Effects of Insulin Deprivation and Replacement on In Vivo Subcutaneous Adipose Tissue Substrate Metabolism in Humans

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The effects of insulin deprivation and replacement on adipose tissue metabolism were investigated in vivo with microdialysis in nine insulin-dependent diabetic patients with no residual insulin secretion. Dialysis probes, implanted in abdominal subcutaneous fat, were continuously perfused, and tissue dialysate concentrations of glycerol (lipolysis index), glucose, lactate, and pyruvate were determined. Comparisons were made with respective metabolite levels in venous plasma. After termination of intravenous insulin infusion, free insulin in plasma fell from 130 to 70 pM. At the same time, glucose levels in plasma and adipose tissue rose in parallel. However, the relative increase in glucose levels was greater in adipose tissue than in blood. On the other hand, the increase in glycerol concentration in adipose tissue (35%) was markedly less than that in venous plasma (250%). Lactate and pyruvate levels in adipose tissue and blood remained unchanged. After the resumption of intravenous insulin, free insulin in plasma rose to ~600 pM. At the same time, the glucose levels in blood and adipose tissue decreased rapidly, and the glycerol concentration in these tissues decreased to 50% of the baseline levels. The lactate and pyruvate levels in subcutaneous tissue increased briefly after insulin replacement, whereas the lactate but not pyruvate levels in blood showed a similar increase. The α- or β-blocking agents phentolamine and propranolol in the ingoing tissue perfusate did not influence tissue glycerol at any time during the experiment. We concluded that insulin-induced changes in circulating metabolites only partly reflect variations in adipose tissue substrate kinetics. During insulin deprivation, glucose is accumulated in the adipose tissue extracellular compartment, probably because of reduced utilization by the adipocytes. The increase in lactate and pyruvate levels in adipose tissue after insulin replacement may be explained by local metabolite production. The lipolytic activity in abdominal subcutaneous adipose tissue is only modestly enhanced during relative insulin deficiency, which is in contrast to overall lipolytic activity. Finally, adrenergic mechanisms do not seem to be involved in the changes in the lipolysis rate, which are induced by hypoinsulinemia and hyperinsulinemia. *Diabetes* 40:666–72, 1991

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Adipose tissue substrate metabolism plays a key role in the regulation of lipid and carbohydrate metabolism in humans. Hydrolysis of adipose tissue triacylglycerols (lipolysis) is an important determinant of the circulating free–fatty acid (FFA) level. The formation of triacylglycerols in the fat cell is dependent on α-glycerophosphate derived from glucose taken up by the adipocyte (1). Moreover, it has been demonstrated in humans that a substantial part of the total glucose disposal after glucose ingestion takes place in adipose tissue (2). Recent data also suggest that human adipose tissue may be a site in which glucose is converted to lactate after glucose intake (3–5). Because postprandial glycogen synthesis in the liver appears largely to be the result of hepatic uptake of lactate and other gluconeogenic substrates (6,7), metabolism in adipose tissue may play an important role in the regulation of carbohydrate homeostasis in humans.

In adults, insulin and catecholamines are the only hormones that have acute and pronounced regulatory effects on the adipose tissue metabolism (8). Insulin stimulates the uptake and intracellular metabolism of glucose and inhibits lipolysis. Catecholamines, on the other hand, stimulate lipolysis and counteract the effect of insulin on glucose metabolism in adipose tissue.

The knowledge of the regulation of human adipose tissue metabolism is principally derived from in vitro studies or measurements of circulating metabolite levels. Recently,

however, we (4,9–11) along with others (5,12,13) introduced the microdialysis sampling technique for in situ investigations of adipose tissue substrate kinetics. With this technique, which was originally used for in situ measurements of biochemical events in brain tissue of experimental animals (for review, see ref. 14), it is possible to continuously monitor intermediary metabolites in the extracellular space of adipose tissue. Moreover, the technique offers a unique possibility to deliver metabolically active hormones or drugs locally through the microdialysis device without causing generalized systemic effects (4,9,11).

