Impaired Insulin Receptor Binding and Postbinding Defects of Adipocytes From Normal and Diabetic Pregnant Women

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SUMMARY

To evaluate the relative contribution of insulin binding and postbinding defects of glucose utilization in peripheral tissue during normal and diabetic pregnancy, we have studied the in vitro insulin action of isolated adipocytes from eight nondiabetic pregnant women and nine pregnant women with insulin-dependent diabetes mellitus who were undergoing cesarian section. The pregnant women were compared with a matched group of normal nonpregnant women undergoing gynecologic surgery. Insulin binding to adipocytes measured at tracer insulin concentration was reduced by 45% ($P < 0.01$) in normal pregnant women and by 30% ($P < 0.02$) in pregnant women with diabetes. In contrast, no changes were found between the three groups in insulin binding to pure monocytes and erythrocytes.

The glucose transport system in fat cells from both groups of pregnant women was characterized by impaired maximal ($P < 0.05$) and half-maximal ($P < 0.05$) response to insulin. When fat cell glucose metabolism was studied, pregnant diabetic women exhibited decreased basal lipogenesis ($P < 0.05$) and decreased maximal responses of lipogenesis and glucose oxidation to insulin stimulation ($P < 0.05$). Similar but less pronounced abnormalities were seen in glucose metabolism of adipocytes from nondiabetic pregnant women.

In conclusion, both in late normal and diabetic pregnancy, insulin binding to adipocytes is significantly reduced and accompanied by decreased insulin sensitivity and reduced maximal insulin responsiveness of glucose transport and by impaired basal and maximally insulin-stimulated glucose metabolism. DIABETES 1986; 35:598–603.

The diabetogenic effect of human pregnancy has not been explained by inappropriate changes in pancreas function[1] or by increased insulin degradation.[2] Attempts to ascertain if changed insulin receptor function contributes to the decreased insulin action of human pregnancy have yielded conflicting results. During late normal pregnancy decreased insulin receptor binding to monocytes[3] and adipocytes[4] has been reported. However, none of the reports revealed when during the menstrual cycle the control subjects were examined. In three other studies of normal pregnancy, insulin binding to blood cells was similar to or higher than that seen during the luteal phase of nonpregnant control women.[5–7]

Recently, we studied insulin binding to monocytes and erythrocytes from pregnant women with insulin-dependent diabetes during the first and third trimester.[8] In the first trimester, insulin binding to both cell types was similar to that in normal nonpregnant women. Moreover, insulin receptor binding remained unchanged during the third trimester even in the face of significantly increased insulin requirement and concomitant hyperinsulinemia.[8] Thus, when estimated from studies of insulin receptors on blood cells, the available literature does not support the view that changed insulin receptor function contributes to the diabetogenicity of human pregnancy.

To study whether the glucose intolerance during pregnancy might be explained through changes in the regulatory mechanisms that control the peripheral insulin action at postbinding steps, we have examined the non-insulin- and insulin-stimulated glucose transport and metabolism in adipocytes from normal nonpregnant (NW) and normal pregnant (NPW) women and from pregnant women with insulin-dependent diabetes mellitus (PWIDD). Moreover, in all three groups of subjects, we studied the comparability of insulin receptors on adipocytes, monocytes, and erythrocytes.

MATERIALS AND METHODS

Subjects. The study comprised 10 NW with no signs or family history of endocrine disorders undergoing gynecologic surgery due to diverse disorders (hysterectomy, sterilization) and 8 NPW and 9 PWIDD who delivered by cesarean section.

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TABLE 1
Clinical data of patients*

	Nonpregnant normal women	Pregnant normal women	Pregnant diabetic women
Number	10	8	9
Age (yr)	31 ± 6	26 ± 5	27 ± 4
Relative body weight	96 ± 11	100 ± 10†	98 ± 10†
Delivery before term (days)		8	21
Dose of insulin before pregnancy (units)			
—Morning			36 ± 10
—Evening			8 ± 5
Dose of insulin during last week before delivery (units)			
—Morning			48 ± 15
—Evening			16 ± 8
Fasting plasma glucose (mmol/L)	5.0 ± 0.6	4.2 ± 1.0	7.5 ± 3.2
Fasting serum insulin (mU/L)	12 ± 7	13 ± 8	16 ± 5
Adipocyte diameter (μm)	74 ± 11	87 ± 6	82 ± 9

*Results are expressed as mean ± 1 SD.
†Before pregnancy.

