Glucose Transport and Metabolism in Adipocytes from Newly Diagnosed Untreated Insulin-dependent Diabetics

Severely Impaired Basal and Postinsulin Binding Activities

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Abstract

Previous studies have shown cellular insulin resistance in conventionally treated insulin-dependent diabetics. To determine whether insulin resistance is also present in insulin-dependent diabetics before the commencement of insulin therapy, we studied nine newly diagnosed untreated insulin-dependent diabetics and nine control subjects. Insulin binding to adipocytes, monocytes, and erythrocytes was normal in the diabetic individuals. Basal (noninsulin stimulated) glucose transport rate was normal, whereas the maximal insulin responsiveness of glucose transport was severely impaired ($P < 0.02$). Insulin sensitivity as judged by left or rightward shifts in the insulin dose-response curves was unchanged. Moreover, the basal lipogenesis rate measured at a glucose concentration of 0.5 mmol/liter was decreased in the diabetics ($P < 0.05$), and the maximal insulin responsiveness of lipogenesis was also reduced ($P < 0.05$).

We conclude that fat cells from untreated insulin-deficient diabetics are insulin resistant. The major defects are (1) reduced maximal insulin responsiveness of glucose transport and conversion to lipids that are postbinding abnormalities, and (2) reduced basal glucose conversion to lipids.

Introduction

Insulin-dependent diabetes mellitus is caused primarily by the lack of or severely reduced production of insulin. However, recent in vivo studies have shown that insulin-dependent diabetic subjects display an impaired insulin effect on the disposal of glucose into peripheral tissues (1–7) as well as decreased inhibitory effect of insulin on hepatic glucose release (1, 5, 6). In vitro studies have shown reduced insulin receptor binding in adipocytes from conventionally treated insulin-dependent diabetics (8, 9). In agreement with the impaired insulin binding, dose-response curves for the insulin effect on glucose transport (8) and antilipolysis (8, 9) were shifted to the right. However, no changes in insulin sensitivity were observed on glucose oxidation and glucose conversion to lipids (8). Impaired basal and maximal insulin action on adipocyte glucose metabolism was found as well (8, 9). It has been suggested that the insulin resistance of insulin-treated diabetic patients might be a result of the insulin treatment itself, and in particular the way of administration of insulin, namely subcutaneous injection, which causes high peripheral levels of insulin as opposed to endogenously released insulin (8, 9). Alternatively, the insulin resistance might be due to the metabolic decompensation of these patients.

The aim of the present study was to determine whether a state of cellular insulin resistance also exists in newly discovered, untreated patients with insulin-dependent diabetes. Hence, we have examined adipocyte insulin binding and action in young untreated insulin-dependent diabetics and in a comparable group of controls. Moreover, we have also measured insulin binding to erythrocytes and monocytes in order to make comparative studies on insulin binding to different cell types.

Methods

Subjects. Nine insulin-dependent diabetics were studied within the first 2 d of admission with newly diagnosed diabetes mellitus before the commencement of diet and insulin therapy. From the day of admission to the day of study, the patients ate the normal hospital diet for nondiabetic subjects (8,500±1,000 kJ, with 40% carbohydrate, 41% fat, and 19% protein). All patients were informed about the nature and possible risks of the study according to Helsinki declaration II and written consent was obtained. Patients in precoma or otherwise needing acute treatment and patients with temperature above 37.5°C and patients in whom diabetes mellitus was secondary to other diseases were not included in the study. During admission (after 1 to 5 d), a glucagon test was performed, which ensured that the patients did have insulin-dependent diabetes mellitus (10) (Table I). The patients were studied in the morning after an overnight fast. A fat biopsy was taken in local anesthesia as previously described (11), and a blood sample was drawn for estimation of insulin receptors on erythrocytes and monocytes as well as estimation of plasma hormones and metabolites.

Nine age- and sex-matched controls were studied in the same way. 3 d before the fat biopsy the volunteers made diet records. Their daily food intake was 8,900±1,500 kJ, with 39% carbohydrate, 43% fat, and 18% protein. The clinical and biochemical data of patients and normal controls are given in Table I.