In this study, we used the microdialysis technique to investigate in situ the effects of acute insulin deficiency and subsequent replacement on subcutaneous adipose tissue substrate kinetics in humans. Microdialysis probes were implanted in the abdominal subcutaneous adipose tissue of subjects with insulin-dependent diabetes mellitus (IDDM) and no residual endogenous insulin secretion who were maintained in a euglycemic state for at least 12 h by means of a variable intravenous infusion of insulin. The probes were continuously perfused, and the outgoing tissue dialysate levels of glycerol (lipolysis index), glucose, lactate, and pyruvate were determined and compared with the corresponding metabolite concentrations in venous blood. After baseline sampling, the intravenous insulin infusion was stopped for 4 h; thereafter, it was recommenced at a fixed rate. Moreover, to evaluate the influence of adrenergic effects on insulin action, α- and β-adrenoreceptor–blocking agents were added to the tissue perfusate. The results demonstrated marked differences between substrate kinetics in adipose tissue and in blood during hypoinsulinemia and hyperinsulinemia.

RESEARCH DESIGN AND METHODS

The study group comprised six men and three women IDDM subjects, aged 28–45 yr (mean ± SE 38 ± 2 yr). Body mass index was 23.3 ± 0.5 kg/m^2. The duration of diabetes averaged 16 ± 1 yr (range 14–19 yr), and in all subjects, the fact that fasting and glucagon-stimulated C-peptide levels were undetectable shows that there was no residual endogenous insulin secretion. The subjects had nonproliferative background retinopathy, but none had proteinuria, hypertension, or autonomic neuropathy. They received no drugs except insulin. Their degree of metabolic control was fairly good; the mean HbA_{1c} was 7.7 ± 0.4% (normal range 3.5–5.0%). The study was approved by the Ethics Committee of the Karolinska Institute. The subjects were given a detailed description of the study, and their consent was obtained.

Microdialysis probe. The microdialysis probe (Carnegie Medicin, Stockholm) has previously been described in detail (15). It consisted of a double-lumen steel cannula with dialysis tubing (0.5 × 10 mm, 20,000-M_r cutoff) glued to its end. The inlet tubing of the probe was connected to a high-precision microinfusion pump (CMA/100 microinjection pump, Carnegie Medicin) and continuously perfused with a sterile solution. The perfusion fluid entered through the inner cannula, passed down to the tip of the probe, streamed upward in the space between the inner cannula and the dialysis membrane, and left the probe through the outer cannula via a side arm from which it is collected.

The in vitro and in vivo (subcutaneous adipose tissue) characteristics of the microdialysis probe have been presented in detail (4,9–11). Briefly, the relative in vitro recovery (dialysate substrate concentration vs. medium substrate concentration × 100) of glycerol, glucose, lactate, and pyruvate increased in a nonlinear fashion at decreasing perfusion velocities. However, at a given perfusion speed, the recovery for each metabolite remained constant over a wide range of substrate concentrations. The metabolite recovery in vivo in subcutaneous adipose tissue (i.e., dialysate substrate concentration vs. metabolite concentration in the extracellular compartment × 100) was lower than the recovery in vitro, which probably occurred because the dialysis membrane was exposed to less fluid in the former situation. However, during steady-state conditions, the tissue dialysate metabolite concentrations remained constant for at least 2 h, which shows, first, that there was no drainage of the substrates from the extracellular compartment to the tissue dialysate, and second, that no hyperemia or edema occurred around the microdialysis probe in the tissue being sampled. Moreover, because enzymes and other large-molecular-weight substances are too large to pass through the dialysis membrane, they are excluded from the dialysate samples. Consequently, the samples can be collected and analyzed without pretreatment, because the compounds, having passed through the dialysis membrane, are protected from enzymatic degradation (16).