Pertinent clinical data for each group of subjects are outlined in Table 1. The PWIDD all had typical ketosis-prone acute-onset, insulin-dependent diabetes mellitus. Three were classified according to White[9] as B, five as D, and one as F. The duration of diabetes was 14 ± 7 yr. Three of the NW were examined in the secretory phase of the menstrual cycle, six were studied in the proliferative phase of the cycle, and one took oral contraceptives. None of the participants took other medications.

The surgery was performed in the morning after at least 8 h fasting. Subcutaneous fat tissue (about 5 g) was obtained from the infraumbilical region at the beginning of surgery. At the same time, a blood sample was drawn. No premedication was given to pregnant patients, whereas NW had morphine chloride and scopolamine. In normal patients the analgetics comprised short-acting barbiturates, halothane, and N_2O. In most cases, suxamethon was added. One NPW and two PWIDD delivered using epidural analgetics (Xylocaine or Marcaine). The rest of the pregnant patients delivered under general anesthesia.

On the day of the delivery the insulin dose of PWIDD was reduced about 50%. About 30 min before and during the delivery in PWIDD, intravenous (i.v.) glucose (278 mmol/L) was infused at a rate of 1 ml/min, whereas no glucose was given before or during delivery/surgery in NPW and NW. The study protocol was approved by the local ethical committee, and informed consent was obtained from all participants.

Diets. During pregnancy PWIDD ate a diet high in starch and restricted in simple carbohydrates and fat with an average energy content of 7100 kJ. NW and NPW consumed unrestricted diets.

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, New Jersey). (^{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). D-U-(^{14}C)glucose (specific activity 333 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, United Kingdom). Tissue and cells were suspended in Hepes buffer (100 mmol/L in the studies of monocyte[10] and erythrocyte[11] binding and 10 mmol/L in the studies of adipocyte binding and action.[12] The pH was adjusted to 7.4 at 37°C in the studies of the fat cells and monocytes and to 7.8 at 37°C in the studies on the erythrocytes.

Insulin receptor binding studies. Details of fat cell isolation and determination of fat cell size and number were as previously described.[12,13] Insulin binding to fat cells (~10^5 cells/ml of cell suspension) was measured in Hepes buffer at 37°C, after incubation for 60 min with A_{14}-(^{125}I)insulin with or without increasing concentrations of unlabeled insulin.[12] To compare insulin binding with 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/L unlabeled insulin (nonspecific binding) averaged 4% of total binding at both 37°C and 15°C. Specific insulin binding was expressed per 30 cm^2 surface area/ml. Degradation of insulin in the medium was <4% at 37°C (trichloroacetic acid solubility after 60 min incubation with 15 pmol/L insulin).

Insulin receptor binding to erythrocytes was determined as previously described[11] with the following modifications. 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 inclined at 45° for 15 min at 37°C. The supernatant was then removed, thus reducing granulocyte contamination of the settled erythrocytes to <0.03 per thousand. After washing, the erythrocytes (at a volume fraction of 0.45) were incubated for 210 min at 15°C in 100 mmol/L Hepes buffer with A_{14}-[^{125}I]insulin with or without native insulin (10 μmol/L).[11] Nonspecific binding averaged 10% of total binding. Specific insulin binding was expressed per 5 × 10^9 cells/ml.

After fractionation of blood on Ficoll-Isopaque, pure monocytes were separated from lymphocytes by exploiting their property of adhering to plastic surfaces at 37°C and detaching again at cold temperatures.[14] In this way, homogeneous suspensions of monocytes were obtained (percentage of monocytes 96.7 ± 1.5, mean ± 1 SD). Monocytes were identified by morphologic and cytochemical criteria,[10] and insulin

binding was performed as previously described.[10] Monocytes (3–8 × 10^6 cell/ml) were incubated for 120 min in 100 mmol/L Hepes buffer at 15°C with tyrosine-A_{14}-[^{125}I]insulin with or without native insulin (10 μmol/L). Nonspecific binding was 22% of total binding. Specific binding was expressed per 5 × 10^6 pure monocytes/ml.

Glucose oxidation. Glucose oxidation was measured by studies of the conversion of the D-U-[^{14}C]glucose to [^{14}C]CO_2 as described earlier.[15] Isolated adipocytes were prepared in a 10-mmol/L Hepes buffer containing 0.5 mmol/L 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/L), and the incubation was continued for 90 min. H_2SO_4 was added and during the subsequent 60 min [^{14}C]CO_2 was collected with phenethylamine as trapping agent.[15] ^{14}C radioactivity was present in an average amount of 23% ± 5 of the non-insulin-stimulated CO_2 release when the incubations were run in the absence of fat cells (blank values). All values for fat cell-produced CO_2 have been corrected for the individual blank value.