Chemicals. Human albumin was obtained from Behring Werke, Marburg, Federal Republic of Germany. Collagenase from clostridium histolyticum, 213 U/mg, was obtained from Worthington Biochemical Corp. (Freehold, NJ). ^{125}I-Monoiodoinsulin with the labeled iodine in tyrosine A_{14} (specific activity, ~250 μCi/μg) was generously donated by Novo Research Institute, Copenhagen, Denmark (12). D-U-[^{14}C]glucose (specific activity, 333 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England. Tissue and cells were suspended in Hepes buffer (100 mmol/liter) in the studies of monocyte (13) and erythrocyte binding (14) and 10 mmol/liter in the studies of adipocyte insulin binding and action (11, 15). The pH was adjusted to 7.4 at 37°C in the studies on the fat cells and monocytes, and to pH 7.8 at 37°C in the studies on the erythrocytes.

Insulin receptor binding studies. Adipose tissue (~10 g) was obtained by open biopsy from the upper quarter of the right gluteal region after a square field had been anesthetized with an epidermal injection of 1% lidocaine without epinephrine. Details about fat cell isolation as well as determination of fat cell size and number have been published previously (11, 15). Insulin binding to fat cells (~10^5 cells/ml of cell suspension)

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was measured in a Hepes buffer at 37°C after incubation for 60 min with tyrosine-A_{14}-labeled ^{125}I-insulin with or without increasing concentrations of unlabeled insulin. In order to compare insulin binding to fat cells and blood cells at the same temperature (insulin binding to blood cells must be measured at subphysiologic temperature to ensure steady state specific binding), we also measured insulin binding at tracer concentrations to fat cells at 15°C with a 120-min incubation period. Cell-associated radioactivity in the presence of 10 μmol/liter unlabeled insulin (nonspecific binding) averaged 4% of total binding both at 37° and 15°C. Specific insulin binding to adipocytes was expressed per 30 cm^2 of surface area per milliliter. Insulin degradation in the medium at 37°C was <4%, measured as TCA solubility after 60 min.

Insulin receptor binding to erythrocytes was determined as described (14) with the following modification. After fractionating the blood once on a Ficoll-Isopaque gradient, the erythrocytes were collected from the bottom of the tubes. The cells were resuspended 1:1 in 0.9% NaCl containing 50 mg/ml dextran 500 T. The tubes were tilted 45° from the vertical for 15 min at 37°C. The erythrocytes then settled and the supernatant containing the granulocytes was removed. In this way, granulocyte contamination was reduced to <0.03/1,000 erythrocytes. After washing, the erythrocytes (at a volume fraction of 0.45) were incubated for 210 min at 15°C in 100 mmol/liter Hepes buffer with tyrosine-A_{14}-labeled ^{125}I-insulin with or without native insulin (10 μmol/liter) as described (14). Nonspecific binding averaged 12% of total binding. Specific insulin binding was expressed per 5×10^9 cells/ml. Pure monocytes were isolated from the mononuclear cell layer obtained by fractionating the blood on a Ficoll-Isopaque gradient, based on the fact that monocytes but not lymphocytes adhere to plastic surfaces at 37°C and detach again in the cold (16). In this way, very homogeneous suspensions of monocytes were obtained (the percentage of monocytes was 97±2, mean±1 SD). Monocytes were identified by morphological and cytochemical criteria (13, 16). Calculation of insulin binding to monocytes was performed as previously described (13). Monocytes ($2.5–8 \times 10^6$ cells/ml) were incubated for 120 min in 100 mmol/liter Hepes buffer at 15°C with tyrosine-A_{14}-labeled ^{125}I-insulin with or without native insulin (10 μmol/liter). Nonspecific binding was 22% of total binding. Specific binding was expressed per 5×10^6 pure monocyte per milliliter.

