Absolute concentrations of glycerol and lactate in human skeletal muscle, adipose tissue, and blood

EVA HAGSTRÖM-TOFT,[1] STAFFAN ENOKSSON,[2]
ERIK MOBERG,[1] JAN BOLINDER,[1] AND PETER ARNER[1]
[1]*Department of Medicine and the Research Center and* [2]*Department of Vascular Surgery, Huddinge University Hospital, Karolinska Institute, S-141 86 Huddinge, Sweden*

Hagström-Toft, Eva, Staffan Enoksson, Erik Moberg, Jan Bolinder, and Peter Arner. Absolute concentrations of glycerol and lactate in human skeletal muscle, adipose tissue, and blood. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E584–E592, 1997.—The absolute concentrations of glycerol and lactate were studied with microdialysis of adipose tissue and skeletal muscle in normal-weight subjects. The basal interstitial glycerol concentration was 232 ± 33, 96 ± 8, and 59 ± 6 μmol/l in fat, muscle, and arterialized plasma, respectively ($P = 0.0002$). This relationship was maintained during both euglycemic hyperinsulinemia, when glycerol decreased in all three compartments, and hypoglycemia, when glycerol first decreased and then increased in fat, muscle, and blood ($P = 0.0001$ for both). Basal interstitial lactate concentrations were similar in adipose tissue (1.1 ± 0.2 mmol/l) and skeletal muscle (1.9 ± 0.4 mmol/l) and higher than in arterialized blood (0.6 ± 0.1 mmol/l, $P = 0.002$). During hyperinsulinemia and hypoglycemia, lactate increased ($P = 0.0001$) and the tissue-blood relationship was maintained ($P = 0.04$). In conclusion, adipose tissue and skeletal muscle mobilize glycerol and lactate at rest. Glycerol and lactate production are influenced by hyperinsulinemia and hypoglycemia in both tissues. Adipose tissue appears to be the major site of glycerol production, whereas skeletal muscle and fat may be equally important for lactate production.

insulin; catecholamines; carbohydrate metabolism; lipolysis; microdialysis

THE RESULTS OF RECENT in vivo studies suggest that in humans, adipose tissue and skeletal muscle have similar roles in the regulation of glucose and lipid metabolism. Lactate mobilization in the periphery (from glucose) was first thought to occur mainly in muscle tissue. However, several groups have since demonstrated significant production in vivo of lactate in subcutaneous adipose tissue (13, 15, 22). Conversely, lipolysis [breakdown of intracellular triacylglycerol (TAG) leading to a mobilization of glycerol and free fatty acids from the tissue] was traditionally considered to occur mainly in fat tissue. It was assumed that intracellular muscle TAG was not mobilized into the bloodstream during lipolysis but used for local intracellular metabolism (30). Extremely high interstitial glycerol concentrations (lipolysis index; 30–40 times those in plasma and similar to those in adipose tissue) were recently demonstrated in skeletal muscle using microdialysis (28). The latter data suggest, first, that lipid mobilization may be as great in muscle as in fat and, second, that the rates of lipolysis in these tissues are 10-fold more rapid than estimated from whole body studies on the overall rates in vivo (10).

Although alternative techniques exist for estimating metabolites in tissues in vivo (13, 26), hitherto only microdialysis has been used to study glycerol and lactate in the interstitial space of human muscle and fat tissue. This technique has several advantages, as discussed (26). It is possible to monitor glycerol and lactate simultaneously in the interstitial space of different tissues during various conditions. At the same time, one can monitor nutritive blood flow in the tissue by adding a flow marker (usually ethanol) to the dialysis solvent. A drawback with the previous microdialysis studies on muscle and fat has been incomplete recovery of substances from the interstitial fluid, because short microdialysis catheters and rapid perfusion rates have been used. We developed a microdialysis method that allows complete recovery of substances in the interstitial fluid, using a microdialysis catheter with a long dialysis membrane combined with a very low perfusate flow rate. With this technique, one can monitor the true glucose concentration in subcutaneous adipose tissue (8).

The relative roles of mobilization of lipids and lactate production in human adipose tissue and skeletal muscle, respectively, are not yet known, nor is it known whether these metabolic processes are regulated in similar or different ways in the two tissues. The aim of this study was therefore *1*) to monitor continuously and simultaneously the true concentrations of lactate and glycerol in subcutaneous adipose tissue and skeletal muscle at rest as well as during conditions that alter lipid and carbohydrate metabolism and *2*) to compare the concentrations of the metabolites in the tissues with the respective circulating concentrations. Healthy nonobese subjects were microdialyzed after an overnight fast, during hyperinsulinemia, with or without hypoglycemia. At the same time, possible changes in tissue blood flow during the experiments were investigated.

METHODS

Subjects

The study group comprised 34 healthy subjects (age 31 ± 2 yr, range 19–53 yr, means ± SE) who participated on one to three separate occasions. They were all of normal weight (body mass index 22.7 ± 0.6 kg/m^2) and drug free. All of them did regular exercise but no one at an athletic level. All experiments were performed in the morning after an overnight fast. The study was approved by the Ethics Committee of Karolinska Institute, Huddinge Hospital, and the subjects were given a detailed description of the procedures before their consent was obtained.

Microdialysis Device

The microdialysis catheter (CMA/60, CMA Microdialysis, Stockholm, Sweden) has been described in detail previously

(35). Briefly, a semipermeable polyamide membrane (30 × 0.62 mm, molecular cut-off 20,000 Da) is glued to the end of double-lumen polyurethane tubing. The perfusion fluid enters the device through the outer lumen, streams down to the microdialysis membrane, and leaves through the inner lumen, from which it is collected. An exchange of substances in the interstitial fluid takes place over the membrane, so that the composition of the dialysate will mirror that of the interstitial fluid (3). In one of the methodological studies, a microdialysis catheter with a cuprophane membrane and thinner outer diameter (CMA/11; 20 × 0.24 mm) was used for comparison. The tubing was connected to a high-precision perfusion pump (CMA/106 for a perfusate velocity 0.3 μl/min and CMA/100 for perfusate velocities of 0.5, 2, and 2.5 μl/min; CMA Microdialysis) and perfused with Ringer solution (8), unless otherwise stated. One to three microdialysis catheters were inserted percutaneously, after local skin anesthesia (EMLA, Astra, Södertälje, Sweden), into the abdominal periumbilical subcutaneous adipose region and into the medial part of the gastrocnemius muscle, respectively. The location of the catheter in the muscle was confirmed by the development of muscle twitches during insertion. The distance between two catheters always exceeded 30 mm.