The patients were admitted to the metabolic ward on the day before the study and received only soluble human insulin subcutaneously before meals. At 2000, an intravenous infusion of soluble insulin (Actrapid Human, Novo, Bagsvaerd, Denmark) was started, and the infusion rate was adjusted by frequently determining the blood glucose levels to maintain euglycemia (5–7 mM). The experiments started at 0800, after an overnight fast, and the subjects remained in the supine position during the study. A dialysis probe was inserted percutaneously, under sterile conditions, into the subcutaneous adipose tissue in the periumbilical region with a steel cannula guide. Anesthesia was unnecessary. The probe was continuously perfused with 5 μl/min Ringer's solution. In seven subjects, two additional dialysis probes were inserted into the same region, and the distance between each probe was always >3 cm. In the latter probes, adrenergic receptor–blocking agents were added as sterile solutions to the ingoing perfusate. These agents included 10^{-4} M propranolol (ICI, Chester, UK) or 10^{-4} M phentolamine (Ciba-Geigy, Basel). Addition of these concentrations of propranolol and phentolamine, respectively, to the tissue perfusate has recently been shown to markedly influence the lipolytic activity (i.e., glycerol levels) in adipose tissue at rest and during exercise (11). In each experiment, 30-min fractions of the outgoing tissue dialysate were collected for analyses of glycerol, glucose, lactate, and pyruvate. The first 30-min fraction was deleted, because methodological experiments have shown a transient rise in tissue dialysate ATP concentrations during the first 15–30 min of tissue dialysis, which is probably due to the initial trauma caused by insertion of the dialysis probe into the subcutaneous adipose tissue (10). After two 30-min baseline samplings, the intravenous insulin infusion was stopped for 4 h. It was then resumed for 2 h at a fixed rate of 0.1 U · kg^{-1} · h^{-1}. In the middle of each 30-min period, blood samples were drawn

from an indwelling polyethylene catheter placed in the cubital vein for the analyses of plasma glycerol, glucose, lactate, and pyruvate. In addition, venous blood samples were collected at −60, 0, 120, 240, and 360 min for the determination of plasma free insulin, catecholamines, and β-hydroxybutyrate. Plasma bicarbonate was measured at 0 and 240 min, respectively.

Analysis of metabolites. Routine methods were used to determine glycerol (17), glucose (18), lactate (19), pyruvate (20), free insulin (21), catecholamines (22), and β-hydroxybutyrate (23) in plasma and for the determination of glycerol (17), lactate (16), and pyruvate (16) in the dialysate.

A new ultrasensitive luminescent method was used for the assay of dialysate glucose (24). The assay was performed at 25°C in a luminometer (LKB-Wallac, Turku, Finland) and involved oxidation by glucose oxidase and mutarotation of glucose. The hydrogen peroxide formed was determined in a reaction catalyzed by hydrogen peroxidase with luminol as the electron donor. First, the sample was incubated (total vol 0.2 ml) for 10 min in 50 mM trisphosphate (pH 7.75) containing 40 U/ml glucose oxidase and 60 U/ml aldose mutarotase. Thereafter, a light-production reaction was initiated by the addition of 50 mM trisphosphate (pH 7.75), 40 μM luminol, 225 nM horseradish peroxidase, and 40 mM diethylenetriaminepentaacetic acid in a total volume of 1 ml. The light emission during the first 106 s of a 2-min incubation was determined. The assay has a linear range between 0.01 and 1 nM glucose. The coefficient of variance for duplicate determinations was only 1.6%. When the method was compared to a routine method (based on glucose oxidase and peroxidase) that was used in previous microdialysis experiments (10), the correlation coefficient for comparison of the two methods was 0.98.

The reported values are means ± SE. Student's paired *t* test was used for statistical evaluation of the results. The total areas under the curves (AUC) were calculated by trapezoidal integration.

RESULTS

After discontinuation of the insulin infusion, plasma free insulin decreased within 2 h by 50–60% ($P < 0.025$; Table 1). Plasma β-hydroxybutyrate gradually increased throughout the insulin-deficient period, and at 4 h, it was enhanced fivefold ($P < 0.005$; Table 1). At the same time, plasma bicarbonate had decreased significantly from 24.3 ± 0.6 to 22.9 ± 0.7 mM ($P < 0.05$). Two hours after the resumption of intravenous insulin administration (0.1 U · kg^{-1} · h^{-1}), the plasma free-insulin concentration had increased to ~600 pM, and the plasma β-hydroxybutyrate levels were markedly suppressed to ~33% of the basal level (Table 1). The plasma catecholamine levels remained unchanged during the insulin-deficient period and after resumption of the insulin infusion (Table 1).