Lipogenesis. Lipogenesis was measured by studies of the conversion of D-U-[^{14}C]glucose to ^{14}C total lipids as described earlier (11). Isolated adipocytes were prepared in a 10 mmol/liter Hepes buffer containing 0.5 mmol/liter glucose (volume fraction 0.05). The cells were preincubated for 45 min at 37°C with or without insulin in increasing concentrations. Then, 0.4 μCi D-U-[^{14}C]glucose was added to each tube (final glucose concentration, 0.5 mmol/liter) and the incubation was continued for 90 min. Then, H_2SO_4 was added, and a Dole extraction was performed and a sample for liquid scintillation counting was taken from the upper phase (11). ^{14}C-radioactivity was present in an average amount of 21±6% of noninsulin-stimulated lipogenesis when incubations were performed in the absence of fat cells (blank values). All values for fat cell-produced total lipids were corrected for the individual blank value.

Studies of glucose transport. Glucose transport was measured as the conversion of D-U-[^{14}C]glucose to total lipids at tracer glucose concentrations (5 μmol/liter). It has been shown that glucose transport is the rate-limiting step for glucose processing at very low glucose concentrations (11, 17, 18).[1] Under these conditions, >80% of the glucose is converted to lipids (17, 18).[1] Therefore, measurements of the conversion rate of D-glucose to total lipids at tracer glucose concentrations will yield an indirect estimation of glucose transport rates (17, 18).[1] Glucose transport was measured as described for lipogenesis with the following modifications: The cells were preincubated in a glucose-free buffer. Then, 0.4 μCi D-U-[^{14}C]glucose (final concentration, 5μmol/liter) was added. The incubation was stopped after 90 min by the addition of H_2SO_4. After this a Dole extraction was performed. Blank values averaged 8±3% of the noninsulin-stimulated lipogenesis under these conditions.

Analytic procedures. Plasma glucose was analyzed with a glucose dehydrogenase method (Merck enzymatic kit, Mannheim, Federal Republic of Germany). Serum insulin was measured by radioimmunoassay (19). Plasma acetoacetate and plasma 3-hydroxybutyrate (ketone bodies) were measured separately by enzymatic micromethod (20). In the Results, the sum is given of the substance concentrations of the two metabolites. Plasma FFA (aliphatic carboxylate C_8 to C_{18}, nonesterified) were assayed according to the method of Itaya and Ui (21). Serum growth hormone was estimated as described by Ørskov et al. (22). The cortisol concentration in urine was determined according to Murphy (23). C-peptide in serum was assayed by the method of Heding (24).

Statistical methods. In the text and tables, data are given as mean±1 SD while the data in the figures represent the mean±1 SEM. An unpaired *t* test was used for comparison between groups. Linear regression analysis was employed in correlation studies using the least-squares method. Ketone bodies were log distributed and hence were log transformed before statistical analysis.

Results

The biochemical data of the patients and normal controls are given in Table I. All patients were nonobese. No significant difference in fat cell diameter was found. The fasting serum insulin levels of diabetics were only slightly decreased, whereas the basal as well as the glucagon-induced rise in serum C-peptide concentration was reduced in all patients, indicating insulin deficiency (10). As expected, the patients had significantly elevated fasting plasma concentration of ketone bodies ($P < 0.001$). However, none of the patients was acidotic. Fasting plasma concentrations of FFA were significantly increased in the diabetics ($P < 0.05$). Two patients had elevated urinary cortisol excretion (>385 mmol/liter), whereas only one had elevated fasting plasma growth hormone concentration (>6 μg/ml).

Table I. Clinical and Biochemical Data in Healthy Controls and Insulin-dependent Diabetics (Mean±1 SD)

	Normals	Diabetics
Sex	4 ♀ 5 ♂	4 ♀ 5 ♂
Age (*yr*)	39±9	38±9
Body weight (*kg*)	69±14	65±16
Fasting plasma glucose (*mmol/liter*)	5.3±0.7	12.8±1.3
Fasting serum insulin (*μU/ml*)	14±4	10±5
C-peptide basal and stimulated*		0.25±0.08
(*nmol/liter*)		0.42±0.14
Fasting plasma ketone bodies (*mmol/liter*)	0.10±0.08	2.08±1.81‡
Fasting plasma FFA (*mmol/liter*)	0.19±0.10	0.39±0.06§
Fat cell diameter (*μm*)	93±15	87±12

* 6 min after intravenous injection of 1 mg glucagon. All diabetics had stimulated C-peptide concentrations below 0.6 nmol/liter (10), thus rendering the patients insulin requiring.
‡ $P < 0.001$.
§ $P < 0.05$.

