glucose, 16·4 % (1 μl min^{-1}), 20·3 % (2 μl min^{-1}) and 20·8 % (4 μl min^{-1}); and lactate, 16·5 % (2 μl min^{-1}) and 36·1 % (4 μl min^{-1}). The coefficients of variation, as calculated from the basal periods of the adenosine experiments (7 probes), were: ethanol outflow/inflow ratio, 3·1 ± 0·5 %; glucose 5·7 ± 1·1 % and lactate 8·3 ± 2·0 %. With 0·5 μl min^{-1} perfusion (as in muscle, see above), the average glucose value of 5 probes was 3·9 ± 0·4 mM during the first hour and 4·3 ± 0·4 mM during the fifth hour.

Effects of increased blood flow in skeletal muscle induced by adenosine perfusion (Fig. 3A and B; 5 probes in 3 subjects)

Ethanol outflow/inflow ratio

When adenosine (515 μM) was included in the microdialysis perfusion medium (at 4 μl min^{-1}) the ethanol outflow/inflow ratio decreased from 0·525 ± 0·029 (during basal conditions) to 0·401 ± 0·033 (to 75·7 % of the basal level) ($P < 0{\cdot}001$). During

the 60 min recovery period the ethanol outflow/inflow ratio rapidly returned to normal (0·517 ± 0·027).

Glucose

The glucose concentration in the collected dialysates, which averaged 0·59 ± 0·06 mM during the basal period increased, on an average, 21 % with adenosine perfusion ($P < 0·001$) and returned to the basal level when adenosine perfusion was terminated (0·58 ± 0·06 mM). The correlation coefficients in this experiment between the dialysate glucose concentration and the ethanol outflow/inflow ratio ($n = 18$) were in the different probes $r = 0·423$ ($P < 0·1$), $r = 0·525$ ($P < 0·05$), $r = 0·538$ ($P < 0·05$), $r = 0·736$ ($P < 0·001$) and $r = 0·747$ ($P < 0·001$). The blood glucose concentration was constant during the experiment, being 5·11 ± 0·11 mM at 2·5 h and 4·95 ± 0·08 mM at 5 h (n.s.).

Lactate

No significant changes in the dialysate lactate concentration occurred during adenosine perfusion, the values averaging 0·41 ± 0·08 mM in the basal period, 0·39 ± 0·07 mM during adenosine perfusion (n.s.) and 0·43 ± 0·07 mM during recovery (n.s.). Blood lactate values were constant during the experiment: 0·90 ± 0·16 mM at 2·5 h and 1·06 ± 0·15 mM at 5 h of the experiment (n.s). There was no significant correlation between the dialysate lactate concentration and that of glucose or the ethanol outflow/inflow ratio.

Effects of circulatory occlusion in skeletal muscle induced by external compression (Fig. 3C and D; 5 probes in 3 subjects)

Ethanol outflow/inflow ratio

The ethanol outflow/inflow ratio before the start of the circulatory occlusion period was 0·405 ± 0·012. This value increased to 0·486 ± 0·013 ($P < 0·001$) during the circulatory occlusion period (an increase of 20 %), while there was no change in the contralateral leg (0·416 ± 0·013). Following this period, the ethanol outflow/inflow ratio returned to close to basal values within 60 min.

Glucose

The glucose concentration of the collected dialysates averaged 0·86 ± 0·09 mM initially and decreased during circulatory occlusion to 0·47 ± 0·05 mM (to 54·7 % of initial values) ($P < 0·01$). The correlation coefficient of the dialysate glucose concentration and the ethanol outflow/inflow ratio (based on all 12 samples collected in this experiment) were in the different probes $r = 0·818$ ($P < 0·01$), $r = 0·797$ ($P < 0·01$), $r = 0·595$ ($P < 0·05$), $r = 0·792$ ($P < 0·01$), and $r = 0·063$ (n.s.). During the circulatory occlusion period, the dialysate glucose concentration in the contralateral leg was 0·77 ± 0·123 mM (n.s. as compared to the pre-occlusion basal period). As with ethanol, the dialysate glucose concentration returned to the basal value within 60 min following the circulatory occlusion. There was no change in the blood glucose concentration as a result of circulatory occlusion (before occlusion, 5·30 mM; during occlusion, 5·14 mM).