The baseline plasma concentration of glucose was 6.6 ± 0.5 mM, and after the insulin infusion was stopped, it increased to a maximum of 12.5 ± 1.3 mM at 3.5 h ($P < 0.001$; Fig. 1*A*). The kinetic pattern of subcutaneous adipose tissue glucose was similar to that in blood (Fig. 1*B*), with a gradual increase in tissue dialysate glucose (200–600 μM) after insulin withdrawal. However, in relative terms, the increase in glucose in adipose tissue was larger than that in blood, and when the relative changes in glucose concentrations in plasma and adipose tissue dialysate were compared, the tissue levels were significantly higher 2–4 h after insulin withdrawal (Fig. 1*C*). Consequently, the AUC for the relative changes in glucose during the insulin-deficient period was ~25% greater in adipose tissue than in blood (43,620 ± 4819 vs. 35,258 ± 2865% × min, $P < 0.025$). When the insulin infusion was restarted, plasma and tissue dialysate glucose concentrations declined in parallel toward baseline values (Fig. 1).

The baseline plasma glycerol concentration was 42.6 ± 7 μM. After termination of the insulin infusion, plasma glycerol increased 2.5-fold within 1 h (Fig. 2*A*). Thereafter, it decreased briefly but remained at a significantly increased level throughout the insulin-deficient period ($P < 0.005$ vs. baseline concentration). Adipose tissue dialysate glycerol, on the other hand, showed only a 35% increase during the insulin-deficient period (Fig. 2*B*), and in relative terms, the increase in glycerol levels was significantly larger in blood than in adipose tissue (Fig. 2*C*). The total AUC for subcutaneous tissue glycerol was significantly smaller than that of plasma glycerol during hypoinsulinemia (29,137 ± 1467 vs. 48,612 ± 7195% × min, $P < 0.025$). When insulin was infused again, both plasma and tissue dialysate glycerol decreased rapidly, and the respective levels were significantly suppressed by ~50% ($P < 0.001$) compared with the corresponding basal levels during the last 1.5 h of the study.

Plasma lactate and pyruvate concentrations did not change significantly after the withdrawal of insulin (data not shown). Likewise, the adipose tissue dialysate concentrations of lactate and pyruvate remained constant during the insulin-deficient period (Fig. 3). After resumption of the insulin infusion, plasma lactate levels increased significantly from 399 ± 47 to 506 ± 55 and 513 ± 88 μM at 285 and 315 min, respectively ($P < 0.05$–0.01). Thereafter, plasma lactate decreased to baseline concentrations. Plasma pyruvate, on the other hand, remained unchanged after the

TABLE 1
Plasma free insulin, β-hydroxybutyrate, and catecholamines before and after withdrawal of insulin for 4 h and after resumption of insulin infusion at a fixed rate of 0.1 U · kg^{-1} · h^{-1} for 2 h

	−1 h	0 h	1 h	4 h	6 h
Free insulin (pM)	108.0 ± 26.4	129 ± 27	48.6 ± 7.8	70.2 ± 25.8	586.8 ± 42.6
β-Hydroxybutyrate (μM)	318 ± 142	312 ± 98	1110 ± 287	1727 ± 311	97 ± 33
Norepinephrine (nM)	1.39 ± 0.11	1.50 ± 0.22	1.21 ± 0.11	1.32 ± 0.18	1.64 ± 0.22
Epinephrine (nM)	0.10 ± 0.02	0.11 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.19 ± 0.03

Values are means ± SE; $n = 9$.