Qualitative estimates of variations in interstitial flow can be made by adding the flow marker ethanol, which is not locally degraded and does not affect the local tissue metabolism, to the perfusate fluid (18). The concentration of ethanol is determined in the ingoing and outgoing dialysis solvent. The ethanol ratio of out to in reflects nutritive blood flow changes. This ethanol technique has recently been validated against the ^{133}Xe washout method in both skeletal muscle (18) and adipose tissue (11). The data during hyperinsulinemia and hypoglycemia are presented as the relative change from the basal outflow-to-inflow ratio, as described previously (19).

Methodological Studies

Recovery. Glycerol and lactate recovery was assessed with the isotope method according to Jansson et al. (24). [U-^{14}C]glycerol and [U-^{14}C]lactate [~3,000 counts·min^{-1} (cpm)·μl^{-1} of each; Amersham Sweden, Solna, Sweden] and hydralazine hydrochloride (0.125 g/l) (Ciba-Geigy, Basel, Switzerland) were added to the perfusion fluid. Hydralazine in the presently used concentration has been shown to markedly stimulate the local blood flow (14). Hence, by securing a constantly high blood flow during the experiment, the influence of blood flow variations on isotope loss is avoided. Thus the label lost will directly reflect substance recovery. One or two microdialysis catheters were inserted in the adipose tissue and skeletal muscle of each subject (4 males, 1 female). The catheters were perfused at 0.3 μl/min with labeled glycerol (n = 5) and labeled lactate (n = 4). Dialysate was collected in six consecutive 45-min fractions, and the radioactivity (cpm) of the perfusate and dialysate was determined. With this technique, the relative loss of labeled substrate across the dialysis membrane is assumed to equal the flux of substrates over the membrane into the perfusion fluid (recovery). It is calculated as

$$\frac{100 \times (\text{cpm}_{\text{in}} - \text{cpm}_{\text{out}})}{\text{cpm}_{\text{in}}}$$

where cpm_{in} is the radioactivity in the perfusion fluid and cpm_{out} is the radioactivity in the dialysate fractions.

Comparison of cuprophane and polyamide dialysis membranes. The cuprophane catheter was placed in a bath of Ringer solution, which was changed every 30 min for 3 h during perfusion with Ringer solution at 2.5 μl/min. Dialysate glycerol was determined in 30-min fractions during 3 h and again after 16 h of in vitro perfusion. The prerinsed cuprophane catheter, one cuprophane catheter that was not rinsed, and one polyamide catheter (30-mm membrane length) were inserted into abdominal adipose tissue (n = 3) or muscle tissue (n = 2) and perfused at 2.5 μl/min. After an equlibration period of 30 min, three 15-min samples were collected.

Substrate Concentrations in Muscle and Adipose Tissue During Basal Conditions

Between one and three microdialysis catheters were inserted in adipose tissue and one to three in muscle, as described above, in 12 subjects (7 males, 5 females). The respective catheters in each tissue were perfused at three perfusate flow rates [0.3 μl/min (n = 9), 0.5 μl/min (n = 9), and 2 μl/min (n = 5)]. In the catheters perfused at 2 μl/min, the perfusion fluid was supplemented with ethanol (50 mM) to estimate variations in blood flow. The subjects were resting in a supine position during the study, and dialysate samples were collected in 45-min fractions for 180 min for the analyses of glycerol, lactate, and ethanol. A retrograde cannula was inserted in a dorsal hand vein, and the hand was placed in a heated box (65°C) for arterialization of venous blood (29). Blood samples were drawn in the middle of each dialysate time fraction. Arterialization was confirmed by blood gas analysis (>95% oxygen saturation).

Substrate Concentrations in Muscle and Adipose Tissue During Hyperinsulinemia

A euglycemic hyperinsulinemic clamp was performed in 11 subjects (6 males, 5 females). Four microdialysis catheters were inserted, two in each tissue region, as described in *Microdialysis Device.* One muscle catheter and one fat catheter were perfused at 0.3 μl/min with Ringer solution alone, and one muscle catheter and one fat catheter were perfused at 2 μl/min, with Ringer solution being supplemented with ethanol (50 mM). Dialysate was collected in 45-min fractions for the analyses of glycerol and lactate and in 15-min fractions for the analysis of ethanol. One cannula was inserted in a cubital vein for infusions, and one cannula was inserted ipsilaterally in a dorsal hand vein for arterialized blood sampling (see *Substrate Concentrations in Muscle and Adipose Tissue During Basal Conditions*) of the metabolites and free insulin concentrations (2 basal samples and at 22.5, 37.5, 52.5, 67.5, and 112.5 min). After a basal sampling period of 90 min, a primed plus continuous (135 min) insulin infusion was started (40 mU·m^{-2}·min^{-1}; Actrapid, NovoNordisk, Copenhagen, Denmark). Circulating glucose was kept at the fasting concentration by means of a variable glucose infusion (200 mg/ml) and bedside blood glucose measurements (HemoCue, HemoCue, Ängelholm, Sweden) every 5 min.

Substrate Concentrations in Muscle and Adipose Tissue During Hypoglycemia

A hypoglycemic hyperinsulinemic clamp was performed in eight subjects (4 males, 4 females, 27 ± 1 yr old). Microdialysis catheters and intravenous cannulas for infusions and blood sampling were inserted, as described for the euglycemic clamp. Ethanol perfusion was performed in six subjects. After 30 min of basal sampling, an intravenous insulin infusion (0.15 U/kg body wt; Actrapid, NovoNordisk, Copenhagen, Denmark) was infused during 60 min. When the concentration of arterialized blood glucose had fallen to <2.5 mmol/l, which took 25–30 min in all subjects, a variable glucose

infusion was started to maintain the blood glucose concentrations at 2.5 mmol/l during the following 30 min. Next, the insulin infusion was terminated while the glucose infusion was continued at a lower rate to obtain a gradual increase in blood glucose to the initial concentration during the following 90 min. Dialysate and plasma were sampled every 15 min, and plasma samples were drawn in the middle of each dialysate sampling period. In addition, plasma catecholamine concentrations were determined (at the same time points as free insulin) to assess the adrenergic counterregulatory response.

Chemical Analysis

Dialysate glycerol and lactate concentrations were determined with enzymatic fluorometric methods, using a tissue sample analyzer that allowed very small sample volumes (CMA/600, CMA Microdialysis). Dialysate ethanol was determined with an enzymatic spectrophotometric method (6), and the dialysate vs. perfusate ethanol ratio was calculated. Plasma glycerol was determined by bioluminescence (17) and blood lactate by an enzymatic, fluorometric method (31). Free insulin in serum was analyzed with a commercial radioimmunoassay kit (Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol (25). Plasma catecholamines were analyzed by high-performance liquid chromatography with electrochemical detection (reference value at rest <0.48 nmol/l) (16). Radioactivity was determined in a liquid scintillation counter (Gammacounter Wizard 1470, Wallac). In methodological experiments, glycerol and lactate were measured in the same dialysate fraction using the CMA/600 and a reference method, respectively (reference 17 for glycerol, reference 31 for lactate). An excellent correlation between the two methods was obtained ($r < 0.97$, slope near 1, intercept near 0).

