Interstitial fluid Concentrations of Glycerol, Glucose, and Amino Acids in Human Quadricep Muscle and Adipose Tissue

Evidence for Significant Lipolysis in Skeletal Muscle

David G. Maggs,* Ralph Jacob,* Francis Rife,* Robert Lange,§ Paola Leone,* Matt J. During,*‡ William V. Tamborlane,* and Robert S. Sherwin*

*Sections of *Endocrinology, ‡Surgery and §Radiology, Yale University, New Haven, Connecticut 06510*

Abstract

To determine the relationship between circulating metabolic fuels and their local concentrations in peripheral tissues we measured glycerol, glucose, and amino acids by microdialysis in muscle and adipose interstitium of 10 fasted, nonobese human subjects during (*a*) baseline, (*b*) euglycemic hyperinsulinemia (3 mU/kg per min for 3 h) and, (*c*) local norepinephrine reuptake blockade (NOR). At baseline, interstitial glycerol was strikingly higher ($P < 0.0001$) in muscle (3710 μM) and adipose tissue (2760 μM) compared with plasma (87 μM), whereas interstitial glucose (muscle 3.3, fat 3.6 mM) was lower ($P < 0.01$) than plasma levels (4.8 mM). Taurine, glutamine, and alanine levels were higher in muscle than in adipose or plasma ($P < 0.05$). Euglycemic hyperinsulinemia did not affect interstitial glucose, but induced a fall in plasma glycerol and amino acids paralleled by similar changes in the interstitium of both tissues. Local NOR provoked a fivefold increase in glycerol ($P < 0.001$) and twofold increase in norepinephrine ($P < 0.01$) in both muscle and adipose tissues. To conclude, interstitial substrate levels in human skeletal muscle and adipose tissue differ substantially from those in the circulation and this disparity is most pronounced for glycerol which is raised in muscle as well as adipose tissue. In muscle, insulin suppressed and NOR increased interstitial glycerol concentrations. Our data suggest unexpectedly high rates of intramuscular lipolysis in humans that may play an important role in fuel metabolism. (*J. Clin. Invest.* 1995. 96:370–377.) Key words: muscle • adipose • insulin • norepinephrine • lipolysis

Introduction

In humans, skeletal muscle and adipose tissue are key regulatory sites of fuel metabolism. In the fasting state, fat is the main source of energy for both skeletal muscle and adipose tissue with the latter being the body's main store of fat energy. It has long been thought that under fasting conditions skeletal muscle is dependent on a supply of fatty acids that arise from the hydrolysis of triacylglycerol in distant fat depots (1), but there is now increasing evidence that skeletal muscle may have independent fat stores which offer an alternative and more immediate source of energy. Moreover, skeletal muscle provides a depot of amino acids that serve as gluconeogenic substrates during fasting and, in the fed state, muscle is the main target organ for insulin stimulated glucose metabolism.

Attempts to characterize the distinct metabolic processes of human skeletal muscle and adipose tissue have been impeded by anatomical limitations. Although adipose tissue is easily accessible and studies using adipose vein cannulation have offered some insights into local metabolism (2, 3) the value of ''isolating'' skeletal muscle through limb (either forearm, calf, or leg) studies is more problematic (4), contributions to net metabolic activity from skin, adipose, bone marrow, and connective tissues confound the interpretation of such studies. Arteriovenous differences across a tissue bed give an indication of ''net'' metabolic activity but may understate the turnover of a substrate to and from the tissue in question. For example, isotope studies have demonstrated that uptake and release of glycerol from the limb occur at a high turnover rate (5) despite a small difference between arterial and venous glycerol concentrations.

Study of the individual contributions of specific tissues to substrate metabolism has been recently enhanced by the development of microdialysis techniques that allow direct sampling of substrate concentrations in the interstital fluid in vivo. The bidirectional nature of the dialysis membrane, also provides the unique opportunity to locally perturb the metabolic milieu by the perfusion of hormones or other agents into the interstitial space. In human peripheral tissues, this interest has mostly concentrated on adipose tissue because of its easy access (6–8). In the current study we used a microdialysis system which enables separate and simultaneous observations in skeletal muscle and adipose tissue of human subjects. It was our aim to characterize fasting interstitial concentrations of glycerol, glucose, and amino acids in the two distinct tissues, and to determine the effects of both systemic hyperinsulinemia and locally administered norepinephrine reuptake blockade on interstitial concentrations of these substrates.

Methods

10 healthy non-obese subjects (5 female; mean age 23.9±1.9 yr, range 18 to 34 yr; mean body mass index 21.3±0.7 kg/m^2, range 18.1 to 24.5 kg/m^2; mean weight 61.9±4.3 kg, range 49.5 to 84.1 kg) who were not taking any medication were enrolled in the project. All gave written informed consent to the study which was approved by the Human Investigation Committee of Yale University. They were studied on four separate occasions over a three week period, each time after an overnight

Address correspondence to Dr. David G. Maggs, Section of Endocrinology, School of Medicine, Yale University, Box 3333, New Haven, CT 06510. Phone: 203-785-4183; FAX: 203-785-6015.

Received for publication 9 September 1994 and accepted in revised form 13 March 1995.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/95/07/0370/08 $2.00
Volume 96, July 1995, 370–377

fast (10 h). The four visits involved (*a*) control study, (*b*) calibration study, (*c*) insulin infusion study, and (*d*) desipramine perfusion study. At the outset of each study, a retrograde cannula was inserted into a vein in the dorsum of the right hand which was positioned in a heated box for sampling of arterialized venous blood (9). A small volume of normal saline (0.9%) was infused through the retrograde intravenous cannula to maintain patency. During the insulin infusion study, a second intravenous catheter was inserted in a contralateral antecubital vein for the infusion of insulin and glucose. Microdialysis probes were inserted into adipose tissue of the anterior abdominal wall as well as skeletal muscle of the anterolateral aspect of the quadricep mass of the left thigh. To account for the acute trauma artifact that occurs after probe insertion, we allowed a 60-min "rest period" immediately after the probes had been inserted before baseline samples were collected. The probes were of a concentric design as previously described (10). In brief, the probes were constructed in our laboratory with 20 mm of exposed cuprophan dialysis membrane (o.d. 250 μm; molecular weight cut-off 5,000 daltons) using vitreous silica inlet and outlet tubing (o.d. 145 μm, i.d. 75 μm). In all studies artificial extracellular fluid (NaCl 135 mM, KCl 3 mM, $MgCl_2$ 1 mM, $CaCl_2$ 1.2 mM, Ascorbate 200 μM, and Na Phosphate buffer of 2 mM adjusted to pH 7.4) was perfused through the probes at a constant rate of 2.5 μl/min. Dialysate was collected on ice in pre-weighed tubes which were weighed again after collection to confirm appropriate flow through the microdialysis probes. In vitro testing of the microdialysis probes confirmed an equal relative recovery of glycerol (76±6%), glucose (68±6%) and amino acids (range 65±5 to 80±8%) when used under conditions that simulated basal in vivo substrate concentrations.