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Fig. 1 depicts competition curves for insulin binding to fat cells measured at 37°C. When expressed in relation to cell surface area concentration, no significant difference between insulin binding to fat cells from normals and newly diagnosed insulin-dependent diabetics was observed. The same was the case when binding was expressed in relation to cell number concentration. Fat cell insulin binding measured at insulin tracer concentration at 15°C as well as insulin binding to erythrocytes and pure monocytes also measured at 15°C was similar in normals and diabetics (Table II).

When adipocyte insulin binding was expressed in relation to cell surface area, no correlations between insulin binding to monocytes and adipocytes or monocytes and erythrocytes were found (Fig. 2). However, a significant inverse correlation existed between erythrocytes and adipocytes ($r = -0.70$, $P < 0.05$, Fig. 2). When insulin binding was expressed in relation to cell number concentration, the negative correlation between erythrocytes and adipocytes still existed ($r = -0.58$, $0.1 > P > 0.05$). On the other hand, no correlations between individual values of cell insulin binding were found when data obtained in the 37°C studies of fat cell insulin binding were used (data not shown). No correlations between insulin binding to fat or blood cells and fasting plasma concentrations of insulin, ketone bodies, or FFA were found.

The basal (noninsulin-stimulated) glucose transport rate into fat cells was identical in diabetics and normal controls (Fig. 3). However, the insulin response above basal levels was severely reduced in fat cells from the diabetic patients as compared with normals (41±25% vs. 101±69%, $P < 0.02$). No difference in insulin sensitivity as expressed as left or rightward shifts in insulin dose-response curves was observed (ED_{50} was 58±25 pmol/liter in normals and 65±30 pmol/liter in the diabetic patients). In the diabetic patients, no correlation was found between percentage insulin response above the basal level of glucose transport and fasting insulin levels ($r = 0.05$, NS), whereas plasma FFA concentrations correlated negatively to percentage insulin response ($r = -0.72$, $P < 0.05$). No correlations between degree of ketosis and glucose transport rates were found. In normal controls, no correlations between insulinemia or ketonemia or plasma FFA levels and glucose transport rates were observed.

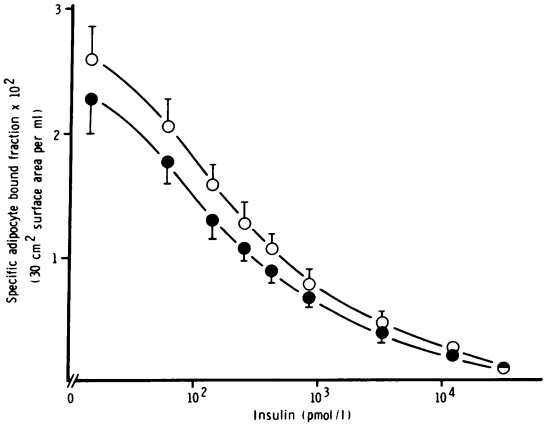

Figure 1. Insulin binding to adipocytes from nine newly diagnosed insulin-dependent diabetic patients (○) and nine control subjects (●). Adipocytes were incubated with 15 pmol/liter ^{125}I-insulin at 37°C for 1 h in the absence or presence of unlabeled insulin in increasing concentrations (mean±1 SEM).

*Table II. Insulin Binding to Adipocytes, Monocytes, and Erythrocytes at 15°C in Healthy Controls and Insulin-dependent Diabetics**

	Normals	Diabetics
Adipocytes (30 cm²/ml)	4.87±1.70	4.59±1.07
Monocytes ($5 \cdot 10^6$/ml)	2.66±0.86	3.09±1.10
Erythrocytes ($5 \cdot 10^9$/ml)	5.69±1.08	5.58±1.70

* Insulin tracer concentration, 15 pmol/liter. Specific insulin bound fractions times 10^2 (mean±1 SD).