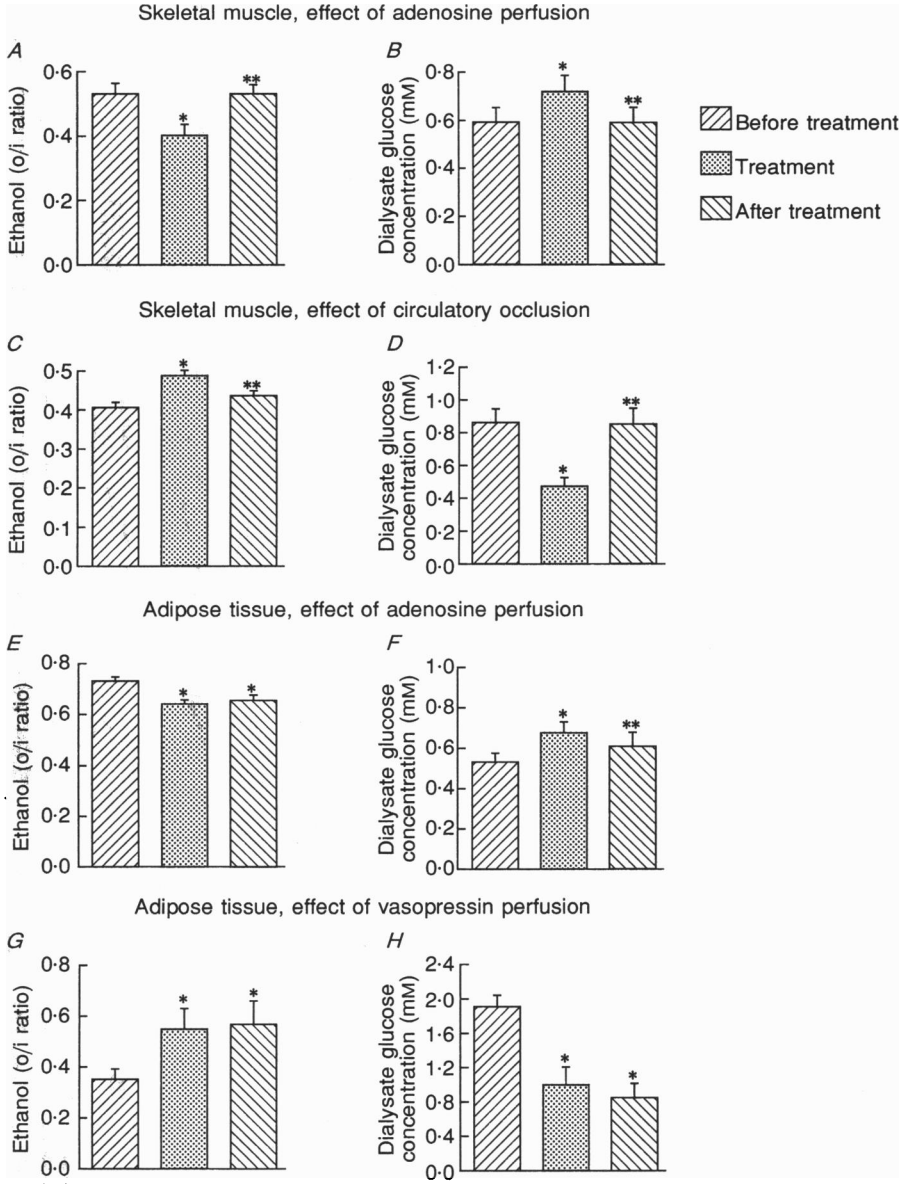

Fig. 3. Effects of blood flow changes on the ethanol outflow/inflow (o/i) ratio and on the dialysate glucose concentrations in skeletal muscle and adipose tissue. Increases in blood flow were induced by microdialysis perfusion with 0·5 mM adenosine, whereas blood flow reductions were induced in muscle by attaching a blood pressure tourniquet (inflated to 200 mmHg) around the proximal part of the thigh and in adipose tissue by microdialysis perfusion with vasopressin (4 IU ml^{-1}). *A* and *B*, 5 probes were perfused at 4 $\mu l\ min^{-1}$ with Krebs–Henseleit buffer (KHB) containing 5 mM ethanol during the basal (0–60 min) and the recovery (120–180 min) periods, and with adenosine included in the perfusion solution during the treatment period (60–120 min). Samples were collected in 10 min fractions during the basal and recovery periods and in 5 min fractions during the treatment period. Values are from 5 consecutive samples in the basal and recovery periods and from 3 consecutive samples in the treatment period. *C* and *D*, 5 probes were perfused at 4 $\mu l\ min^{-1}$ with KHB–ethanol. Dialysate samples were collected in 2 min fractions during the 10 min

Effects of increased blood flow in adipose tissue induced by adenosine perfusion (Fig. 3E and F; 7 probes in 4 subjects)

Ethanol outflow/inflow ratio

Similar to the effect in skeletal muscle, microdialysis perfusion with 515 μM adenosine resulted in a decreased ethanol outflow/inflow ratio (basal, 0·729 ± 0·016; adenosine, 0·641 ± 0·012, $P < 0·001$). Compared to skeletal muscle, the effect in adipose tissue appeared later and the ethanol outflow/inflow ratio was not significantly decreased until the 20–30 min sample following the start of adenosine infusion. The effect tended to be lower than in skeletal muscle, but remained significantly longer (during the entire 60 min recovery period).

Glucose

As in skeletal muscle, the glucose concentration in adipose tissue dialysates increased with adenosine perfusion (basal, 0·51 ± 0·04 mM; adenosine, 0·66 ± 0·05 mM, $P < 0·001$). This increase (28 %) was similar to that recorded in skeletal muscle (21 %). In contrast to the ethanol outflow/inflow ratio, which remained low in the period following adenosine perfusion, the dialysate glucose concentration gradually normalized during the 60 min recovery period (last 10 min sample 0·50 ± 0·09 mM). As for the ethanol data, there was a delayed effect of adenosine on the dialysate glucose concentration compared to its immediate effect on skeletal muscle. The coefficient of correlation between the dialysate glucose concentration and the ethanol outflow/inflow ratio ($n = 20$) was in the different probes, $r = -0·02$ (n.s.), $r = -0·631$ ($P < 0·01$), $r = -0·656$ ($P < 0·001$), $r = -0·724$ ($P < 0·01$), $r = -0·743$ ($P < 0·001$) and $r = -0·897$ ($P < 0·001$). The blood glucose concentration was constant during the experiment, being 5·06 ± 0·14 mM at 2·5 h and 5·03 ± 0·06 mM at 5 h.

Lactate

As in skeletal muscle, there was no significant effect of adenosine on the adipose tissue dialysate lactate concentration (basal period, 0·23 ± 0·03 mM; adenosine perfusion, 0·25 ± 0·03 mM). No significant changes in blood lactate concentration occurred during the experiment: at 2·5 h, 1·04 ± 0·02 mM; at 5 h 1·08 ± 0·05 mM. There was no significant correlation between the changes in the dialysate lactate concentration or those of glucose and the ethanol outflow/inflow ratio.

---

of circulatory occlusion, in 10 min fractions during the 60 min basal period and in 5 min fractions during the 30 min recovery period. Values are from 3 consecutive samples in each period. This experiment was performed immediately after the exercise experiment (following 240 min in Fig. 4), thus explaining the low ethanol and high glucose values before treatment. *E* and *F*, 7 probes in experiments identical to those in *A* and *B*. Values are from 3 consecutive samples in the basal period and 4 consecutive samples in the treatment and recovery periods. *G* and *H*, 3 probes were perfused at 1 μl min^{-1} with KHB–ethanol during the basal (0–60 min) and recovery (150–210 min) periods; during the treatment period (60–150 min) vasopressin was included in the perfusion solution. Samples were collected in 10 min fractions. Values are from 5 consecutive samples in the basal period, 6 consecutive samples in the treatment period and 4 consecutive samples in the recovery period. Values are means ± S.E.M. * and ** denote significant differences from before treatment and treatment periods, respectively ($P < 0·05$).