Magnetic resonance imaging. At the control study, all subjects had cross-sectional magnetic resonance images (MRI) of the mid-thigh and anterior abdominal wall to characterize local anatomy at the two sites of microdialysis probe insertion. Scanning was done on a 1.5 tesla system (SIGNA 1.5; General Electric Medical Systems, Milwaukee, WI). The images were obtained with a T1-weighted imaging sequence, which provides sharp contrast between lipid- and water-containing (muscle) tissues (lipid tissue is bright, while muscle is dark on T1-weighted MR images). This feature was used to measure the thickness of the subcutaneous tissues in the images of the abdomen and the mid-thigh. By measuring the thickness of subcutaneous tissue at the site of muscle probe insertion and knowing the depth catheters were inserted into the thigh we could therefore determine the anatomical position of the microdialysis chamber (distal 20 mm of catheter). The probes were too small to be visualized by MRI so their position could not be confirmed spectroscopically. Also, we estimated levels of adipose tissue within the muscle body itself (i.e., intermuscular adipose tissue or "marbling") by segmenting the images according to MR signal density, a method that gives a qualitative measure of adipose tissue content (11). The percentage of intermuscular adipose tissue is calculated from the image picture elements (pixels) with fat intensity divided by the total number of pixels in the region of muscle tissue selected.

Control study. Plasma and dialysate samples were collected over a 5-h period using the standard flow rate (2.5 μl/min) and perfusate for measurement of glucose, glycerol, amino acids, and catecholamines in the absence of any systemic perturbations. Two microdialysis probes were inserted into each tissue bed and dialysate was collected from each probe at hourly intervals; one pair of probes (in adipose and muscle) was used for the measurement of dialysate substrates while the other pair was used for the measurement of dialysate catecholamines. Plasma samples were collected every hour.

Calibration study. Practical constraints would not allow us to estimate absolute concentrations of substrates in the interstital space of skeletal muscle and adipose tissue by a single calibration procedure. We therefore measured interstitial levels of glycerol and glucose by a no net flux protocol (6), and we used a second calibration procedure, a zero flow rate protocol (12), to measure a full range of acidic and neutral amino acids without the complexities of adding an amino acid "cocktail" to the microdialysis perfusate. However, the two methods compare favorably because in a series of preliminary studies using both calibration procedures in the same subjects, we found that estimates of interstitial concentrations of several key amino acids and glucose were virtually identical regardless of the method applied (unpublished data). The two calibration procedures involved the following: the no net flux protocol is based on the principle that measurement of substrate concentration in dialysate while perfusing the dialysis system with differing concentrations of the substrate in question will allow calculation of absolute interstitial concentration at the point where net exchange across the dialysis membrane is zero and perfusate and dialysate concentrations are equal. For this purpose we added glucose and glycerol to our perfusion medium at predetermined concentrations. Including the use of standard perfusate (where no glucose and glycerol was added), we perfused at four different concentrations of substrates. At each stage, dialysate was collected at 30-min intervals with 30-min "wash-out" periods between each change in perfusate content to allow re-equilibration with the new perfusate concentration. To exclude an order effect, the sequence in which the differing perfusate concentrations were perfused was reversed in half the subjects, however, this had no effect on the data. The zero flow rate protocol is based on the principle that recovery of a substance across the dialysis membrane depends on dialysis flow rate such that at zero flow rate the interstitial space and dialysate fluid are in complete equilibrium. By measuring dialysate concentration of amino acids at different flow rates (between 0.25 to 2.5 μl/min) we were able to calculate absolute interstitial concentration, i.e., the point at which the interstitial space is in complete equilibrium with dialysate, by extrapolating to zero flow rate by nonlinear regression. At each flow rate, dialysate samples were collected to allow volumes of at least 30 μl at the lower flow rates (0.25 and 0.5 μl/min) and 60 μl at the higher flow rates (1.0 and 2.5 μl/min). At each change in flow rate, an appropriate "wash-out" period was allowed to facilitate re-equilibration at the new flow rate. The order in which different flow rates was used was reversed in half the subjects and this had no effect on the data. The study therefore involved the insertion of two microdialysis probes into each tissue bed (i.e., a total of four probes): one pair (in adipose and muscle) was used to carry out the net flux procedure and the other pair to carry out the zero flow rate procedure. At hourly intervals, plasma samples for glucose, glycerol, and amino acids were also collected. Having measured dialysate concentrations and estimated absolute interstitial concentrations of substrates in the two tissue beds we were able to calculate the in vivo recovery of each substrate ([dialysate]/[absolute]) by the microdialysis probes. This allowed us to apply the in vivo recovery from the calibration studies to the dialysate data in the experimental studies and to express substrate dialysate data as absolute interstitial concentrations.

Insulin infusion study. Insulin (3mU/kg per min) was infused intravenously for 3 h combined with a variable glucose (dextrose 20% [wt/vol]) infusion designed to attain euglycemia (5 mM) (13). Plasma samples for glycerol, amino acids, insulin and catecholamines were collected at 30-min intervals during the 1-h baseline and 3-h insulin infusion and further plasma samples were collected at 5-min intervals for measurement of glucose. One microdialysis probe was inserted into each tissue bed for the collection of dialysate and hourly samples were collected at baseline and during insulin infusion for measurement of glucose, glycerol and amino acids.

Desipramine perfusion study. One microdialysis probe was inserted into each tissue bed for the collection of dialysate and Desipramine HCl (100 μM) was added to the microdialysis perfusate in both muscle and adipose tissues at the outset of the study and perfused at 2.5 μl/min for 2 h. Plasma and dialysate samples were collected for measurement of glycerol and catecholamines, and were compared with results from the control study.