Statistics

Data are presented as the means ± SE. The coefficient of variation (CV) was calculated as the SD divided by the mean. Variations over time in the same individual were evaluated with one-factor analysis of variance (ANOVA) repeated measurements. Comparison between the metabolites in the tissue compartments and the circulation over time was performed using two-factor repeated-measurement ANOVA, and individual time segments were compared using factorial ANOVA. Student's paired *t*-test was also used when different time points were compared, and Student's paired or unpaired *t*-test was used when the mean basal metabolite concentrations were compared. Because three compartments (blood, muscle, and fat) were investigated, a Bonferroni correction of the *P* value was used for statistical evaluation of the *t* value (Student's *t*-test).

RESULTS

Methodological Studies

The in vivo recovery of glycerol and lactate in skeletal muscle and adipose tissue at 0.3 μl/min, assessed by the isotope method, is shown in Fig. 1. The clearance of isotope was lower in the first sample but thereafter was stable during the remaining 225 min (CV for samples 2–6 was 0.4–3.3%). During steady state, recovery was >90% for both metabolites, and there was no significant difference in the recovery between the two tissues ($F < 1$).

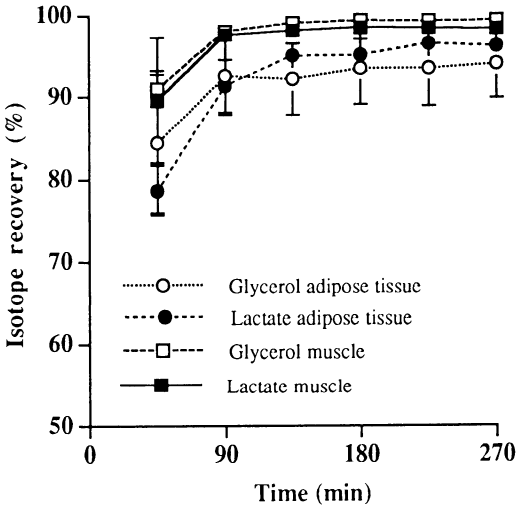


Fig. 1. Isotope recovery of lactate and glycerol in adipose tissue and skeletal muscle. Microdialysis catheters were introduced into abdominal adipose tissue or gastrocnemius muscle and perfused (0.3 μl/min) with [^{14}C]lactate (n = 4 subjects) or [^{14}C]glycerol (n = 5) during basal conditions after an overnight fast. Perfusate was supplemented with hydralazine (0.125 g/l) to secure high and stable blood flow. Recovery was estimated as ratio of label lost in tissue vs. total label (concentration in perfusion fluid). Values are means ± SE.

The in vitro rinsing of the cuprophane membrane showed high concentrations of dialysate glycerol in the initial dialysate samples (2,684 ± 138 μmol/l), with a rapid decline in glycerol concentration, so that glycerol was below the detection limit of the method (<10 μmol/l) after five samples (150 min) in three of the five catheters. However, in the remaining two catheters, the glycerol concentrations in the in vitro dialysate were 180 and 1,188 μmol/l after the study period (16 h). No glycerol was detected during rinsing of the polyamide catheters. Dialysate glycerol from the cuprophane catheters, which were inserted without prerinsing treatment, was increased >100-fold compared with the prerinsed catheters (2,749 ± 45 vs. 24 ± 5 μmol/l). The mean dialysate glycerol during the 45-min sampling was 22 ± 5 and 45 ± 2 μmol/l from the rinsed cuprophane and the polyamide catheters, respectively, in adipose tissue. The respective concentrations in muscle tissue were 13 ± 5 and 35 ± 12 μmol/l.

Absolute Metabolite Concentrations in Adipose Tissue and Muscle

The CV for the experiments performed in the basal state is shown in Table 1. In these experiments, dialysate glycerol and lactate were measured at perfusion

Table 1. *Coefficient of variation for basal tissue and circulating metabolite concentrations and ethanol outflow-to-inflow perfusate ratio*

	Muscle	Adipose Tissue	Blood
Glycerol	11.3 ± 1.1	9.3 ± 1.5	9.7 ± 1.7
Lactate	9.8 ± 1.2	8.9 ± 1.2	6.0 ± 1.5
Ethanol outflow/inflow	10.2 ± 1.4	8.6 ± 3.2	

Values are means ± SE and are expressed as percentage. Outflow/inflow, ratio of outflow to inflow for 45–180 min.

rates of 0.3 μl/min and ethanol was measured at 2 μl/min. Circulating concentrations of lactate and glycerol were stable throughout the experiment. In muscle, the glycerol and lactate dialysate concentrations dropped after the initial sample, and in fat the concentrations increased after the first sample. Thereafter, the dialysate concentrations were stable in both tissues. Because of the deviating concentrations in the first tissue samples, which may have been affected by the traumatic effect of catheter insertion, these samples were not used in calculating the CV and mean basal values. The CV for the ethanol outflow vs. inflow ratio and for the metabolites in the dialysate was ~10% in both tissues, indicating that there were no large alterations in the blood flow or tissue metabolites in the basal state.

Microdialysis was performed at 0.3, 0.5, and 2 μl/min in adipose and skeletal muscle tissue. The dialysate concentrations in the tissues at these perfusion rates and the respective plasma concentrations were compared (Fig. 2). The mean metabolite concentration in 45-min fractions over the 3-h study period is shown. There was no significant difference in the respective metabolite concentrations between 0.3 and 0.5 μl/min flow rates in either tissue. However, for glycerol the adipose tissue concentrations were higher than the skeletal muscle concentrations at respective flow rates ($P \leq 0.03$), whereas the lactate concentrations were similar in the two tissues at both flow rates. Both metabolite concentrations were lower when the tissues were perfused at 2 μl/min compared with the respective concentrations at 0.3 and 0.5 μl/min of perfusion rate except for skeletal muscle lactate (Fig. 2; adipose tissue glycerol, $P = 0.006$ and 0.02; adipose tissue lactate, $P = 0.03$ and 0.007; muscle tissue glycerol, $P = 0.0002$ and 0.03 vs. 0.3 and 0.5 μl/min, respectively, and muscle tissue lactate, not significant; Student's unpaired t-test).