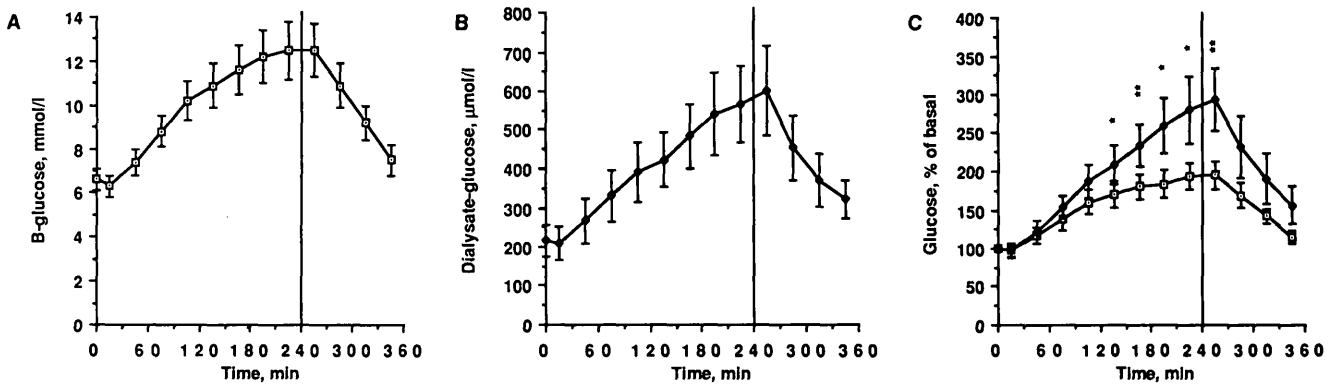

FIG. 1. Effects of insulin deprivation and replacement on glucose in venous plasma (□) and adipose tissue (■). Nine insulin-dependent diabetic subjects, maintained in euglycemic state for at least 12 h by intravenous insulin infusion, were investigated. Dialysis probes were implanted into abdominal subcutaneous adipose tissue and were continuously perfused (5 µl/min) with Ringer's solution. After 30-min equilibration, tissue dialysate was collected in 30-min fractions. Venous plasma samples were simultaneously drawn in middle of each 30-min period. After 60 min of baseline sampling, intravenous insulin infusion was stopped for 4 h. It was resumed for 2 h at fixed rate of 0.1 U · kg^{-1} · h^{-1}. Absolute plasma and tissue dialysate glucose concentrations are given in *A* and *B*, respectively, and respective relative changes from baseline values are given in *C*. *Vertical lines* indicate when insulin infusion was resumed. Values are means ± SE. Results were statistically analyzed with Student's paired *t* test of individual values. *$P < 0.05$; **$P < 0.01$.

insulin infusion was resumed (70–80 µM). In the adipose tissue interstitial compartment, both metabolites increased transiently on the resumption of intravenous insulin (Fig. 3).

The addition of α- and β-adrenergic blocking agents to the tissue perfusate in a pharmacological concentration (10^{-4} M) did not significantly affect the adipose tissue dialysate glycerol concentration during the insulin-deficient period or after insulin replacement (Fig. 4).

DISCUSSION

We used the microdialysis technique to investigate the effects of insulin deprivation and replacement on subcutaneous adipose tissue metabolism in vivo. The withdrawal of insulin for 4 h from IDDM patients with no residual endogenous insulin secretion who were maintained in a euglycemic state by intravenous insulin resulted in diminishing plasma free-insulin levels and increases in plasma glucose, β-hydroxybutyrate, and glycerol concentrations to values like those in previous investigations (25,26). After the reinstitution of intravenous insulin, circulating plasma free insulin was raised to ~600 pM. Concomitantly, the hyperglycemia was rapidly reversed, and plasma glycerol and β-hydroxybutyrate concentrations were markedly suppressed to subbasal values, the latter findings being indicative of pronounced suppression of lipolysis and ketogenesis during the second phase of the experiments.