Substrate and hormone measurements. Dialysate glycerol, glucose and amino acids were measured in duplicate 20-μl aliquots, and catecholamines were measured in duplicate 50-μl aliquots. Plasma and dialysate concentrations of glycerol and glucose were measured by enzymatic assays (Sigma Diagnostics, St. Louis, MO) and total neutral and acidic amino acids by ion exchange chromatography (Dionex, Sunnyvale, CA). Plasma and dialysate catecholamines by a radioenzymatic assay

Table I. Basal Concentrations (mM) of Glycerol, Glucose, and Total Amino Acids in Plasma and Tissue (Muscle and Adipose) Dialysate during the Control, Calibration, and Insulin Infusion Studies

Substrate	Site	Control	Calibration	Insulin infusion
Glycerol	Plasma	0.08±0.01	0.09±0.01	0.08±0.01
	Adipose	0.63±0.10	0.74±0.09	0.61±0.08
	Muscle	0.61±0.14	0.70±0.11	0.57±0.08
Glucose	Plasma	4.83±0.11	4.83±0.11	4.72±0.08
	Adipose	0.71±0.08	0.69±0.07	0.78±0.04
	Muscle	0.63±0.04	0.80±0.12	0.63±0.04
Total amino acids	Plasma	2.09±0.07	1.99±0.09	1.83±0.08
	Adipose	0.44±0.02	0.41±0.03	0.37±0.02
	Muscle	0.49±0.07	0.48±0.06	0.34±0.04

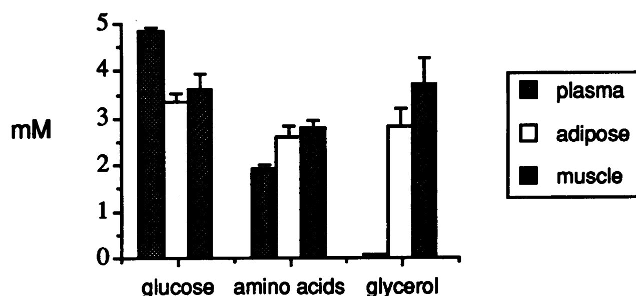

Figure 1. Fasting concentrations (mM) of glucose, amino acids, and glycerol in arterialized plasma (*cross-hatched bars*) and the interstitial space of skeletal muscle (*hatched bars*) and adipose tissue (*open bars*).

(Amersham International plc, UK). The respective intra-assay coefficient of variation (CV), inter-assay CV and limits of detection were as follows: glucose (< 1%, < 1% and 14 μM), glycerol (< 1%, 8.3%, 20 μM), individual amino acids (range 0.5–2.0%, 2–10%, 1–2 μM), epinephrine (18%, 10%, 3 pg/ml), norepinephrine (11%, 5%, 3 pg/ml). Plasma insulin was measured by radioimmunoassay (Diagnostic Systems Laboratories Inc., Webster, TX).

Statistics. Comparisons of baseline values between studies, time dependent changes during the control study and treatment differences between the control, insulin infusion and desipramine studies were done by two way analysis of variance (ANOVA) for repeated measures using the statistical package BMDP (BMDP statistics software, Los Angeles, CA). Data were sphericity tested to determine normal distribution and non-normal data was corrected by Huynd-Feldt factor. The exact level of significance at individual time points was calculated using a paired *t* test, using the variance term for the interaction from ANOVA and a Bonnferoni correction applied for multiple testing. Analysis of net flux data was done by simple linear regression and zero flow rate data by non linear regression, and in both cases regression analyses were tested for goodness of fit. Unless otherwise indicated, results in the text, figures and tables are presented as the mean±standard error of the means (SEM). Tissue substrate data are expressed as dialysate concentrations in Table I, whereas the remaining muscle and adipose interstitial substrate data are expressed in text, tables and figures as estimates of absolute concentrations. Tissue catecholamine data are expressed as dialysate concentrations as we did not estimate absolute interstitial concentrations of catecholamines during the calibration study.

Results

Magnetic resonance images. Total thickness of skin and subcutaneous tissue at the two sites of microdialysis catheter insertion were as follows: abdomen (mean 12.3±2.2 mm and range 3.8–26.8 mm) and leg (mean 4.3±1.0 mm and range 2.0–12.4 mm). In each case, the leg catheter was inserted at right angles to the skin surface to a depth of 35 mm in the anterolateral aspect of the thigh. As the dialysis chamber was situated in the distal 20 mm of the catheter we concluded that in all cases the dialysis chamber of the skeletal muscle catheter was positioned in the body of the quadricep muscle and not in subcutaneous fat. The oblique insertion of the adipose probe into the body of subcutaneous fat of the abdominal wall assured appropriate positioning in adipose tissue. 2 of the 10 subjects (both female) had measurable intermuscular adipose tissue within the quadriceps muscle where fat pixels accounted for 15 and 21% of the total pixels in muscle respectively, whereas the remaining subjects had no measurable intermuscular adipose tissue. The two subjects with intermuscular adipose tissue did not differ from the remainder of the group either by morphometric or basal biochemical indices, or with regard to responses to the experimental interventions.

Control and calibration studies. There was no significant difference in baseline measurements of plasma and dialysate glycerol, glucose and total amino acids between studies (Table I) and during the 5 h control study there was no change with time in plasma and dialysate levels of glycerol, glucose, amino acids and catecholamines, nor with plasma levels of insulin (data not shown). As shown in Fig. 1, the calculated absolute interstitial concentrations of glycerol in muscle and adipose tissue were 3.7±0.5 (range 1.2–7.7) mM and 2.8±0.4 (range 2.0–3.5) mM, respectively. There was no statistical difference between the two tissue beds, but the tissue concentrations were markedly higher than that measured in arterialized plasma (0.087±0.008 mM). Interstitial glucose concentrations in muscle and adipose tissue were 3.6±0.2 (range 2.1–4.8) and 3.3±0.3 (range 2.0–4.5) mM, respectively, and again there was no difference in concentrations between the two tissue beds. However, the levels calculated for interstitial glucose were significantly lower than those measured in arterialized plasma (4.83±0.11 mM). Total amino acid concentrations in muscle and adipose tissue were similar, and were significantly higher than plasma concentrations (Fig. 1). Table II lists the individual amino acid concentrations in plasma and the interstitial space of muscle and adipose tissue and highlights significant differences between the two tissue beds and plasma. In particular, levels of taurine, glutamine and alanine (Fig. 2), as well as glutamate and phenylalanine were significantly higher in muscle interstitium than in adipose tissue and plasma. Aspartate and serine concentrations, on the other hand, were higher in adipose tissue than in muscle and plasma.