As judged by the results obtained with the recovery experiments, values for metabolites in dialysate collected at a perfusion rate of 0.3 μl/min seemed to represent almost full recovery (isotopic experiments showed >90% recovery). Furthermore, there was no increase in dialysate metabolite concentrations when perfusion speed was decreased from 0.5 to 0.3 μl/min. Therefore, microdialysates collected at 0.3 μl/min were considered to represent absolute values for tissue metabolites. The respective tissue concentrations at this perfusion rate and the blood concentrations were compared. For glycerol, the adipose tissue concentrations (~230 μmol/l) were significantly higher than the muscle concentrations (~100 μmol/l), which in turn were significantly higher than the concentrations in blood (~60 μmol/l; $P \leq 0.02$ by Student's paired t-test). For lactate, muscle concentrations (~1.9 mmol/l) did not differ significantly from the respective adipose tissue concentrations (~ 1.1 mmol/l; $P = 0.09$). However, the concentrations in both these tissues were significantly higher than the circulating concentrations (~0.6 mmol/l; $P < 0.05$).

Metabolite Concentrations During the Hyperinsulinemic Euglycemic Clamp

The concentration of fasting plasma free insulin was 46 ± 8 pmol/l, and, during the insulin infusion, concentrations rose to a mean of 434 ± 24 pmol/l. Plasma glucose was stable at the fasting concentration (5.5 ± 0.1 mmol/l) during the 135-min glycemic clamp (CV 8.2 ± 1.5%). The glycerol concentrations in all three compartments decreased after insulin infusion ($P = 0.0001$ by one-factor ANOVA repeated measurements; Fig. 3*A*). The tissue differences observed in the basal state (Fig. 2) were maintained during the clamp experiments. Thus adipose tissue glycerol was two- to threefold higher before and during hyperinsulinemia than muscle glycerol, which in turn was about twice as high as in the circulation ($P \leq 0.04$ using factorial ANOVA or Student's paired t-test at each time point). During the first 45 min, the relative decrease in plasma glycerol was larger in plasma (50%) compared with adipose tissue (10%) and muscle (25%; $P < 0.05$), but thereafter there was no significant difference in relative terms between the compartments.

The lactate concentrations rose significantly in the three compartments during insulin infusion ($P = 0.0001$ by one-factor repeated ANOVA; Fig. 3*B*). Throughout

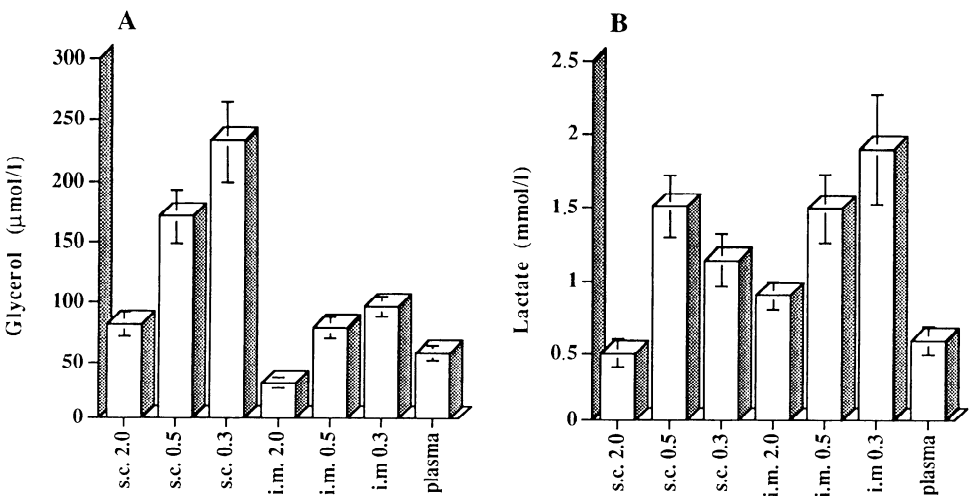


Fig. 2. Mean glycerol (*A*) and lactate (*B*) concentrations in adipose tissue and skeletal muscle tissue dialysate and blood during basal conditions. Microdialysis catheters were implanted in abdominal adipose tissue and gastrocnemius muscle and were perfused at 0.3 and 0.5 μl/min for 3 h ($n = 6$ subjects). Dialysate was sampled in 45-min fractions and dialysate glycerol and lactate concentrations were determined. Arterialized blood samples were collected in middle of each dialysate fraction. In a group of 5 subjects, microdialysis catheters were inserted as described above and perfused at 2 μl/min. Mean concentrations (45–180 min) were calculated (means ± SE).

the experiment the concentrations in adipose tissue and muscle dialysates were two to three times higher than in blood ($P < 0.05$ or less by comparison with factorial ANOVA at each time-point), which was a similar blood-to-tissue ratio as in the experiments in the basal state (Fig. 2). No significant difference between adipose tissue or muscle lactate was recorded before or during hyperinsulinemia.

The ethanol outflow-to-inflow ratio did not change significantly during the hyperinsulinemic clamp, indicating that the nutritive blood flow was not markedly altered (Fig. 3*C*).

Table 2. *Insulin and catecholamine concentrations during hypoglycemic hyperinsulinemic clamp*

	Basal	Peak	112 Min
Plasma insulin	32 ± 3	708 ± 72†	34 ± 2
Plasma epinephrine	<0.3§	3.6 ± 0.6†	0.7 ± 0.1†
Plasma norepinephrine	1.5 ± 0.1	3.0 ± 0.5*	1.8 ± 0.1

Values are means ± SE in pmol/l. *$P < 0.05$, †$P < 0.005$ by Student's paired *t*-test when compared with basal. §Epinephrine concentrations were below limit of detection (0.3 pmol/l) in 7 subjects.

Metabolite Concentrations During the Hyperinsulinemic Hypoglycemic Clamp

After insulin infusion, plasma glucose fell from 5.1 ± 0.1 to 2.6 ± 0.1 mmol/l. By the end of the recovery period, the plasma glucose had returned to fasting concentrations. The insulin concentrations as well as the adrenergic counterregulatory response are depicted in Table 2. The insulin concentrations were stable during the infusion (CV 6.5 ± 1.5%) and 1 h after termination of the infusion the concentrations had returned to baseline. Epinephrine peaked in close association with the glucose nadir at concentrations >10-fold above the baseline (50–70 min after start of the insulin infusion), and the concentrations were still increased 1 h after the end of the insulin infusion. For norepinephrine, the increase was only about twofold, and the peak value occurred 30–110 min after the start of the insulin infusion.