After the withdrawal of insulin, plasma and adipose tissue dialysate glucose rose in parallel, but the increase in glucose in subcutaneous fat was greater than that in blood. The latter may suggest an accumulation of glucose in the adipose tissue extracellular compartment during insulin deficiency. This is in contrast to studies in nondiabetic subjects that show that, after postprandial elevation of the circulating glucose levels, the kinetic pattern of subcutaneous tissue glucose closely resembles that in venous blood (10,13,14). Hence, it may be suggested that our observation of retention of glucose in the adipose tissue extracellular space mainly reflected a decreased local glucose utilization due to the insulin-deficient state rather than an increased influx of the hexose from the circulation.

Contrary to what was expected, the increase in glycerol in abdominal subcutaneous adipose tissue was much less pronounced than that in blood after insulin withdrawal. Because glycerol, in contrast to FFA, is not reutilized by human fat cells (27), this finding indicates a sustained suppression of the lipolytic activity in abdominal subcutaneous fat at a

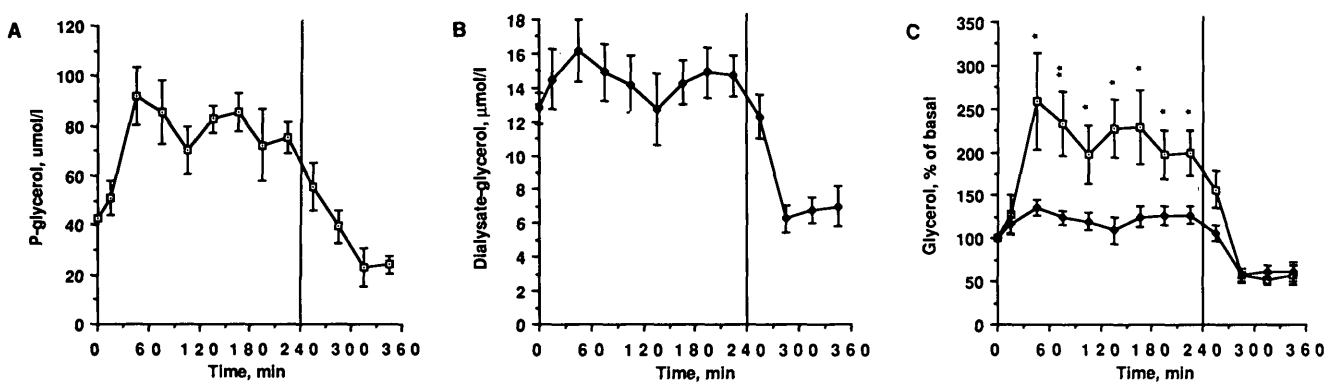

FIG. 2. Effects of insulin deprivation and replacement on glycerol in plasma (□) and subcutaneous adipose tissue (■) in 9 insulin-dependent diabetic subjects. See Fig. 1 for details.