Insulin infusion and desipramine perfusion studies. During the insulin infusion study, plasma insulin increased from 8±2 μU/ml (basal) to 215±10 μU/ml (infusion) and was stable throughout the infusion period. Plasma levels of epinephrine did not change from basal values (40±9 pg/ml) and there was a small but insignificant rise in norepinephrine above baseline (from 236±29 pg/ml to 277±47 pg/ml after 180 min). As shown in Fig. 3, the variable glucose infusion maintained plasma glucose at 5 mM and adipose and muscle interstitial concentrations of glucose did not change from respective basal values during the study. The insulin infusion produced a 55%

Table II. Plasma and Interstitial (Adipose and Muscle) Concentrations (μM) of Amino Acids Estimated during the Calibration Study

Amino acid	Plasma	Adipose	Muscle
Alanine	244±27	248±22	333±35*‡
Asparagine	45±3	52±3	56±4*
Aspartate	9±1	62±6*§	43±4*
Citrulline	28±1	51±5*	48±11*
Cysteine	49±2	34±4	34±4
Glutamate	42±4	43±8	66±9*‡
Glutamine	506±30	529±56	672±80*‡
Glycine	194±15	565±92*	400±48*
Isoleucine	53±5	65±7	70±10
Leucine	128±6	149±12	165±19*
Methionine	20±2	18±3	14±2
Phenylalanine	50±2	58±3*	72±7*‡
Serine	98±7	195±19*§	125±9*
Taurine	29±2	76±12*	165±19*‡
Threonine	124±9	163±6*	185±17*
Tyrosine	53±3	54±4	62±15
Valine	216±10	222±14	257±25
BCAA	396±19	436±30	492±53*
Total	1914±83	2613±152*	2800±213*

BCAA, branch chain amino acids (isoleucine, leucine, valine). * Higher than plasma concentration ($P < 0.05$). ‡ Higher than interstitial adipose concentration ($P < 0.05$). § Higher than interstitial muscle concentration ($P < 0.05$).

reduction in plasma glycerol concentration and glycerol levels in muscle and adipose interstitium declined in a similar fashion (Fig. 4). Total amino acid levels in plasma were also suppressed by insulin ($P < 0.01$) as were interstitial concentrations of amino acids in muscle ($P < 0.05$) and adipose tissue ($P < 0.01$) (percent of baseline: plasma 68±6, muscle 78±7, adipose 70±11). Individual amino acids were also suppressed by insulin in a similar proportional fashion in plasma and both tissues. Local perfusion of both tissue beds with desipramine resulted in interstitial glycerol concentrations that were five-fold higher (muscle and adipose tissue, $P < 0.001$) and dialysate norepinephrine levels that were twofold higher (muscle and adipose tissue, $P < 0.01$) (Fig. 5) than respective levels observed during the control study. Also, the local perfusion of both tissue beds with desipramine had no significant effect on

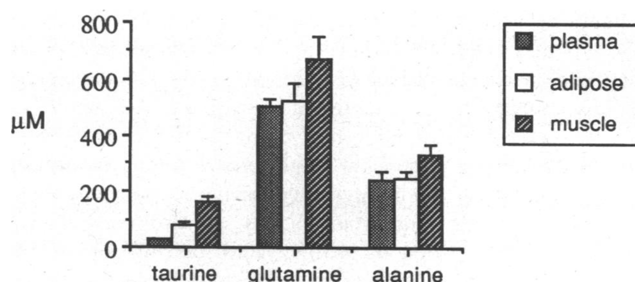

Figure 2. Fasting concentrations (μM) of taurine, glutamine, and alanine in arterialized plasma (*cross-hatched bars*), and the interstitial space of skeletal muscle (*hatched bars*) and adipose tissue (*open bars*).

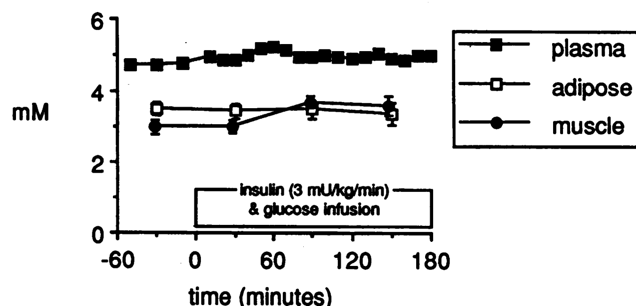

Figure 3. Glucose concentration (mM) in arterialized plasma (*closed squares*) and the interstitial space of skeletal muscle (*closed circles*) and adipose tissue (*open squares*) at baseline (−60 to 0 min) and at each hour during hyperinsulinemia (0 to 180 min).

concentrations of glycerol and catecholamines in plasma, nor with dialysate concentrations of epinephrine (data not shown).

Discussion

In the current study we used a novel microdialysis system that allowed us to measure the concentrations of key metabolic substrates in the interstitial space of skeletal muscle as well as adipose tissue in healthy human volunteers. Our findings are most notable for the markedly elevated glycerol levels we observed in the interstitial fluid of skeletal muscle when related to the levels in plasma and adipose tissue interstitium. This finding is unexpected, first, because the 30–40-fold concentration difference between the interstitial space and plasma was

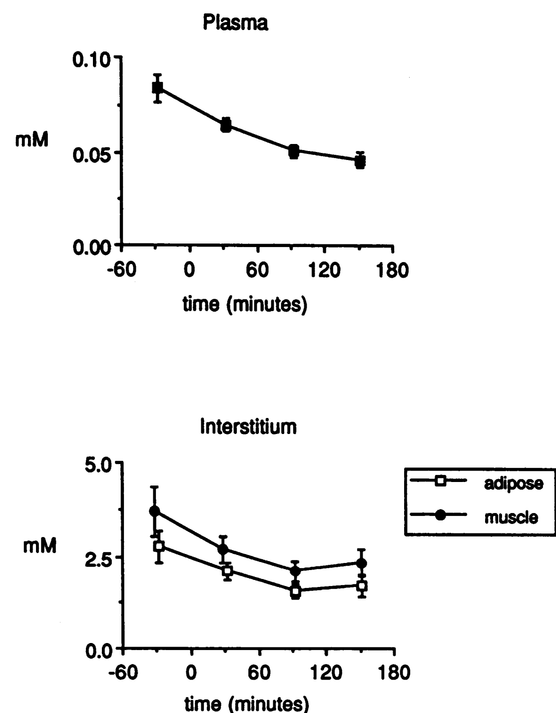

Figure 4. Glycerol concentration (mM) in arterialized plasma (*closed squares*) and the interstitial space of skeletal muscle (*closed circles*) and adipose tissue (*open squares*) at baseline (−60 to 0 min) and at each hour during hyperinsulinemia (0 to 180 min).