Glucose conversion to total lipids measured at a glucose concentration of 0.5 mmol/liter, where steps distal to glucose transport are rate limiting, is depicted in Fig. 4. A significant decrease in noninsulin-stimulated lipogenesis was found in fat cells from the diabetic patients ($P < 0.05$). Moreover, the insulin response above basal levels was very low (35±23% vs. 72±41% in normals, $P < 0.05$). Actually, in two patients, no insulin response was detectable. The absolute lipogenesis rate in maximally insulin-stimulated cells was thus markedly reduced (to 40% of that in normals, Fig. 4). Due to the low insulin responses, changes in insulin sensitivity could not be estimated in these studies. No correlations between basal or maximally insulin-stimulated lipogenesis rates and fasting concentrations of insulin or ketone body or FFA were found in any of the groups.

Discussion

Normal insulin binding in patients with untreated insulin-dependent diabetes mellitus. The present study demonstrates that insulin binding to fat cells from insulin-requiring diabetics before the institution of insulin therapy is normal. The unaltered insulin binding in this study as opposed to the reduced binding in insulin-treated diabetics (8, 9) supports the hypothesis that the reduced insulin binding in the latter studies is an acquired defect, probably secondary to subcutaneous insulin therapy, which causes high peripheral levels of insulin (8). The insulin binding to erythrocytes and monocytes was also unaltered in the untreated, insulin-dependent diabetic patients corroborating previous studies (25).

Monocytes and erythrocytes have been widely used as tools in clinical studies of insulin receptors, and receptor data from

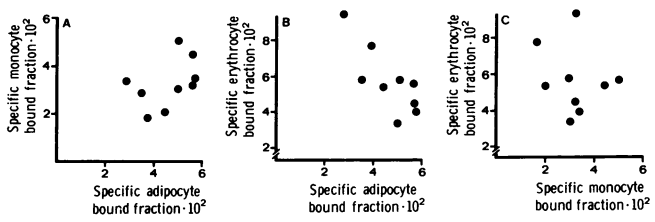

Figure 2. Interrelationships between insulin binding to adipocytes, monocytes, and erythrocytes from nine newly diagnosed insulin-dependent diabetic patients. Cells were incubated with 15 pmol/liter radioactive insulin at 15°C. Insulin binding to adipocytes, monocytes, and erythrocytes was expressed to 30 cm² surface area/ml, 5×10^6 monocytes/ml, and 5×10^9 erythrocytes/ml, respectively.

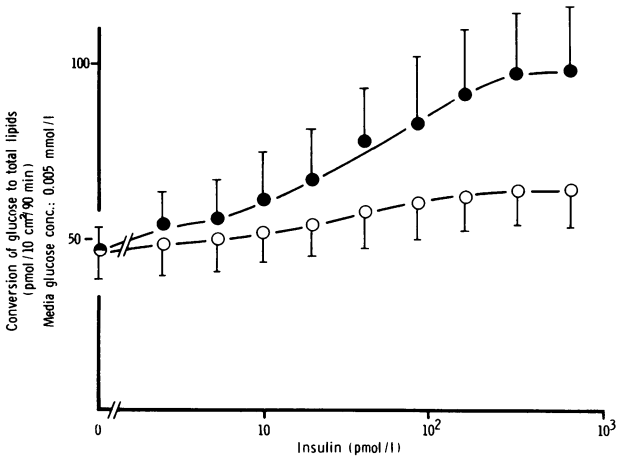

Figure 3. Glucose transport rates in adipocytes from nine diabetic patients (○) and nine control subjects (●). Transport was measured as lipogenesis at tracer glucose concentration (5 μmol/liter). Adipocytes were preincubated in a glucose-free Hepes buffer at 37°C without or with insulin in the indicated concentrations for 45 min. Then labeled glucose was added, and the incubation was continued for 90 min (mean±1 SEM).

blood cells have been extrapolated to target cells for insulin. The validity of this extrapolation has been said to be supported by several studies (26, 27). However, Soll et al. (26) merely demonstrated that lymphocytes and adipocytes from obese mice showed lower insulin binding values than did lymphocytes and adipocytes from normal mice. Olefsky et al. (27) found decreased mean binding to monocytes and adipocytes from obese subjects. However, a positive correlation was found only after pooling data from obese and normal subjects. In previous studies of normal subjects (11) or long-term insulin-treated diabetics (8) we found no consistent correlations between insulin binding to erythrocytes, monocytes, and adipocytes. Taylor et al. (28) found no relationship between monocyte and adipocyte insulin binding in a variety of clinical situations including normal subjects, insulin-treated diabetics, and patients with cirrhosis of the liver.