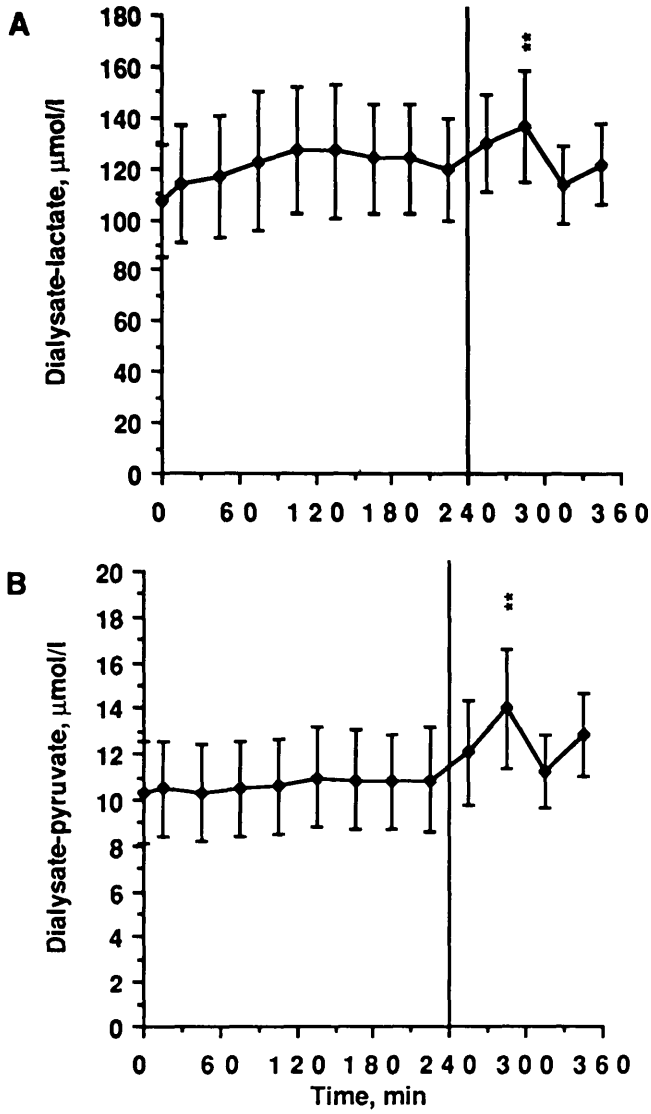

FIG. 3. Effects of insulin deprivation and replacement on lactate (*A*) and pyruvate (*B*) concentrations in subcutaneous adipose tissue in 9 insulin-dependent diabetic subjects. See Fig. 1 for details. **$P < 0.01$ vs. baseline.

circulating insulin concentration of ~70 pM. It is possible, but not likely, that the diversity in glycerol responses between venous blood and adipose tissue is due to a reduced utilization of glycerol in muscle and liver during insulin deficiency. Alternatively, it may be explained by variations in the antilipolytic effect of insulin between different adipose tissue depots; studies in vitro have repeatedly revealed regional differences in the antilipolytic potency of insulin (28–30). Moreover, it is well established that the antilipolytic effect occurs at a lower insulin concentration than is required to stimulate glucose metabolism (31,32). Thus, the weak lipolytic response we found in abdominal subcutaneous fat may be due to the remaining insulin concentration having been high enough to offset the lipolytic activity in this particular region. On the other hand, after insulin replacement, both the plasma and adipose tissue glycerol levels were rapidly suppressed and reached a stable plateau of ~50% of the respective basal levels. This observation is consistent with earlier reports that, at an insulin concentration of ~600 pM, the rate of adipose tissue lipolysis is maximally inhibited (32).

It has been demonstrated in vitro that the antilipolytic effect of insulin can to some extent be mediated by interactions with adrenoreceptors (33). With respect to the influence of adrenergic interactions, we failed to show any difference in the glycerol levels of adipose tissue dialysate during hypoinsulinemia or hyperinsulinemia, whether α- or β-adrenoreceptor–blocking agents were added to the tissue perfusate. The concentrations of propranolol and phentolamine in the perfused fluid that were used in these experiments have recently been shown to markedly influence glycerol kinetics in adipose tissue in microdialysis experiments performed at rest and during physical exercise (11). Thus, in vivo changes in adipose tissue lipolysis during hypoinsulinemia or hyperinsulinemia do not seem to involve adrenergic mechanisms. However, it should be noted that our studies were performed at rest. Whether adrenergic effects interact with insulin during situations of increased sympathetic activity, e.g., in exercise, remains to be established.

It might be argued that the relative unresponsiveness of abdominal subcutaneous adipose tissue to insulin withdrawal, and to adrenoreceptor-blocking agents in situ, may be related to the chronic diabetic state. However, this seems unlikely, because IDDM patients are usually characterized by increased lipolytic activity and enhanced adipocyte β-adrenoreceptor sensitivity (34).