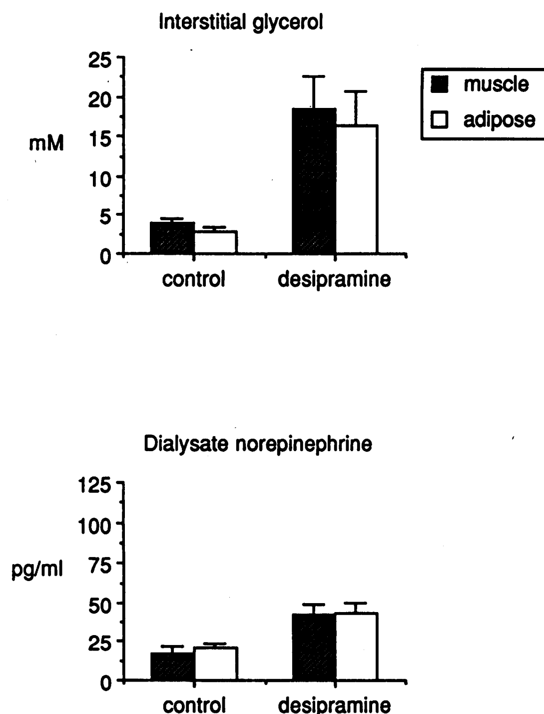

Figure 5. Interstitial glycerol (mM) and dialysate norepinephrine (pg/ml) concentrations in the interstitial space of skeletal muscle (*hatched bars*) and adipose (*open bars*) tissue during the control and desipramine perfusion studies.

much greater than the relative concentration differences we determined for other substrates; and secondly, glycerol concentrations in muscle were as elevated as the levels in adipose tissue. Such high interstitial glycerol concentrations are probably due to the existence of triacylglycerol hydrolysis and reuptake within both muscle and adipose tissue (14). This is potentially advantageous as it amplifies the potential resources to mobilize free fatty acids under conditions of stress (15), sympathetic nervous stimulation (16) or fasting (17). The high concentration difference between the interstitial space of adipose tissue and the circulation is consistent with the large arteriovenous difference for glycerol (arterial ~ 50 μM, venous ~ 200 μM) seen across adipose tissue of fasted humans (3) resulting from the hydrolysis of intracellular triacylglycerol by hormone sensitive lipase. The free fatty acids released in concert are either oxidized intracellularly or passed into the systemic circulation, whereas the majority of glycerol is released and not used locally because of the weak activity of glycerol kinase in adipose tissue (18). Other workers have estimated concentrations of glycerol in the interstitial space of human adipose tissue using microdialysis and have reported interstitial levels that were much closer to plasma concentrations (7, 19, 20) than those reported here. The discrepancy between our findings and these studies may be explained by fundamental differences in the physical properties of the respective microdialysis systems including the dialysis membrane, the size and dimensions of the probe, the nature of the perfusate and the flow rate. For example, Arner et al. (19) used a microdialysis system with a dialysis membrane which had a higher molecular weight cut-off (20,000 daltons) and a fourfold larger cross-sectional area, Ringer's solution as the perfusate which they perfused at a higher rate (5.0 μl/min) and a lower calculated in vivo recovery of glycerol (7%). Without direct comparisons of the respective microdialysis systems, it is difficult to ascertain why our estimations of interstitial glycerol should be so much higher than those reported by others, however, it is interesting to note that our microdialysis probe has a smaller cross-sectional area than those used by others. It is likely that smaller probes cause less of a perturbation of the interstitial environment and minimal depletion of local substrates which are more readily replenished by convection from adjacent tissues, thus allowing closer equilibration with the interstitial space. Whereas, larger probes create a potential for a false space around the probe that reaches a closer equilibrium with the more dynamic microcirculation rather than the slower mixing interstitial pool. In addition, we are confident that the high dialysate glycerol levels we observed are not an artifact of our "in-house" method of probe construction. In vitro testing failed to demonstrate that the collecting system or the membrane itself were sources of contaminant glycerol. Also, we have used the same probes in human brain, a tissue where one would expect negligible lipolytic activity, and are unable to detect glycerol in the dialysate (unpublished data) suggesting that the high glycerol we observe in adipose and muscle dialysate does indeed arise from the tissues.

The observation that glycerol levels within muscle interstitium were as high as levels in adipose tissue is particularly noteworthy. While these findings were unexpected, recent studies have confirmed earlier suggestions that there are significant fat deposits in skeletal muscle. Using proton magnetic resonance spectroscopy of human skeletal muscle, two lipid compartments containing free fatty acids and triacylglycerol have been identified (21). One compartment is thought to reflect adipose tissue within the body of the muscle (intermuscular adipose tissue), as it had similarities with subcutaneous adipose tissue, and the other was attributed to the presence of intracellular triacylglycerol stores. Metabolic studies also suggest that skeletal muscle has lipid stores which are an important source of energy during fasting and exercise. In the fasting state, skeletal muscle derives most of its energy from fat oxidation (1), yet the total lipid oxidation that occurs is substantially greater than the free fatty acid supply from the circulation, implying that lipid is oxidized from local muscle stores (22). Moreover, studies using labelled isotopes indicate that human skeletal muscle has a high turnover of both fatty acids and glycerol (23) and, more specifically, that there is a high glycerol exchange across the forearm in the absence of a significant efflux of glycerol (5), findings that might be expected to lead to the high glycerol concentrations we observed in the interstitial space of muscle. Although little is known about the metabolism of glycerol in muscle, the combination of high interstitial concentrations and low net efflux suggests that the rate of local metabolism must be substantial. Glycerol may be phosphorylated by glycerol kinase, an enzyme that exists in skeletal muscle of vertebrates (24), but its specific activity remains to be established in humans. Alternatively, muscle glycerol consumption may occur through phosphorylation by pathways independent of glycerol kinase. This occurs in adipose tissue (25), but evidence for the existence of such pathways in muscle is uncertain. Finally, glycerol may also be used through the action of local glycerol dehydrogenase, an enzyme that has been isolated in human muscle (26).

The great disparity between glycerol levels in muscle interstitium and plasma raises the question of whether the muscle probe was positioned correctly. However, MRI determined local

anatomy and the paucity of subcutaneous fat at the point of insertion confirmed that the muscle probes were positioned in muscle rather than subcutaneous fat. Practical constraints did not allow us to discern whether the glycerol in muscle dialysate had arisen from muscle tissue itself or from intermuscular adipose tissue that was "marbled" within the muscle body. To minimize the possible influence that intermuscular adipose tissue may have had on our findings, we purposefully studied lean subjects. Despite this, 2 of the 10 subjects had detectable intermuscular adipose tissue, indicating the presence of significant fat marbling. However, this had little impact on our findings as their data was no different from the remainder of the group. This would suggest that either intermuscular adipose tissue was a minor contributory factor to the high levels of glycerol in muscle interstitium or that intermuscular adipose tissue was present in all subjects, and the major source of glycerol, but was not detectable by our scanning method. It is intriguing to speculate that the presence of intermuscular adipose tissue in obese subjects may result in even higher interstitial glycerol levels in muscle. Such changes might be expected to interfere with insulin stimulated glucose metabolism (27) and contribute to the insulin resistance so commonly observed in obese subjects.