With regard to metabolites, glycerol concentrations (Fig. 4*A*) fell significantly during the insulin infusion and increased significantly after hypoglycemia in fat, muscle, and blood ($P = 0.0001$ by one-factor ANOVA repeated measurements). However, in muscle tissue the increase in glycerol concentrations appeared 15 min earlier than in adipose tissue. Hence, the tissue differences for glycerol (fat > muscle) observed in the basal state and in the euglycemic clamp experiments were not present during the first 30 min after termination of the insulin infusion (at 67–97 min in Fig. 4*A*). Thereafter, the relationship among the three compartments (fat > muscle > plasma) was restored ($P \leq 0.03$

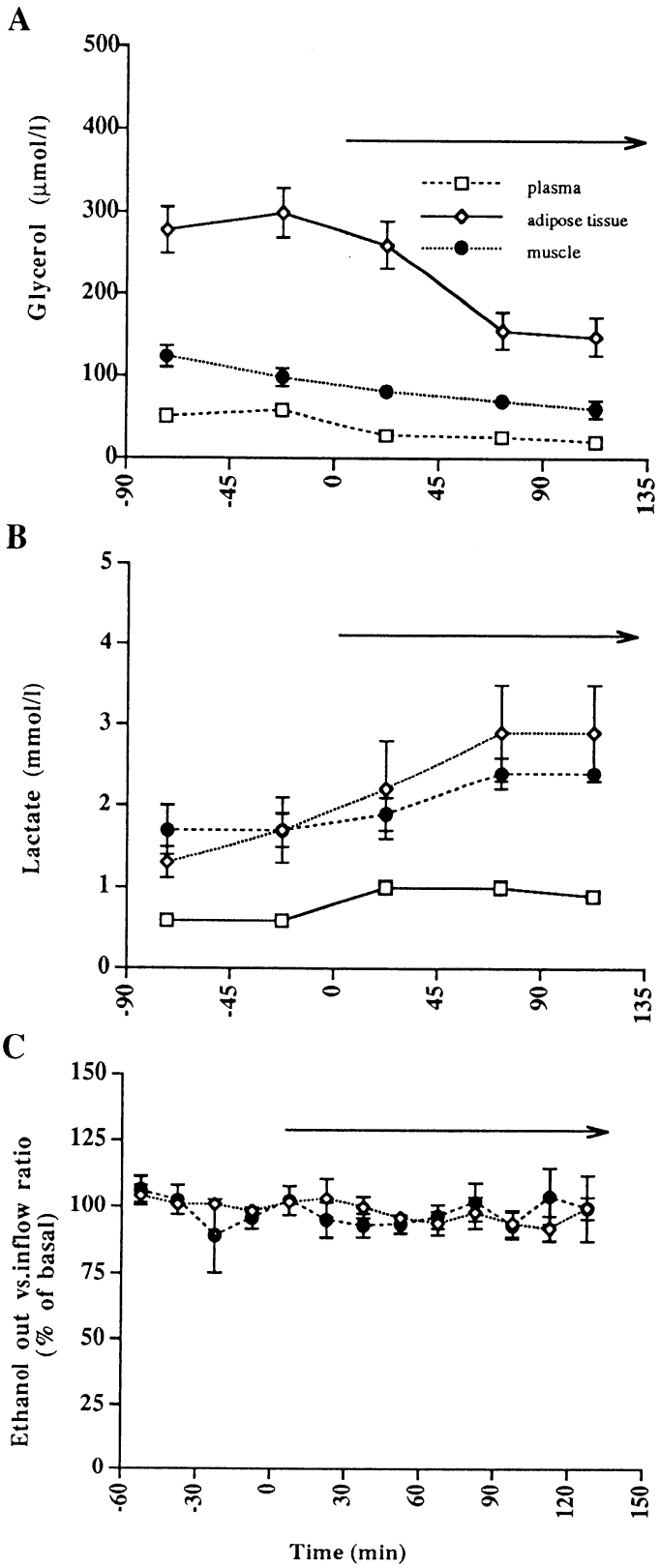
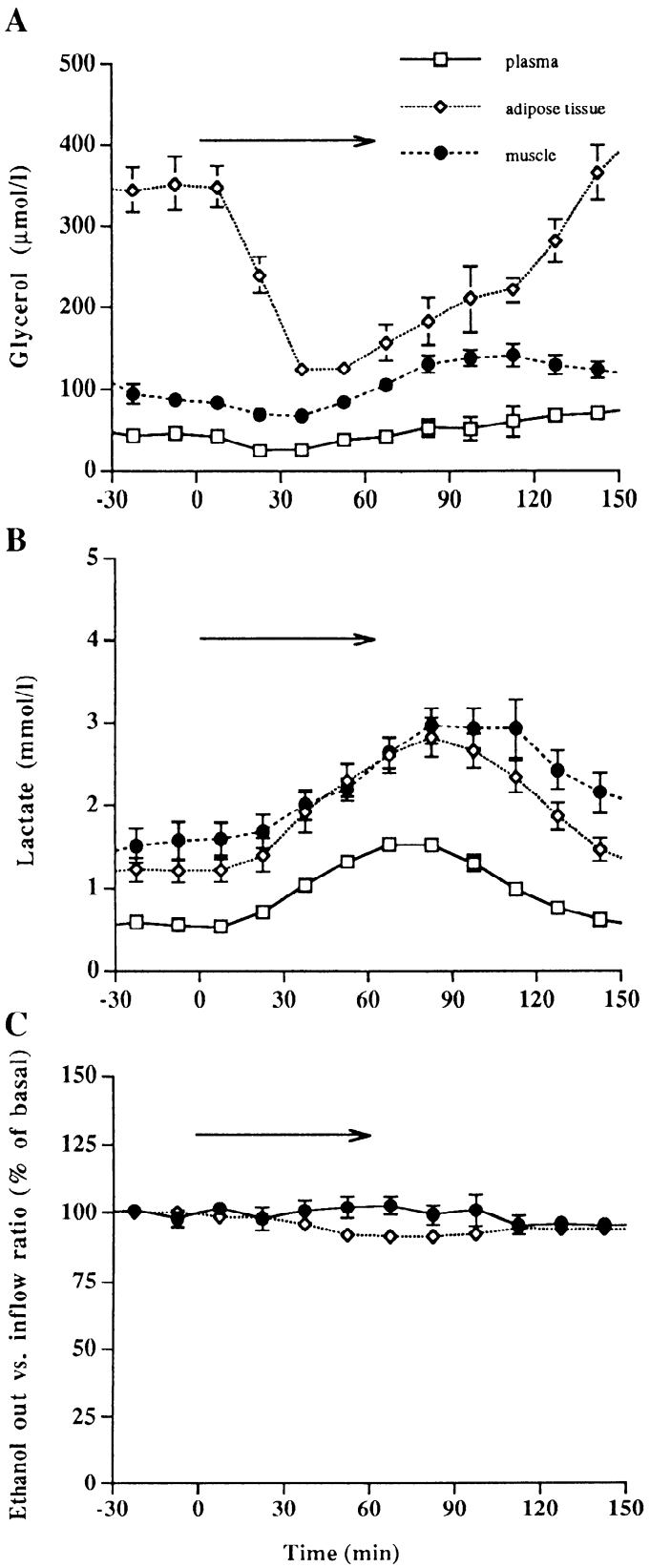