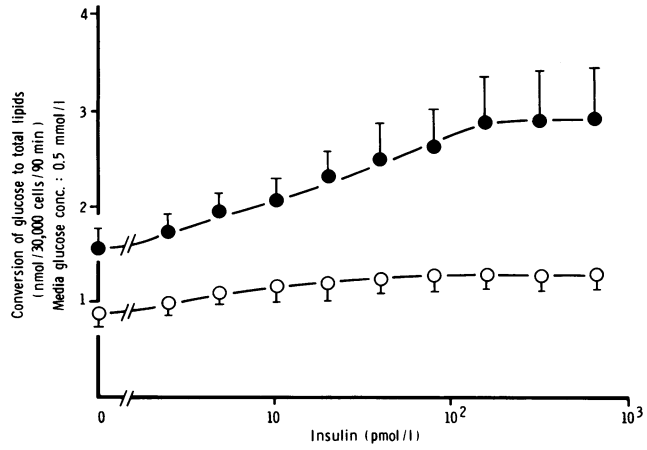

Figure 4. Lipogenesis in adipocytes from nine diabetic patients (○) and nine control subjects (●). Lipogenesis was measured as described in the legend to Fig. 3 in a Hepes buffer containing 0.5 mmol/liter glucose (mean±1 SEM).

Recently, in a study of newly diagnosed noninsulin-dependent diabetics, we failed to find significant correlations between blood cells and fat cells (29). In the present study, monocyte and adipocyte binding did not correlate, but erythrocyte and adipocyte insulin binding was significantly negatively correlated. Taken together, this indicates that the extrapolation of individual insulin-binding values from blood cells to fat cells should be avoided.

Impaired maximal insulin responsiveness of glucose transport and metabolism in adipocytes from untreated diabetics. The most striking defect in fat cells from insulin-dependent diabetics before the start of insulin treatment is a severely blunted or abolished responsiveness to insulin of glucose transport and metabolism. These are postbinding phenomena, probably due to the depletion of glucose carriers (30–32) and a lack of enzyme responsiveness in the glycolytic pathways. Since such defects are reversed by insulin treatment (8), these are acquired defects. They may represent adaptive changes in the cells to the catabolic situation with insulin deficiency. The similarities to the results in fasting human subjects (33), in whom the same reduced insulin responsiveness was found, should be emphasized.

Comparisons to in vivo studies of insulin action in patients with newly diagnosed insulin-dependent diabetes mellitus. Adipose tissue is only responsible for a minor fraction of total body glucose uptake (34). Therefore, our results should be compared with appropriate in vivo studies. Recently, two groups have investigated in vivo insulin action in newly diagnosed diabetics using the euglycemic clamp technique. Nankervis et al. (35) found that nontreated insulin-requiring diabetics were characterized by elevated basal hepatic glucose production and hepatic insensitivity to insulin, as well as markedly impaired glucose disposal to peripheral tissues. Yki-Järvinen et al. (36), who studied newly diagnosed diabetics after 2 wk of insulin therapy, found similar defects. Thus, our findings of decreased maximal insulin responsiveness in fat cells are compatible with these reports.

Possible factors responsible for the insulin resistance in patients with untreated insulin-dependent diabetes mellitus. Ketoacidosis is a well known cause of insulin resistance clinically as well as experimentally (37, 38). Although our patients had significantly higher levels of ketone bodies than did normals, the absolute level of ketonemia was not high, and none of our patients was acidotic. No relationship between the levels of plasma ketones and insulin binding or action was observed.