Further support for the presence and significance of high rates of intramuscular lipolysis is provided by the dynamic changes in glycerol concentration within the interstitial space of muscle during the insulin and desipramine studies. In humans, earlier studies using microdialysis have demonstrated potent effects of insulin (8, 28) and altered sympathetic nervous activity (8, 29, 30) on glycerol levels in adipose tissue. Our data indicate that muscle lipid responds qualitatively to the opposing effects of insulin and sympathetic nervous stimulation in a nearly identical fashion. During hyperinsulinemia, interstitial glycerol decreased in skeletal muscle as well as adipose tissue, suggesting suppressed lipolysis. Whereas, the local perfusion of both tissue beds with desipramine, a norepinephrine reuptake blocker, produced a rapid fivefold elevation in interstitial glycerol, indicating a marked increase in local lipolysis in both tissues. The specific metabolic processes through which insulin might reduce interstitial glycerol in muscle include: an increase in glycerol utilization via phosphorylation followed by either oxidation or glycogen synthesis, an increase in free fatty acid esterification or a suppression of triacylglycerol hydrolysis. However, in the absence of a knowledge of the existence, function and regulation of the enzymes in muscle necessary to facilitate glycerol utilization (see earlier) or triacylglycerol hydrolysis/re-esterification this question cannot be resolved with any certainty. It has been established that adipose tissue lipolysis is under tight neural and hormonal control whereby sympathetic stimulation increases lipolysis by activating an intracellular hormone sensitive lipase (HSL) by the stimulation of a cAMP-dependent protein kinase (31). Conversely, insulin inhibits the phosphorylation and therefore the activity of HSL, in part, by reducing cAMP levels (32). Only recently, this same enzyme has been identified and characterized in small quantities in muscle tissue (33, 34) and it is hypothesized that it plays a role in intracellular triacylglycerol hydrolysis working in concert with extracellular muscle lipoprotein lipase on the endothelial surface (35). However, the physiological importance and the potential regulation of muscle HSL has yet to be characterized. In the rat heart (36) and isolated myocytes (37) an adrenergic and therefore cAMP dependent activation of intracellular lipase has been identified but the local regulatory effect of insulin in muscle has not been determined.

It is also important to consider the influence locally altered blood flow has on these data as it is recognized that in vivo recovery of substrates by microdialysis is potentially influenced by changes in local blood flow as well as local metabolic activity. This has been highlighted by an adaptation of the microdialysis technique using ethanol clearance from microdialysis perfusate as an estimate of local nutritive blood flow (i.e., increased ethanol clearance indicates increased local blood flow) (38). Accepting the qualitative nature of this technique, ethanol clearance in human thigh muscle and adipose tissue (39) is altered by the local administration of vasoconstrictor and vasodilator agents (via the microdialysis probe) or arterial occlusion, and that the marked alterations in local blood flow produced are positively correlated with relatively small changes in in vivo glucose recovery of glucose. The question of how local blood flow alters in vivo recovery of glycerol has not been directly addressed although it is theorized (40) that increases in local blood flow might increase the clearance of glycerol from the tissue bed thereby lowering in vivo recovery. In the absence of a measure of blood flow in this study, it is uncertain to what degree blood flow changes may influence dialysate glycerol during insulin and desipramine administration. In muscle, the fall in dialysate glycerol during hyperinsulinemia might be attributed, in part, to an insulin-mediated increase in muscle blood flow which has been reported in some (41) but not all (42) studies. However, this possibility is difficult to reconcile with muscle arteriovenous changes that occur during hyperinsulinemia (42): if the observed reduction in interstitial levels of glycerol occurs predominantly through an increase in blood flow and not through suppressed lipolysis then one would expect an increase in glycerol efflux from the tissue bed during hyperinsulinemia. In addition, it is likely that the changes in muscle blood flow following insulin administration are more subtle than the marked changes in blood flow induced in the above studies (38, 39) where some impact on in vivo recovery was reported for glucose. Thus, insulin-mediated vascular changes probably had little effect on glycerol recovery from muscle interstitium. In adipose tissue, it seems even less likely that insulin-mediated blood flow changes may confound the glycerol data. Indeed, studies have shown that insulin has little or no effect on local blood flow (43, 44) while lowering interstitial glycerol by $\sim 50\%$ (44). In both tissues, locally perfused desipramine induced a significant increase in norepinephrine accompanied by a large increment in local glycerol. This finding is consistent with norepinephrine's beta-adrenergic stimulatory properties and is corroborated by the fact that local perfusion of adipose tissue with norepinephrine induces a similar large increment in glycerol in adipose tissue (30). It should be emphasized that a change in glycerol of this magnitude ($\sim$ fivefold) could only be accounted for by a change in metabolic activity since accompanying changes in blood flow (whether alpha- or beta-adrenergic mediated) would be expected to have a minor effect on dialysate glycerol measurements.

An additional potential limitation of the study relates to the fact that we used the in vivo recovery of substrates calculated during the calibration procedure to "derive" interstitial concentrations in separate experiments. However, basal dialysate concentrations were equal at all visits (except for the desipramine study where desipramine was added at the outset and appropriate comparisons could not be made) suggesting that studies

started under similar basal conditions. Also, the control study confirmed that there was no time-dependent shift from baseline in both plasma and dialysate levels of any substrate, thus suggesting that the experiments were carried out under stable conditions and that there was no significant depletion of substrates by the microdialysis probes from the tissues.

We also observed substantial concentration gradients between the interstitial space and the circulation for glucose and amino acids. Both muscle and adipose interstitial glucose levels were 60–70% of arterialized plasma values, data that are in keeping with glucose levels measured in human adipose tissue by a variety of techniques, including microdialysis with direct glucose sensing, ultrafiltration and equilibration (45). In all cases interstitial glucose was estimated to be ~ 50% of concurrent plasma values and this supports the concept of glucose extraction by peripheral tissues, even under fasting conditions (46). The presence of such a concentration gradient for glucose has important implications for the use of glucose sensing devices positioned in the subcutaneous space. However, in contrast to our findings, other studies have measured interstitial glucose by microdialysis and estimate concentrations near or equal to plasma venous levels (6, 47–49). Again these discrepancies may reflect differences between the microdialysis systems in those studies and the probes used in this study, or may be in part explained by the comparisons they drew with venous plasma levels of glucose which is known to be ~ 10% lower than arterial levels (50). The existence of concentration gradients is further underlined by the large concentration gradients we observed between the interstitial space and plasma for some key amino acids. However, the concentration gradients for these amino acids were reversed, in keeping with efflux of amino acids from the tissue beds. Specifically, taurine levels were two fold higher in muscle than in adipose tissue. Taurine is thought to play an important osmoregulatory role in skeletal muscle (51), and high levels have been reported in the intracellular (52) and interstitial space of muscle (53). Also, glutamine and alanine levels in muscle interstitium were significantly higher than the concentrations in adipose interstitium and plasma. These findings are consistent with organ balance studies (54–56) demonstrating that glutamine and alanine account for a major proportion of the amino acids released by skeletal muscle and provide further evidence that the dialysate substrate concentrations were representative of the interstitial space of the muscle bed.