Effects of decreased blood flow in adipose tissue induced by vasopressin perfusion (Fig. 3G and H; 3 probes in 2 subjects)

Ethanol outflow/inflow ratio

The average basal value for the ethanol outflow/inflow ratio was $0{\cdot}345 \pm 0{\cdot}039$. When the perfusion medium was supplemented with 4 IU ml^{-1} of vasopressin, this value increased to $0{\cdot}545 \pm 0{\cdot}081$ ($P < 0{\cdot}001$) and remained at this level ($0{\cdot}562 \pm 0{\cdot}095$) during the 60 min recovery period following cessation of vasopressin perfusion.

Glucose

The dialysate glucose concentration in the basal period averaged $1{\cdot}90 \pm 0{\cdot}13$ mM (blood glucose concentration, $5{\cdot}07 \pm 0{\cdot}01$ mM). As a result of the vasopressin perfusion, this value decreased to 52·6 % of basal levels ($1{\cdot}01 \pm 0{\cdot}21$ mM, $P < 0{\cdot}001$) and remained at this level during the 60 min period following cessation of the vasopressin perfusion (at 44·5 % of the initial value). The dialysate ethanol outflow/inflow ratio and the dialysate glucose concentration correlated significantly, the correlation coefficient ($n = 21$) in the different probes being $-0{\cdot}847$ ($P < 0{\cdot}001$), $-0{\cdot}840$ ($P < 0{\cdot}001$) and $-0{\cdot}955$ ($P < 0{\cdot}001$).

Lactate

The dialysate lactate concentration in the basal period averaged $0{\cdot}90 \pm 0{\cdot}07$ mM (blood lactate concentration, $1{\cdot}18 \pm 0{\cdot}015$ mM). As a result of the vasopressin perfusion, this value increased by 22·2 % (to $1{\cdot}10 \pm 0{\cdot}11$ mM, $P < 0{\cdot}001$) and remained at this level during the 60 min period following cessation of the vasopressin perfusion. The dialysate lactate concentration correlated significantly with the ethanol outflow/inflow ratio ($n = 21$), the correlation coefficients in the different probes being $r = -0{\cdot}96$, $r = -0{\cdot}843$ and $r = -0{\cdot}848$ respectively ($P < 0{\cdot}05$). The increase in dialysate lactate concentration was not appreciably changed in two probes in which the dialysis perfusion medium was also supplemented with 5 mM glucose (no glucose, 1·04, 1·19 mM; glucose supplemented, 1·26, 1·20 mM).

Effects of increased blood flow in skeletal muscle induced by exercise (Fig. 4; n = 5 probes from 3 subjects)

Ethanol outflow/inflow ratio

In the basal state, the ethanol outflow/inflow ratio averaged $0{\cdot}500 \pm 0{\cdot}025$ and was decreased to $0{\cdot}366 \pm 0{\cdot}018$ (to 73·2 % of basal) and $0{\cdot}303 \pm 0{\cdot}019$ (to 60·6 % of basal) with intermittent isometric leg contractions at 20 and 40 % of the maximum voluntary contraction (MVC) force, respectively ($P < 0{\cdot}001$). The 60 min recovery period was not sufficient to restore the ethanol outflow/inflow to pre-exercise values.

Glucose

Although the blood glucose concentration remained constant during the exercise bouts (basal, $4{\cdot}98 \pm 0{\cdot}14$ mM; 20 % of MVC, $5{\cdot}11 \pm 0{\cdot}16$ mM; 40 % of MVC, $4{\cdot}99 \pm 0{\cdot}21$ mM), there were dramatic effects on the glucose concentration in the collected muscle dialysates (basal, $0{\cdot}75 \pm 0{\cdot}15$ mM; 20 % of MVC, $1{\cdot}20 \pm 0{\cdot}08$ mM; 40 % of MVC, $1{\cdot}57 \pm 0{\cdot}10$ mM). On average, there was a 64·4 % increase at 20 % of MVC