The circulating levels of lactate and pyruvate remained stable during the insulin-deficient period. This accords with the findings of Miles et al. (25), who studied insulin deficiency in ketosis-prone diabetic patients. Moreover, the subcutaneous adipose tissue dialysate levels of lactate and pyruvate remained constant after insulin withdrawal. However, on re-

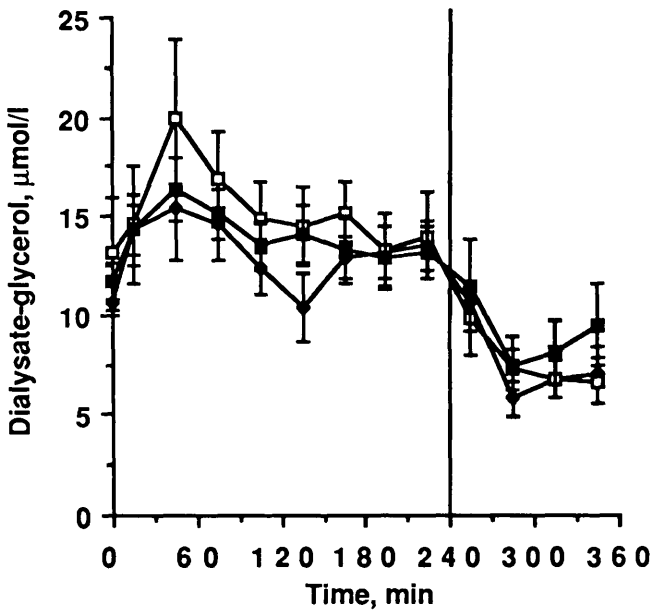

FIG. 4. Effects of α- and β-adrenoreceptor blockade on glycerol concentrations in subcutaneous adipose tissue during insulin deficiency and after insulin replacement. Three dialysis probes were implanted into abdominal subcutaneous adipose tissue in 7 insulin-dependent diabetic subjects. One probe was perfused with Ringer's solution alone (●), and others were perfused with either 0.1 mM phentolamine (■) or 0.1 mM propranolol (□). See Fig. 1 for details.

sumption of the insulin infusion, there was a transient increase in lactate and pyruvate concentrations in adipose tissue, whereas only lactate increased in blood. Thus, the increase of lactate and pyruvate in adipose tissue may not solely represent an enhanced influx of the metabolites from the arterial circulation to the interstitial space. Instead, it may be explained by the in situ production and release of lactate and pyruvate, secondary to increased intracellular glucose availability after correction of the insulin-deficient state. However, the latter interpretation is speculative and needs further clarification.

Note that absolute concentrations of respective metabolites in adipose tissue are unknown in our experiments. It is impossible to use in vitro recovery to calculate the absolute adipose tissue metabolite levels, because the recovery in vivo in adipose tissue is lower than the recovery in vitro (10–12). However, it is possible to indirectly determine by a calibration technique the true tissue metabolite concentrations with microdialysis during steady-state conditions (10,12). With the latter method, the tissue is perfused through the microdialysis probe with increasing metabolite concentrations, and the outgoing dialysate concentration that is in equilibrium with the ingoing perfusate level is calculated; this concentration reflects the absolute tissue concentration (10,12). Using this method, we and others have found the concentrations of glucose, lactate, and pyruvate in subcutaneous fat to be similar to or slightly higher than those in venous blood (4,5,10,12,13), whereas the tissue glycerol concentration was shown to be 2–3 times higher than the venous plasma level (9). Unfortunately, this method for estimating the true tissue metabolite concentrations is very time-consuming. In addition, perfusion of the tissue with supranormal concentrations of the metabolites during the calibration may increase the interstitial levels of these substances and thereby artificially influence the results of a subsequent kinetic experiment. Therefore, it was impossible to include this methodology in our investigations. Moreover, the local adipose tissue blood flow was not measured during hypoinsulinemia and hyperinsulinemia. Hence, the data do not allow any quantitative calculations to be made.

It is becoming increasingly evident that adipose tissue metabolism plays a crucial role in the maintenance of lipid and carbohydrate homeostasis in humans. However, our knowledge of the regulation of these processes is mainly based on in vitro studies or whole-body measurements. Clearly, our data demonstrate that insulin-induced changes in circulating metabolites only partly reflect metabolism in a target tissue for insulin. Moreover, as was shown in this study, the microdialysis technique offers unique prospects for studying these events in vivo by simultaneous determinations of different metabolites during systemic and/or in situ manipulation of the hormonal milieu.

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