Fig. 3. Adipose tissue, skeletal muscle, and circulating glycerol (*A*) and lactate (*B*) concentrations during a hyperinsulinemic (40 $mU \cdot m^{-2} \cdot min^{-1}$) euglycemic clamp (135 min). Microdialysis catheters were inserted as described in legend to Fig. 2 and perfused at 0.3 μl/min. Additional catheters were perfused at 2 μl/min with ethanol (50 mmol/l) for determination of variations in blood flow (*C*). Samples were collected every 15 min and expressed as percentage of basal ratio (basal period = −45–0 min). Basal absolute ethanol ratio was 0.48 ± 0.02 in adipose tissue and 0.18 ± 0.01 in muscle. Arrows indicate insulin + glucose infusion. Values are means ± SE; $n = 11$ subjects.

by factorial ANOVA or paired *t*-test). In relative terms (%basal glycerol) the decrease in adipose tissue glycerol was prolonged and more profound, compared with the respective decrease in muscle tissue (at 52.5 min glycerol was 35% of basal in adipose tissue vs. 80% in muscle and plasma, $P = 0.005$, and at 82.5 min adipose tissue glycerol was 53 vs. 125% in muscle and 115% in plasma, $P = 0.013$). There was a continuous increase in plasma and adipose tissue glycerol, whereas skeletal muscle glycerol peaked and then decreased during the last hour.



The lactate concentrations increased gradually during the insulin infusion and during 30–45 min after the hypoglycemia nadir and decreased thereafter in fat, muscle, and blood [$P = 0.0001$ by one-factor repeated ANOVA (Fig. 4*B*)]. Throughout the experiment, the concentrations in fat and muscle were two to three times higher than the respective concentrations in blood ($P < 0.003$ or less by one-factor factorial ANOVA for individual time points). Just as in the basal state and in the euglycemic clamp experiments, no significant difference was found between adipose tissue and skeletal muscle lactate concentrations throughout the experiment.

The statistical evaluation of interstitial blood flow with the ethanol out-to-inflow ratio (Fig. 4*C*) revealed that the nutritive blood flow increased in adipose tissue during the hypoglycemic clamp procedure, as shown by a decreasing relative ethanol out- vs. inflow ratio (one-factor repeated ANOVA $F = 2.8$, $P = 0.002$). No significant change in the ethanol ratio was observed in skeletal muscle.

DISCUSSION

In the present study it was possible to measure the absolute concentrations of glycerol and lactate in subcutaneous adipose tissue and skeletal muscle. The results of the isotope recovery experiments show a high and stable recovery (>90%) of both metabolites with the presently used microdialysis device in both tissues. This means that near-true concentrations of lactate and glycerol should be obtained, and thus the uncertainty of recalculations from incomplete recovery experiments is avoided. In the following discussion dialysate metabolite concentrations will therefore be referred to as tissue concentrations. The low recovery found in the initial sample is probably due to transient tissue disturbances caused by the catheter insertion and speaks in favor of adding an equilibration period before starting basal sampling in microdialysis experiments.

With regard to glycerol, a fixed relationship among the three compartments was recorded during the various experimental conditions. In the basal state, adipose tissue concentrations were about four times higher than plasma concentrations, which is somewhat higher

Fig. 4. Adipose tissue, skeletal muscle, and arterialized plasma concentrations of glycerol (*A*) and lactate (*B*) during a 60-min hyperinsulinemic (0.15 U·kg^{-1}·min^{-1}) hypoglycemic clamp ($n = 8$ subjects). Microdialysis catheters were implanted, and dialysate and arterialized blood were collected as described in legends to Figs. 2 and 3, except that 15-min intervals were applied for sampling. Variations in tissue blood flow ($n = 6$) were assessed with ethanol outflow vs. inflow ratio and expressed as percentage of basal ratio (basal ethanol ratio was 0.58 ± 0.05 in adipose tissue and 0.25 ± 0.03 in muscle; *C*). Arrows indicate insulin infusion. Values are means ± SE.

than previous estimates of the glycerol concentration using microdialysis with incomplete recovery (4, 23) or an adipose venous cannulation technique (13). Skeletal muscle glycerol concentrations were about twice the concentrations in the circulation and lower than those in adipose tissue in the basal state. When hormonal regulators of lipolysis were manipulated, the changes in glycerol (lipolysis index) were similar in all three compartments. During hyperinsulinemia, decreasing concentrations were observed, and after hypoglycemia, which induces a catecholamine response, there was an increase in the glycerol concentration. The antilipolytic effect seemed more pronounced in adipose tissue, whereas, during the initial recovery period after hypoglycemia, a more rapid increase in tissue glycerol was observed in muscle compared with fat. However, essentially the tissue differences in glycerol concentrations were maintained in these various conditions, strongly indicating that active lipid mobilization occurs both in adipose tissue and skeletal muscle during hyperinsulinemia and hypoglycemia.

Hence, effects of the major hormones regulating lipolysis in humans, insulin and catecholamines, appear to be similar in the tissues. Thus we found that insulin inhibits lipid mobilization in both skeletal muscle and adipose tissue, whereas the lipolysis is stimulated by hypoglycemic counterregulation (catecholamine burst) in both tissues. It is not possible in this study to establish whether lipids mobilized from skeletal muscle originate from intracellular lipid droplets or from intermuscular fat (30). However, the subjects investigated were all lean, so the intermuscular adipose tissue content should be low, according to previous findings with magnetic resonance imaging (34). Furthermore, the reason for the different time course in skeletal muscle and adipose tissue can only be speculated on, e.g., an influence by these hormones on lipoprotein lipase in skeletal muscle cells.

A fixed relationship among lactate concentrations in the three tissues was also observed during the various types of investigations. The basal lactate concentrations in the three compartments differed, since muscle and adipose tissue concentrations were two to three times higher than the blood concentrations. However, no major differences between fat and muscle tissue were observed. During hyperinsulinemia and insulin-induced hypoglycemia, there was a similar increase of lactate in adipose tissue, muscle, and blood, and the relationship among lactate concentrations in the three compartments (muscle = fat > blood) was maintained. Altogether, these are unambiguous signs of significant lactate production in both skeletal muscle and adipose tissue. Furthermore, the regulation of lactate concentrations by hyperinsulinemia and catecholamine stimulation (after hypoglycemia) seems to be similar in the two tissue beds. The findings of adipose tissue lactate production during a hyperinsulinemic clamp are in conformity with data using the arteriovenous-balance technique (9). However, when the arteriovenous-balance technique was applied over deep forearm tissues (mainly muscle), there were no signs of lactate release during a glucose clamp, and in the basal state the tissue took up lactate (9). The only clear explanation for this divergence in results is that different skeletal muscle regions were studied with different techniques.