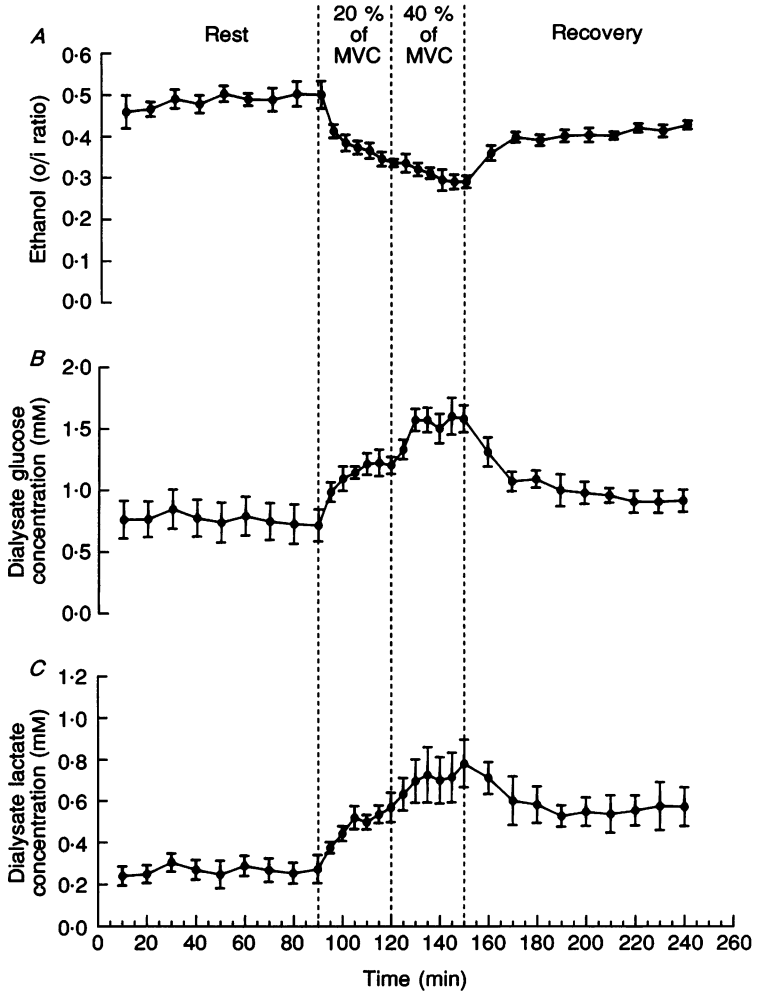

Fig. 4. Influence of exercise on the ethanol outflow/inflow ratio (*A*) and on the dialysate glucose (*B*) and lactate (*C*) concentrations. Subjects performed intermittent isometric thigh muscle contractions (5 s contractions with 10 s of rest) at 20 and 40 % of their maximal voluntary isometric contraction force. Microdialysis probes were placed in the thigh muscle and perfused at 4 μl min^{-1} with Krebs–Henseleit buffer containing 5 mM ethanol. After the 90 min basal period, the subjects exercised for 30 min at each exercise intensity. Dialysate samples were collected in 10 min fractions during the basal and recovery periods and in 5 min fractions during the exercise period. Values are means ± S.E.M. for 5 probes in 3 subjects. In this figure the average basal outflow/inflow ratio is lower and the dialysate glucose concentration is higher than in the other experiments. This is entirely explained by the 2 probes of 1 subject (o/i ratio, 0·44 and 0·45; dialysate glucose concentration, 1·20 and 0·99 mM).

and a 114 % increase with exercise at 40 % of MVC ($P < 0{\cdot}001$). The dialysate glucose concentration was highly correlated with the ethanol outflow/inflow ratio, the correlation coefficient ($n = 30$) in the different probes being $r = -0{\cdot}853$ ($P < 0{\cdot}001$), $r = -0{\cdot}886$ ($P < 0{\cdot}001$), $r = -0{\cdot}906$ ($P < 0{\cdot}001$), $r = -0{\cdot}925$ ($P < 0{\cdot}001$),

$r = -0{\cdot}925$ ($P < 0{\cdot}001$). As was the case with the ethanol outflow/inflow ratio, the dialysate glucose concentration was not restored to the pre-exercise value during the 60 min recovery period.

Lactate

The pre-exercise lactate concentration in the collected dialysates was $0{\cdot}27 \pm 0{\cdot}06$ mM. This may be compared with the blood lactate concentration of $1{\cdot}14 \pm 0{\cdot}29$ mM. At the two levels of exercise, the blood lactate concentration was only slightly changed to $1{\cdot}18 \pm 0{\cdot}20$ mM at 20 % and $1{\cdot}27 \pm 0{\cdot}10$ mM at 40 % of MVC. Simultaneously, the dialysate lactate concentration increased to $0{\cdot}52 \pm 0{\cdot}05$ and $0{\cdot}71 \pm 0{\cdot}12$ mM ($P < 0{\cdot}001$) and was still elevated 60 min following the termination of exercise.

DISCUSSION

The use of microdialysis in human skeletal muscle and adipose tissue

The use of microdialysis in humans, especially in human skeletal muscle, had been anticipated to involve additional problems compared to its use in the anaesthetized rat on which most previous work had been performed (see Introduction). For example, it was feared that voluntary or involuntary contractions might continually disturb diffusion conditions around the probe. However, the present results do not indicate a high degree of variability in microdialysis samples from skeletal muscle. During basal-period sampling, we found coefficients of variation of dialysate glucose and lactate concentrations of the order of 8–10 % for both skeletal muscle and abdominal adipose tissue, or around 20 % if the right and left sides were compared. Similar figures (9–10 %) were reported for the adipose tissue dialysate lactate concentration in a study in the rat over 2·5 h using another type of probe (Hagström, Arner, Ungerstedt & Bolinder, 1990). During the intermittent isometric contractions, the coefficients of variation for glucose and lactate were, in fact, somewhat lower than in the resting state. The present results thus demonstrate that microdialysis allows stable monitoring of the interstitial space in skeletal muscle, both at rest and during isometric contractions. Evidently some damage to muscle fibres and vessels cannot be avoided upon insertion of a microdialysis probe. In spite of this, the examples given below clearly show that the microdialysis technique is highly useful in detecting physiological responses. This may be explained by the fact that the damage is located to the cells and vessels in the immediate vicinity of the probe but that a large part of the tissue section mirrored by the probe is undamaged. In the present study vasoactive compounds administered via the probe gave the expected responses and we showed that the intensity-dependent lactate formation with exercise could be detected locally via the microdialysis probe, but not in the blood samples. In addition, when muscle blood flow was changed, the interstitial glucose concentration, as mirrored by the microdialysis samples, changed in a way which could be explained in physiological terms. In a parallel study with local insulin injections, we have seen that we can profoundly increase the cellular glucose uptake in the local area close to the probe, while the blood glucose concentration remains unchanged.

Figure 5 illustrates in a simplified manner the major compartments that influence the substance concentrations as 'seen' by the microdialysis probe located

in the interstitial space of skeletal muscle or adipose tissue. In the case of glucose, the supply is mediated by the blood flow and removal occurs via cellular glucose uptake and via drainage through the microdialysis probe. The transfer of glucose from capillary to tissue is known to be flow limited at resting blood flows (Crone &

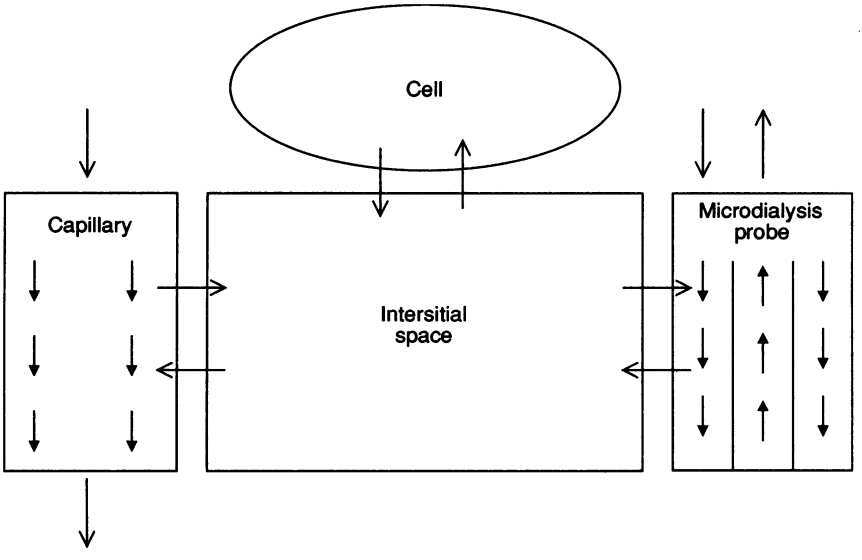

Fig. 5. A simplified illustration of the major compartments influencing the substance concentrations detected by the microdialysis probe located in the interstitial space of skeletal muscle or adipose tissue.