Lipogenesis. Lipogenesis was measured as the conversion of D-U-[^{14}C]glucose to ^{14}C total lipids. The experimental procedure was the same as that described for CO_2 production. After CO_2 collection had been terminated, a Dole extraction was performed, and a sample for liquid scintillation counting was taken from the upper phase.[15] ^{14}C radioactivity was present in an average amount of 16% ± 4 of the non-insulin-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/L). It has been shown that glucose transport is the rate-limiting step for glucose processing at very low glucose concentrations.[16,17] Under these conditions more than 80% of the glucose is converted to lipids.[16,17] 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.[16,17] 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 5 μmol/L) 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 non-insulin-stimulated lipogenesis under these conditions.

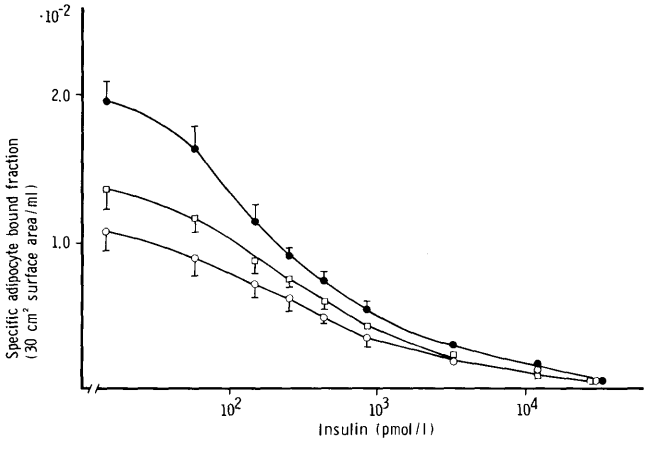

FIGURE 1. Specific [^{125}I]insulin binding to adipocytes from 10 nonpregnant normal women (●), 9 pregnant insulin-dependent diabetic women (□), and 8 pregnant normal women (○). Insulin binding was measured at 37°C as described in MATERIALS AND METHODS (mean ± SEM).

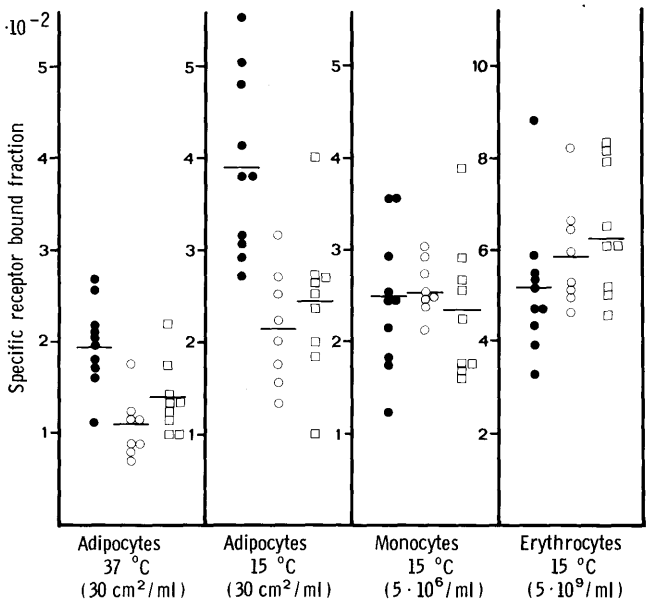

FIGURE 2. Individual values of specific ([^{125}I]insulin binding at tracer insulin concentrations to adipocytes (at 37°C and 15°C), monocytes, and erythrocytes (at 15°C) from 10 nonpregnant normal women (●), 8 pregnant normal women (○), and 9 pregnant insulin-dependent women (□). Bars denote mean values.

Analytic methods. Plasma glucose was analyzed with a glucose dehydrogenase method (Merck enzymatic kit, Darmstadt, Federal Republic of Germany). In nondiabetic subjects, serum insulin was measured with a radioimmunoassay technique.[18] Free insulin in serum of diabetic subjects was measured with the same assay after the serum had been incubated at 37°C for 150 min and subsequently precipitated with polyethylene glycol.[19]

Statistical methods. In text and tables, data are given as the mean ± 1 SD (or range when indicated), whereas data in the figures represent the mean ± 1 SEM. Significant differences between groups were assessed by Mann-Whitney's test. In correlation studies, Spearman's test was employed.