Plasma counterregulatory hormones were also measured in order to evaluate their role in the event of insulin resistance (39–41). However, only two of our patients had elevated urinary cortisol excretions, and one had elevated fasting plasma growth hormone concentrations. These patients were comparable to the rest of the group in all aspects. Therefore, it seems unlikely that the insulin unresponsiveness found in fat cells from our patients is caused primarily by these hormones. Catecholamines are also able to induce insulin resistance in vivo in man (42) and in vitro in isolated adipocytes (43). Actually, we did not measure serum catecholamines in our diabetics, but previous studies (44) have shown that the serum levels of catecholamines parallel the degree of metabolic derangement. Since none of our patients was acidotic or severely ketotic, it is unlikely that catecholamines were the major cause of insulin resistance in the diabetics for the present study.

Noninsulin-stimulated (basal) glucose transport and metabolism in fat cells from patients with insulin-dependent diabetes

mellitus. No change in basal glucose transport rate in adipocytes from our diabetic patients was found, whereas basal glucose metabolism was severely depressed in the same cells. The interpretation of the depressed basal lipogenesis rate relative to the normal basal transport rate in fat cells from the untreated diabetics depends on whether transport or posttransport steps are regarded as being rate limiting for glucose metabolism. In our hands, transport is not rate limiting for basal glucose metabolism even in adipocytes from normal subjects (11).[1] This is discussed in detail elsewhere.[1] Shortly, it is based on the following observations: When medium glucose concentration is increased from tracer only (5 μmol/liter where transport is rate limiting [11, 18]) to 0.5 mmol/liter by addition of unlabeled glucose, the cellular uptake of radioactive glucose decreases to about one-third, whereas the transport rate is only slightly decreased (K_m for glucose transport is 7 mmol/liter). The same decrease in cellular uptake is found if glucose is replaced by 2-deoxyglucose (which is phosphorylated but not further metabolized) at the same concentration, and/or 2-deoxyglucose is used as tracer. Thus, the decrease in cellular hexose uptake must be caused by competition at the level of phosphorylation and not by escape of transported glucose converted to metabolic products. Moreover, total lipids comprise >80% of the intracellular radioactivity at tracer concentration (11, 18) as well as at a glucose concentration of 0.5 mmol/liter (11)[1] in human fat cells. The decreased basal lipogenesis rate in fat cells from untreated diabetics at this glucose concentration is therefore caused by a true decrease in metabolism rate and not by a shift in rate-limiting steps from transport to posttransport steps.

Although glucose conversion to total lipids is the most important metabolic pathway for glucose at this glucose concentration, other pathways such as lactate production may become of relative more importance at higher glucose concentrations (18).

The depressed basal rate of lipogenesis is not an insulin receptor or a postreceptor phenomenon, since it is present in cells not acutely exposed to insulin. Yet, the possibility exists that the impaired basal metabolic activity is a reflection of the insulin-deficient state, especially the metabolic deterioration and catabolic changes that follow insulin deficiency. This interpretation agrees with the findings in insulin-deficient streptozotocin-treated rats (45). Recently, however, it was shown that acute hyperinsulinism in normal subjects, induced by 6 h of insulin infusion, also leads to an impaired basal lipogenesis rate in adipocytes (46). Also, after chronic insulin therapy for several years, the basal glucose metabolism of fat cells from conventionally treated diabetics is severely depressed (8). It has been hypothesized that this depression may be induced by the chronic peripheral hyperinsulinemia (8). This idea is supported by studies of the long-term effects of continuous insulin infusion therapy on adipocyte metabolism in insulin-dependent diabetics where a significant aggravation of basal glucose conversion to lipids has been found (47). Thus, experimental evidence exists that suggests that both hypo- and hyperinsulinemia may induce impaired basal glucose metabolism in human adipocytes.

We conclude that in fat cells from untreated insulin-dependent diabetic subjects, insulin binding is normal, whereas the insulin responsiveness is markedly reduced at both glucose transport and glucose metabolism level. The basal glucose metabolism rate is also reduced. The observed abnormalities are most probably caused by insulin deficiency and accompanying catabolic deterioration.

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