Levitt, 1984), and therefore small variations in the relationship between blood flow and glucose consumption will lead to corresponding variations in the interstitial glucose concentration. Assuming a resting blood flow of 2–4 ml $(100\ g)^{-1}\ min^{-1}$ in skeletal muscle and adipose tissue (Lassen, Lindbjerg & Munck, 1964; Lesser & Deutsch, 1967), the glucose supply is 10–20 nmol $(100\ mg)^{-1}\ min^{-1}$. Approximately 10 % of this supply is taken up by the muscle tissue (Crone & Levitt 1984; Wallberg-Henriksson, 1987) and significantly less by adipose tissue (Frayn, Coppack, Humphreys & Whyte, 1989). The volume of tissue drained by the microdialysis probe is unknown, but 1 mm around the probe is a reasonable guess, based on previous studies in the rat brain employing microelectrodes stereotactically positioned close to the microdialysis probe (Benveniste, Hansen & Ottosen, 1989) or on theoretical predictions (Bungay, Morrison & Dedrick, 1990). This would equal 100 mg of tissue, from which approximately 2 nmol of glucose are drained per minute (see Table 1) by the microdialysis probe in muscle and adipose tissue. Therefore, under the conditions of the present study, it is clearly possible that the probe drainage of glucose is not insignificant compared to the cellular glucose uptake and tissue supply. Everything else being constant, it is therefore not surprising that a change in blood flow dramatically affects the glucose concentration in the collected dialysate, as shown in Figs 3 and 4. This is in accordance with the finding in the rat that when the supply of a compound is arrested at death, the dialysate concentration

rapidly decreases (Ståhle, 1991). In the present experiments, the changes in the ethanol outflow/inflow ratio were accompanied by inverse changes in the dialysate glucose concentration. The only exception to this was found in the recovery period after the adenosine perfusion in adipose tissue. This might be explained by the known stimulatory effect of adenosine on adipocyte (but not on muscle) glucose uptake (Smith, Kuroda & Simpson, 1984). Although this inevitable supply–removal relationship involved in microdialysis may be looked upon as a drawback of the technique, it can also be advantageous since it makes it possible artificially to influence the balance between supply and consumption for a specific compound in the interstitial space. One example of this approach is given in the present study (see the last section of the Discussion).

The present data on ethanol clearance revealed that the outflow/inflow ratio is clearly lower in skeletal muscle than in adipose tissue at all dialysate flow rates (Table 1). In all probability, this indicates that the blood flow in the para-umbilical adipose tissue is lower than in the resting thigh muscle. The outflow/inflow ratio increased linearly with increasing microdialysis flow rate in skeletal muscle, but not in adipose tissue. This is explained by the higher outflow/inflow ratios recorded in adipose tissue than in skeletal muscle, leading to near maximal outflow/inflow ratios being reached at lower microdialysis flow rates. The solubility of ethanol is roughly ten times higher in water than in fat. This means that in both tissues, ethanol will move predominantly by diffusion via the interstitial water space and be rapidly removed by the capillaries. The data of Wozniak, Pert, Mele & Linnoila (1991), given in the following paragraph, support this notion. The radial diffusion of ethanol through cells would be higher in muscle, due to its higher water content, and this is likely to have contributed to the higher ethanol clearance in this tissue. However, the dialysate glucose concentration is similar in the two tissues, which may indicate that a lower blood flow in the subcutaneous adipose tissue, compared to skeletal muscle, is balanced by a lower basal glucose uptake. This notion is strongly supported by available estimations based on arteriovenous glucose determinations in adipose tissue and muscle (Wallberg-Henriksson, 1987; Frayn *et al.* 1989). Another difference between the two tissues was detected regarding the absolute amount of lactate removed by the dialysis probe. This variable increased progressively with increasing dialysis flow rates in muscle (although not as much as expected from *in vitro* results) but levelled off in adipose tissue at 1–2 μl min^{-1} at approximately half the muscle value. This may be an indication that a smaller lactate pool is available to the probe in adipose tissue than in muscle (the physiological implications of this are discussed in the last section).

The use of ethanol in monitoring skeletal muscle blood flow

In a previous study (Hickner *et al.* 1991), we showed, in the rat, that the clearance of ethanol from the probe into the tissue responds to blood flow fluctuations in the localized region around the microdialysis probe. Ethanol is not metabolized in muscle and adipose tissue (Lieber, 1977) and few have been able to demonstrate a direct effect of ethanol on local metabolism *in vivo*. When metabolic effects have been reported (Williams, 1965; Lange & Sobel, 1983; Cussó, Vernet, Cadefau & Urbano-Marquez, 1989), the results have been demonstrated only *in vitro* and in

preparations exposed to levels of ethanol in excess of 20 mM. In addition, the available data indicate that the present 5 mM ethanol perfusion at the most only minimally affects tissue blood flow (Fewings, Hanna, Walsh & Whelan, 1966). This is supported by our findings in the rat (Hickner *et al.* 1992) that the percentage ethanol clearance was identical whether 5 mM, 50 mM or 1 M ethanol was included in the perfusion medium. In the present study, muscle dialysate glucose concentration (at 4 μl min^{-1} perfusion flow rate) was 0·73 ± 0·14 mM before and 0·72 ± 0·12 mM after ethanol inclusion in the perfusion medium. In a recent study in which brain tissue was perfused with [^{14}C]ethanol, Wozniak *et al.* (1991) showed evidence of very rapid diffusion of ethanol through the tissue, accompanied by rapid and efficient removal via the vasculature. This resulted in low tissue ethanol concentrations; the mean ethanol concentration 0·5 mm from the probe being only 6 % of that in the perfusion medium. In addition the fraction of the perfused ethanol found in the brain tissue immediately on cessation of a 20 min perfusion was very small (0·88 %). In the present study the ethanol outflow/inflow ratio in human skeletal muscle and adipose tissue was found to respond readily to changes in blood flow induced by exercise or circulatory occlusion (muscle) and by pharmacological agents included in the microdialysis perfusion solution (muscle and adipose tissue). Some indication of the sensitivity of the ethanol technique may be obtained by comparing the difference between the very low blood flows during circulatory occlusion or vasopressin infusion and those recorded in the basal state with a blood flow of the order of 2–4 ml (100 g)$^{-1}$ min $^{-1}$ in skeletal muscle and adipose tissue (Lassen *et al.* 1964; Lesser & Deutsch, 1967). At present, the blood flow results obtained by ethanol perfusion are qualitative. However, an accompanying study (Hickner, Bone, Ungerstedt, Jorfeldt & Henriksson, 1993) clearly shows that the blood flow results obtained by the microdialysis ethanol technique closely matches those obtained with the ^{133}Xe wash-out technique.