The present data do not tell exactly whether it is adipose or skeletal muscle tissue that plays the dominant role in the mobilization of lipids and lactate to circulation. To quantify the mobilization of metabolites, the absolute rate of the nutritive blood flow needs to be determined. The ethanol perfusion technique used in this study detects only qualitative variations of the flow in the individual tissue. According to previous methodological studies (11, 19), the method should detect a variation of 50% or more in tissue flow. Our data indicate that no major variations occurred in the nutritive blood flow in either tissue in the basal state or during hyperinsulinemia. During insulin-induced hypoglycemia, the blood flow increased in adipose tissue, whereas it was unchanged in skeletal muscle during these conditions. The adipose tissue data from the hypoglycemia experiments are in accordance with previous findings showing increased adipose tissue blood flow during hypoglycemia using the ethanol technique (7) and the xenon washout technique (12). For muscle tissue it is not clear if changes in the nutritive blood flow occur during hypoglycemia in humans. In rats an increased blood flow was observed using the ethanol technique (19). In humans only whole limb or forearm blood flow (1, 2, 20) has been recorded showing increased blood flow. However, these measurements may include tissues other than muscle as well as changes attributable to factors other than altered nutritive blood flow.

The difference between arterial and tissue concentrations was similar in adipose tissue and skeletal muscle for lactate but ~2.5-fold higher in fat compared with muscle for glycerol. Ethanol perfusion showed no major (>50%) variations in the interstitial blood flow during the different experimental conditions except for the small adipose tissue blood flow increase during hypoglycemia. If the resting tissue blood flow rate is assumed not to be substantially lower in adipose tissue than in skeletal muscle [muscle blood flow ~1 $ml \cdot 100\,g^{-1} \cdot min^{-1}$ (18, 27), adipose tissue blood flow 1–7 $ml \cdot 100\,g^{-1} \cdot min^{-1}$ (11, 21, 27) by the xenon washout technique], lipolytic activity should be substantially higher in adipose tissue than in skeletal muscle after an overnight fast, during hyperinsulinemia, and during hypoglycemia. Taking the increase in adipose tissue blood flow into account only strengthens the role of adipose tissue for lipid mobilization. With regard to the rate of lactate production, on the other hand, it can be assumed to be similar in the two tissues during the various conditions investigated in the study. The present conclusions are based on the results in subcutaneous abdominal adipose tissue and the gastrocnemius muscle. Hence, it cannot be excluded that regional differences in this aspect exist. Nor can a possible influence of sex be ignored. The number of subjects studied was not large enough to permit analysis according to gender.

The present results regarding tissue glycerol concentrations are not in accordance with those found recently by Maggs et al. (28), who studied skeletal muscle and adipose tissue glycerol concentrations using a microdialysis method, in which a short catheter and rapid perfusion speed were used, leading to incomplete recovery. In that study, substantially higher concentrations were found in the tissues (30–40 times higher than in blood) compared with our results. Unlike our findings, similar glycerol concentrations in the two tissues were observed. The cause of these discrepancies remains unclear. The divergent results could hardly be caused by regional tissue differences. Maggs et al. investigated a different skeletal muscle region but used the same adipose tissue region as we did. A possible reason for the high glycerol concentrations is that absolute concentrations could not be measured in the study by Maggs et al. Instead, recalculations of the dialysate glycerol were performed by use of the estimated in vivo recovery obtained from calibration experiments. The high glycerol concentrations could also be artifactual. The dialysis membranes used by Maggs et al. are prepared with glycerol. We found extremely high glycerol concentrations when rinsing the type of cuprophane microdialysis membrane that was used by Maggs and colleagues. After thorough rinsing of the membranes, the in situ glycerol concentrations were lower than the concentrations found with the membrane type used in the present study, which is reasonable, since that membrane is 10 mm longer and thus should lead to a higher recovery at the same perfusion rate. In addition, in a number of other studies adipose tissue glycerol concentrations are reported to be similar to those presently found (150–200 μmol/l) when both the no-net-flux calibration method (4, 21) and the zero-flow calibration (5) are used. Finally, recent data speak strongly against tissue glycerol concentrations as high as 3 mmol/l (33). Glycerol was infused intravenously to reach circulating concentrations of ~500 μmol/l. This elevation of plasma glycerol led to an increase in adipose tissue glycerol concentrations and stimulated muscle uptake of glycerol, which could not possibly occur if the tissue concentrations were as high as 3 mmol/l.

In conclusion, in both adipose tissue and skeletal muscle a mobilization of significant amounts of lipids and lactate seems to occur during resting conditions. Insulin inhibits lipid mobilization and stimulates lactate production in both tissues, whereas hypoglycemia increases both lipid and lactate mobilization in these tissues; little if any change in tissue blood flow occurs during these conditions. Adipose tissue seems to be the dominant source of glycerol production, whereas the two tissues may contribute to an equal extent to lactate production.

We gratefully acknowledge the excellent technical assistance of L. Dungner, K. Hertel, B.-M. Leijonhufvud, C. Sjøsberg, E. Sjøslin, and K. Wåhlén.

This study was supported by grants from the Swedish Medical Research Council, Swedish Athletes Research Council, Karolinska Institute, Novo Nordisk Pharma, and the Foundations of Tore Nilsson, Thuring, Soderberg, Osterman, Martin Rind, and Magnus Bergvall. CMA Microdialysis is acknowledged for generous support.

Address for reprint requests: P. Arner, Dept. of Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.

Received 24 December 1996; accepted in final form 8 May 1997.