To conclude, we have characterized the metabolic milieu of the interstitial space of human skeletal muscle and adipose tissue, two important sites of regulatory fuel metabolism. Under fasting conditions, levels of important fuel substrates within peripheral insulin sensitive tissues are quite different from their respective plasma concentrations but are in keeping with other studies demonstrating glucose extraction by both tissues, glycerol efflux from adipose tissue, and the release of some key amino acids from skeletal muscle. In both tissues, insulin lowers interstitial levels of glycerol as well as amino acids, whereas, local norepinephrine reuptake blockade results in a dramatic increase in interstitial glycerol concentrations. The presence of high glycerol concentrations in the interstitial space of muscle raises the interesting possibility that muscle tissue is bathed in a lipid rich medium which is regulated by local hormonal changes and autonomic system activity and acts as a direct source of energy independent of the circulation. In view of the fact that lipid metabolism is thought to affect other metabolic processes in muscle such as glucose (27) and amino acid (57) metabolism, our findings further highlight the possible impact such a lipid rich milieu has on local muscle fuel metabolism in health and disease.

Acknowledgments

We are grateful for the invaluable assistance of the staff of the General Clinical Research Center, Aida Groszmann and Andrea Belous of the Core Laboratory for hormone analysis and Karen Davis for the construction of the microdialysis probes. We are also thankful for the assistance of Sarah Maggs for the preparation of the manuscript.

This work was supported in part by grants from the National Institutes of Health (DK-20495, RR-125, and DK-45735) and the American Diabetes Association.

References

1. Zierler, K. 1976. Fatty acids as substrates for heart and skeletal muscle. *Circ. Res.* 38:459–63.

2. Coppack, S., K. Frayn, S. Humphreys, P. Whyte, and T. Hockaday. 1990. Arteriovenous differences across human adipose and forearm tissues after overnight fast. *Metabolism.* 39:384–90.

3. Coppack, S., R. Fisher, G. Gibbons, S. Humphreys, M. Mcdonough, J. Potts, and K. Frayn. 1990. Postprandial substrate depositions in human forearm and adipose tissues in vivo. *Clin. Sci.* 79:339–48.

4. Simonsen, L., J. Bulow, J. Madsen, F. Hermensen, and A. Astrup. 1993. Local forearm and whole body respiratory quotient in humans after an oral glucose load. *Acta Physiol. Scand.* 147:69–75.

5. Elia, M., K. Kahn, G. Calder, and A. Kurpal. 1993. Glycerol exchange across the human forearm assessed by a combination of tracer and arteriovenous exchange techniques. *Clin. Sci.* 84:99–104.

6. Lonnroth, P., P.-A. Jansson, and U. Smith. 1987. A microdialysis method allowing characterisation if intercellular water space in humans. *Am. J. Physiol.* 253:E228–31.

7. Jansson, P., U. Smith, and P. Lonnroth. 1990. Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. *Am. J. Physiol.* 258:E918–22.

8. Hagstrom-Toft, E., P. Arner, U. Johansson, L. Eriksson, U. Ungerstedt, and J. Bolinder. 1992. Effect of insulin on human adipose tissue metabolism in situ. Interactions with beta-adrenoceptors. *Diabetologia.* 35:664–70.

9. McGuire, E., J. Helderman, J. Tobin, R. Andres, and R. Bergman. 1976. Effects of arterial versus venous sampling on glucose kinetics in man. *J. Appl. Physiol.* 41:65–73.

10. During, M., and D. Spencer. 1992. Adenosine: A potential mediator of seizure arrest and postictal refractoriness. *Ann. Neurol.* 32:618–624.

11. Staten, M., W. Totty, and W. Kohrt. 1989. Measurement of fat distribution by magnetic resonance imaging. *Invest. Radiol.* 24:345–349.

12. Jacobson, I., M. Sandberg, and A. Hamberger. 1985. Mass transfer in brain dialysis devices-a new method for the estimation of extracellular amino acids concentration. *J. Neurosci. Meth.* 15:263–268.

13. De Fronzo, R., J. Jordan, T. Andres, and R. Andres. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* 237:E214–223.

14. Tagliaferro, A., S. Dobbin, R. Curi, B. Leighton, L. Meeker, and E. Newsholme. 1990. Effects of diet and exercise on the in vivo rates of the triglyceride-fatty acid cycle in adipose tissue and muscle of the rat. *Int. J. Obesity.* 14:957–971.

15. Newsholme, E. 1977. The regulation of intracellular and extracellular fuel supply during sustained exercise. *Ann. NY Acad. Sci.* 301:81–91.

16. Brooks, B., and J. Arch. 1983. Effect of some hormones on the rate of triacylglycerol/fatty-acid substrate cycling in adipose tissue of the mouse in vivo. *Biosci. Rep.* 3:263–7.

17. Wolfe, R., E. Peters, S. klein, O. Holland, J. Rosenblatt, and H. Gary. 1987. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am. J. Physiol.* 252:E189–196.

18. Vaughan, M. 1961. The metabolism of adipose tissue in vivo. *J. Lipid Res.* 2:293–316.

19. Arner, P., E. Kriegholm, P. Engfeldt, and J. Bolinder. 1990. Adrenergic regulation of lipolysis in situ at rest and during exercise. *J. Clin. Invest.* 85:893–898.

20. Jansson, P., A. Larsson, U. Smith, and P. Lonnroth. 1992. Glycerol production in subcutaneous adipose tissue in lean and obese humans. *J. Clin. Invest.* 89:1610–1617.

21. Schick, F., B. Eismann, W.-I. Jung, H. Bongers, M. Bunse, and O. Lutz.

1993. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Mag. Res. Med.* 29:158–167.

22. Dagenais, G., R. Tancredi, and K. Zierler. 1976. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. *J. Clin. Invest.* 58:421–431.

23. Coppack, S., R. Judd, and J. Miles. 1993. Uptake and release of glycerol and free fatty acids by human adipose and muscle tissue in vivo. *Diabetes.* 42 (Suppl. 1):38A (Abstract).

24. Newsholme, E., and K. Taylor. 1969. Glycerol kinase activities from vertebrates and invertebrates. *Biochem. J.* 112:465–474.

25. Vaughan, M., and D. Steinberg. 1965. Glyceride biosynthesis, glyceride breakdown and glycogen breakdown in adipose tissue: mechanisms and regulation. *In* Handbook of Physiology. A. E. Renold and G. F. Cahill, editors. American Physiological Society (section 5, Adipose Tissue). Washington, DC. 239–251.

26. Hagenfeldt, L., and J. Wahren. 1968. Human forearm metabolism during exercise. II. Uptake, release and oxidation of individual fatty acids and glycerol. *Clin. Lab. Invest.* 21:263–276.