In the present study, we found that, in the basal state, the ethanol outflow/inflow ratio correlated negatively with the dialysate glucose concentration. The slope of the regression lines decreased in both tissues with increasing microdialysis flow rates, i.e. a constant change in the ethanol outflow/inflow ratio had progressively less influence on the dialysate glucose concentration. In most subsequent experiments, we used a flow rate of 4 μl min^{-1}, i.e. the highest of the three flow rates where regression lines were obtained. In the 4 μl min^{-1} experiments, the variance of the dialysate glucose concentration during the basal period sampling was not significantly decreased when the values were corrected on the basis of the previously obtained glucose–ethanol relationship.

Other approaches to control recovery in microdialysis experiments

At the present microdialysis flow rates, the measured glucose concentrations in the collected dialysates are much lower than the actual concentrations in the interstitial fluid (i.e. the 'recovery' is much less than 100 %; Lönnroth *et al.* 1987; Henriksson, Rosdahl, Fuchi, Oshida & Ungerstedt, 1990). The low recovery conforms with all previous microdialysis studies and is due to the interplay between the removal of substances by the dialysis probe, as discussed in the first section of the Discussion, and the restrictions posed by the tissue diffusion coefficient,

to which tissue tortuosity and the volume fraction made up of interstitial fluid are important determinants (Nicholson & Phillips, 1981). Several 'internal standards' have been proposed for controlling recovery in microdialysis experiments (Alexander, Grothusen & Schwartzman, 1988; Larsson, 1991; Kurosawa, Hallström & Ungerstedt, 1991; Scheller & Kolb, 1991) and, in addition, several equations have been proposed to allow calculation of the actual *in vivo* concentration of a specific substance from the concentration in the collected dialysate (Jacobson, Sandberg & Hamberger, 1985; Lerma, Herranz, Herreras, Abraira & Martin del Rio, 1986; Amberg & Lindefors, 1989; Benveniste, Hansen & Ottosen, 1989; Morrison, Bungay, Hsiao, Ball, Mefford & Dedrick, 1991). It is evident that these techniques, in some instances, may be of great value. However, they generally do not account for the fact that the concentration of a substance in the tissue immediately surrounding the microdialysis probe is governed by a delicate balance between supply and removal of that substance, or in some instances, of its metabolic precursor. In addition, a few attempts have been made to monitor blood flow in the immediate region around a microdialysis probe. Van Wylen, Willis, Sodhi, Weiss, Lasley & Meutzer (1990) determined local cardiac blood flow by H_2 clearance via a platinum wire within a dialysis fibre, whereas P. A. Jansson, A. Larsson, U. Smith & P. Lönnroth (personal communication) used ^{133}Xe clearance measurements in the subcutaneous adipose tissue contralateral to the microdialysis sites.

Significance of the dialysate lactate determinations

In a recent microdialysis study, de Boer, Postema, Plijter-Groendijk & Korf (1991) estimated the rat muscle interstitial lactate concentration to be 1·9 mM, which was clearly higher than the estimated value in (central) venous plasma. The present muscle dialysate lactate concentration, at 1 μl min^{-1}, was significantly higher than in adipose tissue and generally higher than in venous whole blood, but probably lower than in plasma, as plasma lactate values are higher than the corresponding values in whole blood (+ 46 %, Stainsby & Eitzman, 1988). When the microdialysis flow rate was decreased to 0·5 μl min^{-1}, the muscle dialysate lactate concentration was, however, clearly higher than the estimated plasma value. Based on arteriovenous lactate concentration measurements, Yki-Järvinen, Bogardus & Foley (1990) found that, in the fasting state, skeletal muscle contributed to the plasma lactate concentration, but they produced evidence that after meals the plasma lactate concentration may be determined by the rate of glucose disposal in tissues other than muscle. Still, the microdialysis results regarding this problem must be used with caution, since it cannot be ruled out that the microdialysis probe may lead to falsely high resting lactate values, e.g. by causing glycolytic stimulation through damage to or irritation of the surrounding muscle cells.

Previously, it was shown that, provided that 2·5 mM glucose was included in the perfusion medium, the adipose tissue dialysate lactate concentration was also higher than in blood (Jansson, Smith & Lönnroth, 1990). On this basis, it was concluded that abdominal adipose tissue produces lactate both in the postabsorbtive state and after meals. The present microdialysis data provide further insight into this matter. Firstly, the present study shows that, although adipose tissue may be important as a lactate producer, it is of less importance,

quantitatively, than skeletal muscle. This can be stated since, with increasing microdialysis flow rates, the absolute amount of lactate which could be removed by the microdialysis probe levelled off at a flow rate of 1–2 μl min^{-1} in adipose tissue. At 4 μl min^{-1} the amount of lactate removed by the probe had not yet levelled off in skeletal muscle and was more than twice as high as in adipose tissue. Interestingly, the amount of lactate removed from adipose tissue levelled off at 0·8–1·0 nmol min^{-1}, which, with the probe draining 100 mg of tissue (as estimated in the first Discussion section) fits in well with the estimation by Jansson *et al.* (1990) that the adipose tissue lactate release is $\approx$ 1 nmol (100 mg)$^{-1}$ min^{-1}. In canine subcutaneous adipose tissue *in situ*, Fredholm (1971) showed that lactate was being released at arterial concentrations up to 1·7 mM. Another significant finding of the present study is that when the artificially low interstitial glucose concentration was increased with the adenosine-induced hyperaemia, skeletal muscle or adipose tissue dialysate lactate concentrations were not increased. This indicates that in neither tissue was blood glucose a quantitatively significant source of lactate formation and seems to be at odds with the finding by Jansson *et al.* (1990) that glucose must be included in the microdialysis perfusion medium to obtain normal lactate values. With tissue ischaemia, induced by vasopressin administration, the dialysate lactate concentration increased in adipose tissue even though the dialysate (and probably the interstitial) glucose concentration was almost halved. This may indicate that the extra lactate released from adipose tissue in this condition may be secondary to increased glycogenolysis, as has previously been shown during sympathetic nerve stimulation (Fredholm & Karlsson, 1970).