REFERENCES

1. **Abrahamson, D. L., N. Schkloven, M. N. Margolis, and I. A. Mirsky.** Influence of massive doses of insulin on peripheral blood flow in man. *Am. J. Physiol.* 128: 124–132, 1939.
2. **Allwood, M. J., J. Ginsburg, and A. Paton.** The effect of insulin hypoglycaemia on blood flow in intact and sympathectomized extremities in man. *J. Physiol.* 139: 97–107, 1957.
3. **Arner, P., and J. Bülow.** Assessment of adipose tissue metabolism in man—comparison of Fick and microdialysis techniques. *Clin. Sci. (Lond.)* 82: 247–256, 1993.
4. **Arner, P., E. Kriegholm, P. Engfeldt, and J. Bolinder.** Adrenergic regulation of lipolysis in situ at rest and during exercise. *J. Clin. Invest.* 85: 893–898, 1990.
5. **Barbe, P., L. Millet, J. Galitzky, M. Lafontan, and M. Berlan.** In situ assessment of the role of the $beta_1$-, $beta_2$- and $beta_3$-adrenoceptors in the control of lipolysis and nutritive blood flow in human subcutaneous adipose tissue. *Br. J. Pharmacol.* 117: 907–913, 1996.
6. **Bernst, E., and J. Gutman.** Determination of ethanol with alcohol dehydrogenase and NAD. In: *Methods of Enzymatic Analysis*, edited by H. U. Bergmeyer. New York: Academic, 1974, vol. 3, p. 1499–1505.
7. **Bolinder, J., S. Sjöberg, and P. Arner.** Stimulation of adipose tissue lipolysis following insulin-induced hypoglycaemia: evidence of increased beta-adrenoceptor-mediated lipolytic response in IDDM. *Diabetologia* 39: 845–853, 1996.
8. **Bolinder, J., U. Ungerstedt, and P. Arner.** Microdialysis measurement of the absolute glucose concentration in subcutaneous adipose tissue allowing glucose monitoring in diabetic patients. *Diabetologia* 35: 1177–1180, 1992.
9. **Coppack, S. W, K. N. Frayn, S. M. Humphreys, H. Dhar, and T. D. R. Hockady.** Effects of insulin on human adipose tissue metabolism in vivo. *Clin. Sci. (Lond.)* 77: 663–670, 1989.
10. **Coppack, S. W., M. D. Jensen, and J. M. Miles.** In vivo regulation of lipolysis in humans. *J. Lipid Res.* 35: 177–193, 1994.
11. **Fellländer, G., B. Linde, and J. Bolinder.** Evaluation of the microdialysis ethanol technique for monitoring of subcutaneous adipose tissue blood flow in humans. *Int. J. Obes.* 20: 220–226, 1996.
12. **Fernquist-Forbes, E., B. Linde, and R. Gunnarsson.** Insulin absorption and subcutaneous blood flow in normal subjects during insulin-induced hypoglycaemia. *J. Clin. Endocrinol. Metab.* 67: 619–623, 1988.
13. **Frayn, K. N., S. W. Coppack, S. M. Humphreys, and P. L. Whyte.** Metabolic characteristics of human adipose tissue in vivo. *Clin. Sci. (Lond.)* 76: 509–516, 1989.
14. **Galitzky, J., M. Lafontan, J. Nordenström, and P. Arner.** Role of vascular alpha-2 adrenoceptors in regulating lipid mobilization from adipose tissue. *J. Clin. Invest.* 91: 1997–2003, 1993.
15. **Hagström, E., P. Arner, U. Ungerstedt, and J. Bolinder.** Subcutaneous adipose tissue: a source of lactate production after glucose ingestion in humans. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E888–E893, 1990.
16. **Hallman, H., L. E. Farnebo, B. Hamberg, and G. Jonsson.** A sensitive method for determination of plasma catecholamines using liquid chromatography with electrochemical detection. *Life Sci.* 23: 1049–1052, 1978.
17. **Hellmér, J., P. Arner, and A. Lundin.** Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal. Biochem.* 177: 132–137, 1989.
18. **Hickner, R. C., D. Bone, U. Ungerstedt, L. Jorfeldt, and J. Henriksson.** Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and ^{133}Xe clearance. *Clin. Sci. (Lond.)* 86: 15–25, 1994.
19. **Hickner, R. C., U. Ungerstedt, and J. Henriksson.** Regulation of skeletal muscle blood flow during acute insulin-induced hypoglycemia in the rat. *Diabetes* 1340–1344, 1994.
20. **Hilsted, J., F. Bonde-Petersen, M.-B. Nørgaard, M. Greniman, N. J. Christensen, H.-H. Parving, and M. Suzuki.**

Haemodynamic changes in insulin-induced hypoglycaemia in normal man. *Diabetologia* 26: 328–332, 1984.

21. **Jansson, P.-A., A. Larsson, U. Smith, and P. Lönnroth.** Glycerol production in subcutaneous adipose tissue in lean and obese humans. *J. Clin. Invest.* 89: 1610–1617, 1992.
22. **Jansson, P.-A., U. Smith, and P. Lönnroth.** Evidence for lactate production by human adipose tissue in vivo. *Diabetologia* 33: 253–256, 1990.
23. **Jansson, P.-A., U. Smith, and P. Lönnroth.** Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E918–E922, 1990.
24. **Jansson, P.-A., T. Veneman, N. Nurjahan, and J. Gerich.** An improved method to calcualte adipose tissue interstitial substrate recovery for microdialysis studies. *Life Sci.* 54: 1621–1624, 1994.
25. **Kuzuya, M., P. M. Blix, O. L. Horowitz, D. F. Steiner, and A. H. Rubinstein.** Determinations of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes* 26: 22–29, 1977.
26. **Lafontan, M., and P. Arner.** Application of in situ microdialysis to measure metabolic and vascular responses in adipose tissue. *Trends Pharmacol. Sci.* 17: 309–313, 1996.
27. **Linde, B., P. Hjemdahl, U. Freyschuss, and A. Juhlin-Dannfelt.** Adipose tissue and skeletal muscle blood flow during mental stress. *Am. J. Physiol.* 256 (*Endocrinol. Metab.* 19): E12–E18, 1989.
28. **Maggs, D. G., R. Jacob, F. Rife, R. Lange, P. Leone, M. J. During, W. V. Tamborlane, and R. S. Scherwin.** Interstitial fluid concentrations of glycerol, glucose, and amino acids in human quadriceps muscle and adipose tissue. *J. Clin. Invest.* 96: 370–377, 1995.
29. **McGuire, E. A. H., J. H. Helderman, J. D. Tobin, R. Andres, and R. Berman.** Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J. Appl. Physiol.* 41: 65–73, 1976.
30. **Oscai, L. B., D. A. Essig, and W. K. Palmer.** Lipase regulation of muscle triglyceride hydrolysis. *J. Appl. Physiol.* 69: 1571–1577, 1990.
31. **Passoneau, J. V.** Lactate: fluorometric method. In: *Methods of Enzymatic Analysis*, edited by H. V. Bergmeyer. New York: Academic, 1974, vol. 3, p. 1468–1472.
32. **Pickup, J. C.** Sampling and sensing blood glucose. *Lancet* 342: 1068, 1993.
33. **Samra, J., C. L. Ravell, S. L. Giles, P. Arner, and K. N. Frayn.** Interstitial glycerol concentration in human skeletal muscle and adipose tissue is close to the concentration in blood. *Clin. Sci. (Lond.)* 90: 453–456, 1996.
34. **Schick, F., B. Eismann, W.-I. Jung, H. Bongers, M. Bunse, and O. Lund.** Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn. Reson. Med.* 29: 158–167, 1993.
35. **Tossman, U., and U. Ungerstedt.** Microdialysis in the study of extracellular concentrations of amino acids in the rat brain. *Acta Physiol. Scand.* 128: 9–14, 1986.