27. Randle, P., P. Garland, C. Hales, and E. Newsholme. 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* i:785–789.

28. Hagstrom-Toft, E., P. Arner, B. Naslund, U. Ungerstedt, and J. Bolinder. 1991. Effects of insulin deprivation and replacement on in vivo subcutaneous adipose tissue substrate metabolism in humans. *Diabetes.* 40:666–672.

29. Arner, P., E. Kriegholm, and P. Engfeldt. 1990. In situ studies of catecholamine-induced lipolysis in human adipose tissue using microdialysis. *J. Pharm. Exp. Ther.* 254:284–288.

30. Arner, P., E. Kriegholm, and P. Engfeldt. 1991. In vivo interactions between beta-1 and beta-2 adrenoceptors regulate catecholamine tachyphylaxia in human adipose tissue. 259:317–322.

31. Nilsson, N., P. Stralfors, G. Fredrikson, and P. Belfrage. 1980. Regulation of adipose tissue lipolysis: effects of noradrenaline and insulin on phosphorylation of hormone-sensitive lipase and on lipolysis in intact rat adipocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 111:125–130.

32. Londos, C., R. Honnor, and G. Dhillon. 1985. cAMP-dependent protein kinase and lipolysis in rat adipocytes. III. Multiple modes of insulin regulation of lipolysis and regulation of insulin responses by adenylate cyclase regulators. *J. Biol. Chem.* 260:15139–15145.

33. Holm, C., P. Belfrage, and G. Fredrikson. 1987. Immunological evidence for the presence of HSL in rat tissue other than adipose tissue. *Biochem. Biophys. Res. Commun.* 148:99–105.

34. Small, C., A. Garton, and S. Yeaman. 1989. The presence and role of hormone-sensitive lipase in heart muscle. *Biochem. J.* 258:67–72.

35. Oscai, L., D. Essig, and K. Palmer. 1990. Lipase regulation of muscle triglyceride hydrolysis. *J. Appl. Physiol.* 69:1571–1577.

36. Palmer, W., R. Caruso, and L. Oscai. 1981. Possible role of lipoprotein lipase in the regulation of endogenous triacylglycerols in the rat heart. *Biochem. J.* 198:159–166.

37. Palmer, W., and T. Kane. 1983. Hormone-stimulated lipolysis in cardiac myocytes. *Biochem. J.* 216:241–243.

38. Hickner, R., H. Rosdahl, I. Borg, U. Ungerstedt, L. Jorfeldt, and J. Henriksson. 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 Physiol. Scand.* 143:355–356.

39. Rosdahl, H., U. Ungerstedt, L. Jorfeldt, and J. Henriksson. 1993. Interstitial glucose and lactate balance in human skeletal muscle and adipose tissue studied by microdialysis. *J. Physiol. (Lond.).* 471:637–657.

40. Hagstrom-Toft, E., P. Arner, H. Wahrenberg, A. Wennlund, U. Ungerstedt, and J. Bolinder. 1993. Adrenergic regulation of human adipose tissue metabolism in situ during mental stress. *J. Clin. Endocrinol. Metab.* 76:392–398.

41. Laakso, M., S. Edelman, G. Brechtel, and A. Baron. 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J. Clin. Invest.* 85:1844–1852.

42. Natali, A., G. Buzzigoli, S. Taddei, D. Santora, M. Corri, R. Pedrinelli, and E. Ferrannini. 1990. Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes.* 39:490–500.

43. Madsen, J., and A. Malchow-Moller. 1983. Effects of glucose, insulin and nicotinic acid on adipose tissue blood flow in rats. *Acta Physiol. Scand.* 118:175–180.

44. Johansson, U., P. Arner, J. Bolinder, E. Hagstrom-Toft, U. Ungerstedt, and L. Eriksson. 1993. Influence of insulin on glucose metabolism and lipolysis in adipose tissue in situ in patients with liver cirrhosis. *Eur. J. Clin. Invest.* 23:837–844.

45. Schmidt, F., W. Sluiter, and A. Schoonen. 1993. Glucose concentration in subcutaneous extracellular space. *Diab. Care.* 16:695–700.

46. Kelley, D., J. Reilly, T. Veneman, and L. Mandarino. 1990. Effects of insulin on skeletal muscle glucose storage, oxidation, and glycolysis. *Am. J. Physiol.* 258:E923–929.

47. Jansson, P., J. Fowelin, U. Smith, and P. Lonnroth. 1988. Characterisation by microdialysis of intercellular glucose level in subcutaneous tissue in humans. *Am. J. Physiol.* 255:E218–220.

48. Bolinder, J., E. Hagstrom, U. Ungerstedt, and P. Arner. 1989. Microdialysis of subcutaneous adipose tissue in vivo for continuous glucose monitoring in man. Scand. *J. Clin. Lab. Invest.* 49:465–474.

49. Bolinder, J., U. Ungerstedt, and P. Arner. 1992. Microdialysis measurements of the absolute glucose concentration in subcutaneous adipose tissue allowing glucose monitoring in diabetic patients. *Diabetologia.* 35:1177–1180.

50. Liu, D., E. Moberg, M. Kollind, P.-E. Lins, U. Adamson, and I. Macdonald. 1992. Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. *Diabetologia.* 35:287–290.

51. Nieminen, M.-L., L. Tuomisto, E. Solatunturi, L. Eriksson, and M. Paasonen. 1988. Taurine in the osmoregulation of the Brattleboro rat. *Life Sci.* 42:2137–2143.

52. Arfvidsson, B., H. Zachrisson, A.-C. Moller-Loswick, A. Hyltander, R. Sandstrom, and K. Lundholm. 1991. Effect of systemic hyperinsulinemia on amino acid flux across human legs in postabsorptive state. *Am. J. Physiol.* 260:E46–52.

53. Lehmann, A. 1989. Effects of microdialysis-perfusion with anisoomotic media on extracellular amino acids in the rat hippocampus and skeletal muscle. *J. Neurochem.* 53:525–535.

54. Felig, P., and J. Wahren. 1971. Amino acid metabolism in man. *J. Clin. Invest.* 50:2703–2714.

55. Elia, M., G. Neale, and G. Livesy. 1985. Alanine and glutamine release from the human forearm: effects of glucose administration. *Clin. Sci.* 69:123–133.

56. Frayn, K., K. Khan, S. Coppack, and M. Elia. 1991. Amino acid metabolism in human subcutaneous adipose tissue in vivo. *Clin. Sci.* 80:471–474.

57. Ferrannini, E., E. Barrett, S. Bevilacqua, R. Jacob, M. Walesky, R. Sherwin, and R. DeFronzo. 1986. Effect of free fatty acids on blood amino acid levels in humans. *Am. J. Physiol.* 250:E686–694.