In conclusion, the present results demonstrate that the microdialysis technique permits chemical monitoring of the interstitial space in skeletal muscle both at rest and during isometric contractions. The ethanol technique may be used to detect variations in the nutritive tissue blood flow, which is often necessary in microdialysis experiments. The present data show that a change in blood flow dramatically affects the glucose concentration in the collected dialysate, a likely consequence of probe glucose drainage artificially shifting the balance between the supply and the consumption of interstitial glucose. The present microdialysis results are compatible with a lower blood flow, glucose consumption and lactate release in the abdominal adipose tissue than in resting skeletal muscle. Furthermore, the data indicate that skeletal muscle is a more significant contributor to the plasma lactate pool than adipose tissue and that in neither tissue is blood glucose a significant source of lactate formation.

This work was supported by the Swedish Medical Research Council (project B87-14X-07917), the Karolinska Institute, the Magnus Bergvall Foundation and the Research Council of the Swedish Sport Federation. We thank Georg Goertz for constructing the connecting device between inlet tubing and pump and for drawing Fig. 1, Margaret Hickner, Ann-Chatrine Samuelsson and Berit Sjöberg for technical assistance, and Robert Hickner, Anna-Karin Collin and Hans Karlsson for valuable help and fruitful discussions.

REFERENCES

ALEXANDER, G. M., GROTHUSEN, J. R. & SCHWARTZMAN, R. J. (1988). Flow dependent changes in the effective surface area of microdialysis probes. *Life Sciences* **43**, 595–601.

AMBERG, G. & LINDEFORS, N. (1989). Intracerebral microdialysis: II. Mathematical studies of diffusion kinetics. *Journal of Pharmacological Methods* **22**, 157–183.

ARNER, P., BOLINDER, J., ELIASSON, A., LUNDIN, A. & UNGERSTEDT, U. (1988). Microdialysis of adipose tissue and blood for *in vivo* lipolysis studies. *American Journal of Physiology* **255**, E737–742.

BENVENISTE, H., HANSEN, A. J. & OTTOSEN, N. S. (1989). Determination of brain interstitial concentrations by microdialysis. *Journal of Neurochemistry* **52**, 1741–1750.

BERNT, E. & GUTMANN, I. (1974). Determination with alcohol dehydrogenase and NAD. In *Methods of Enzymatic Analysis 3*, ed. BERGMEYER, H. U., pp. 1499–1505. Verlag Chemie GmbH, Weinheim, FRG.

BOLINDER, J., HAGSTRÖM, E., UNGERSTEDT, U. & ARNER, P. (1989). Microdialysis of subcutaneous adipose tissue in vivo for continous glucose monitoring in man. *Scandinavian Journal of Clinical and Laboratory Investigation* **49**, 465–474.

BUNGAY, P. M., MORRISON, P. F. & DEDRICK, R. L. (1990). Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sciences* **46**, 105–119.

CRONE, C. & LEVITT, D. G. (1984). Capillary permeability to small solutes. In *Handbook of Physiology*, section 2, *The Cardiovascular System*, vol. VI, *Microcirculation*, part 1, ed. RENKIN, E. M. & MICHEL, C. C., pp. 461–466. American Physiological Society, Bethesda, MD, USA.

CUSSÓ, R., VERNET, M., CADEFAU, J. & URBANO-MARQUEZ, A. (1989). Effects of ethanol and acetaldehyde on the enzymes of glycogen metabolism. *Alcohol and Alcoholism* **24**, 291–297.

DE BOER, J., POSTEMA, F., PLIJTER-GROENDIJK, H. & KORF, J. (1991). Continuous monitoring of extracellular lactate concentration by microdialysis lactography for the study of rat muscle metabolism in vivo. *Pflügers Archiv* **419**, 1–6.

DELGADO, J. M. R., DEFEUDIS, F. V., ROTH, R. H., RYUGO, D. K. & MITRUKA, B. K. (1972). Dialytrode for long-term intracerebral perfusion in awake monkeys. *Archives Internationales Pharmacodynamie et de Therapie* **198**, 9–21.

FEWINGS, J. D., HANNA, M. J. D., WALSH, J. A. & WHELAN, R. F. (1966). The effects of ethyl alcohol on the blood vessels of the hand and forearm in man. *British Journal of Pharmacology and Chemotherapy* **27**, 93–106.

FRAYN, K. N., COPPACK, S. W., HUMPHREYS, S. M. & WHYTE, P. L. (1989). Metabolic characteristics of human adipose tissue in vivo. *Clinical Science* **76**, 509–516.

FREDHOLM, B. B. (1971). The effect of lactate in canine subcutaneous adipose tissue in situ. *Acta Physiologica Scandinavica* **81**, 110–123.

FREDHOLM, B. B. & KARLSSON, J. (1970). Metabolic effects of prolonged sympathetic nerve stimulation in canine subcutaneous adipose tissue. *Acta Physiologica Scandinavica* **80**, 567–576.

HAGSTRÖM, E., ARNER, P., UNGERSTEDT, U. & BOLINDER, J. (1990). Subcutaneous adipose tissue: a source of lactate production after glucose ingestion in humans. *American Journal of Physiology* **258**, E888–893.

HENRIKSSON, J., ROSDAHL, H., FUCHI, T., OSHIDA, Y. & UNGERSTEDT, U. (1990). The use of microdialysis for the in vivo study of skeletal muscle glucose metabolism. In *Muscle and Motility 2*, ed. MARÉCHAL, G. & CARRARO, U., pp. 365–370. Intercept Ltd, Andover, Hants.

HICKNER, R. C., BONE, D., UNGERSTEDT, U., JORFELDT, L. & HENRIKSSON, J. (1993). Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and ^{133}Xe clearance. *Clinical Science* (in the Press).

HICKNER, R. C., ROSDAHL, H., BORG, I., UNGERSTEDT, U., JORFELDT, L. & HENRIKSSON, J. (1991). Ethanol may be used with the microdialysis technique to monitor blood flow changes in skeletal muscle: dialysate glucose concentration is blood flow dependent. *Acta Physiologica Scandinavica* **143**, 355–356.

HICKNER, R. C., ROSDAHL, H., BORG, I., UNGERSTEDT, U., JORFELDT, L. & HENRIKSSON, J. (1992). The ethanol technique of monitoring local blood flow changes in rat skeletal muscle: implications for microdialysis. *Acta Physiologica Scandinavica* **146**, 87–97.

JACOBSON, I., SANDBERG, M. & HAMBERGER, A. (1985). Mass transfer in brain dialysis devices – a new method for the estimation of extracellular amino acids concentration. *Journal of Neuroscience Methods* **15**, 263–268.

JANSSON, P.-A., SMITH, U. & LÖNNROTH, P. (1990). Evidence for lactate production by human adipose tissue in vivo. *Diabetologia* **33**, 253–256.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über Harnstoffbilding im Tierkörper. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **210**, 33–66.

KUROSAWA, M., HALLSTRÖM, Å. & UNGERSTEDT, U. (1991). Changes in cerebral blood flow do not directly affect in vivo recovery of extracellular lactate through microdialysis probe. *Neuroscience Letters* **126**, 123–126.

LANGE, L. G. & SOBEL, B. E. (1983). Mitochondrial dysfunction induced by fatty acid ethyl esters, myocardial metabolites of ethanol. *Journal of Clinical Investigation* **72**, 724–731.

LARSSON, C. I. (1991). The use of an "internal standard" for control of the recovery in microdialysis. *Life Sciences* **49**, PL73–78.

LASSEN, N. A., LINDBJERG, J. & MUNCK, O. (1964). Measurement of blood-flow through skeletal muscle by intramuscular injection of xenon-133. *Lancet* i, 686–689.

LERMA, J., HERRANZ, A. S., HERRERAS, O., ABRAIRA, V. & MARTIN DEL RIO, R. (1986). In vivo determination of extracellular concentration of amino acids in the rat hippocampus. A method based on brain dialysis and computerized analysis. *Brain Research* **384**, 145–155.

LESSER, G. T. & DEUTSCH, S. (1967). Measurement of adipose tissue blood-flow and perfusion in man by uptake of ^{85}Kr. *Journal of Applied Physiology* **23**, 621–630.

LIEBER, C. S. (1977). Metabolism of ethanol. In *Metabolic Aspects of Alcoholism*, ed. LIEBER C.S., pp. 1–29. MTP, Lancaster, UK.

LÖNNROTH, P., JANSSON, P.-A. & SMITH, U. (1987). A microdialysis method allowing characterization of intercellular water space in humans. *American Journal of Physiology* **253**, E228–231.

LOWRY, O. H. & PASSONNEAU, J. V. (1972). A collection of metabolite assays. In *A Flexible System of Enzymatic Analysis*, chap. 9, pp. 174–177 and 194–201. Academic Press, Inc., New York.

MORRISON, P. F., BUNGAY, P. M., HSIAO, J. K., BALL, B. A., MEFFORD, I. N. & DEDRICK, R. L. (1991). Quantitative microdialysis: analysis of transients and application to pharmacokinetics in brain. *Journal of Neurochemistry* **57**, 103–119.

NICHOLSON, C. & PHILLIPS, J. M. (1981). Ion diffusion modified by tortuosity and volume fraction in extracellular microenvironment of the rat cerebellum. *Journal of Physiology* **321**, 225–257.

SCHELLER, D. & KOLB, J. (1991). The internal reference technique in microdialysis: a practical approach to monitoring dialysis efficiency and to calculating tissue concentration from dialysate samples. *Journal of Neuroscience Methods* **40**, 31–38.

SMITH, U., KURODA, M. & SIMPSON, I. A. (1984). Counter-regulation of insulin-stimulated glucose transport by catecholamines in the isolated rat adipose cell. *Journal of Biological Chemistry* **259**, 8758–8763.

STÅHLE, L. (1991). Drug distribution studies with microdialysis: I. Tissue dependent difference in recovery between caffeine and theophylline. *Life Sciences* **49**, 1835–1842.

STAINSBY, W. N. & EITZMAN, P. D. (1988). Roles of CO_2, O_2 and acid in arteriovenous $[H^+]$ difference during muscle contractions. *Journal of Applied Physiology* **65**, 1803–1810.

UNGERSTEDT, U. (1991). Microdialysis – principles and applications for studies in animals and man. *Journal of Internal Medicine* **230**, 365–373.

UNGERSTEDT, U. & PYCOCK, C. (1974). Functional correlates of dopamine neurotransmission. *Bulletin der Schweizerischen Akademie der Medizinischen Wissenshaften* **1278**, 1–13.

VAN WYLEN, D. G. L., WILLIS, J., SODHI, J., WEISS, R. J., LASLEY, R. D. & MENTZER, R. M. JR (1990). Cardiac microdialysis to estimate interstitial adenosine and coronary blood flow. *American Journal of Physiology* **258**, H1642–1649.

WALLBERG-HENRIKSSON, H. (1987). Glucose transport into skeletal muscle. Influence of contractile activity, insulin, catecholamines and diabetes mellitus. *Acta Physiologica Scandinavica* **131**, suppl. 564, 30.

WILLIAMS, H. E. (1965). In vitro effects of ethanol on carbohydrate metabolism. *Clinical Research* **13**, 336.

WOZNIAK, K. M., PERT, A., MELE, A. & LINNOILA, M. (1991). Focal application of alcohols elevates extracellular dopamine in rat brain: a microdialysis study. *Brain Research* **540**, 31–40.

YKI-JÄRVINEN, H., BOGARDUS, C. & FOLEY, J. E. (1990). Regulation of plasma lactate concentration in resting human subjects. *Metabolism* **39**, 859–864.