RESULTS

Insulin binding to adipocytes at 37°C. The competition curves for specific insulin binding expressed per surface area concentration are depicted in Figure 1. At tracer insulin concentration (15 pmol/L) the insulin binding to fat cells from NPW and PWIDD was reduced by 45% ($P < 0.01$) and by 30% ($P < 0.02$), respectively, when compared with NW. Because the fat cells from the NW were smaller than cells from the pregnant groups (Table 1), these differences were less significant when the data were expressed per cell number concentration (reductions in tracer insulin binding of 30%, $P < 0.02$, and 21%, $P < 0.05$, were observed in NPW and

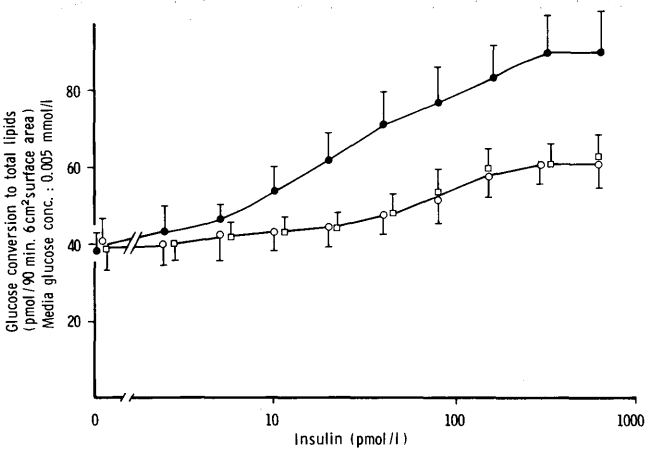

FIGURE 3. D-U-[^{14}C]glucose transport into adipocytes as estimated by the conversion of tracer glucose (0.005 mmol/L) to total lipids at 37°C. Adipocytes from 10 nonpregnant normal women (●), 9 pregnant insulin-dependent diabetic women (□), and 8 pregnant normal women (○). Results are mean ± SEM.

PWIDD, respectively). The insulin concentrations causing half-maximal displacement of tracer insulin were higher in NPW (346 ± 132 pmol/L, $P < 0.05$) and in PWIDD (333 ± 192 pmol/L, $P < 0.05$) than in NW (202 ± 89 pmol/L). These findings suggest impaired apparent receptor affinity in pregnant women. No significant correlations were found between adipocyte insulin binding at tracer insulin concentrations or the insulin concentrations giving half-maximal displacement of tracer insulin and the fasting plasma insulin concentrations in any of the groups.

Comparative studies of [^{125}I]insulin binding to adipocytes, monocytes, and erythrocytes at 15°C. At a subphysiologic temperature (15°C), insulin binding to adipocytes from the three categories of women showed the same pattern as at 37°C, with significantly depressed values for tracer-bound insulin in both NPW ($P < 0.001$) and PWIDD ($P < 0.01$) (Figure 2) when expressed per unit surface area. The binding was also depressed when expressed per cell number concentration ($P < 0.02$). In contrast, no change was found between the three groups in insulin binding to erythrocytes and pure monocytes (Figure 2). Comparative studies of insulin receptors on adipocytes, monocytes, and erythrocytes from the three groups of subjects measured at 15°C at tracer insulin concentration (15 pmol/L) showed no significant relationships. Neither did comparative studies in the same individuals of insulin binding to adipocytes measured at 37°C and insulin binding to blood cells measured at 15°C.

Adipocyte glucose transport. D-U-[^{14}C]glucose conversion to total lipids was measured under conditions (final glucose concentration: 0.005 mmol/L) where transport, and not intracellular metabolic steps, was rate limiting.[17] There were no significant differences between groups in basal non-insulin-stimulated glucose transport when the data were expressed per unit surface area (Figure 3). When expressed per cell number concentration, a small, insignificant decrease was observed in the pregnant groups. It was found that the insulin concentration giving half-maximal glucose transport (40 ± 21 pmol/L in NW) was significantly higher in both NPW (64 ± 30 pmol/L, $P < 0.05$) and PWIDD (63 ± 15 pmol/L, $P < 0.05$), suggesting an impaired insulin sensitivity (Figure 3). The maximally insulin-stimulated glucose transport in percentage above basal value was 99 ± 33% in NW and 53 ± 31% ($P < 0.02$) in NPW and 67 ± 34% ($P < 0.05$) in PWIDD (Figure 3). The latter findings suggest additional post-binding defects of adipocyte glucose transport in pregnant women.

Adipocyte glucose metabolism. D-U-[^{14}C]glucose conversion to total lipids was measured at a final glucose concentration of 0.5 mmol/L where intracellular metabolic steps were rate limiting for human adipocyte glucose utilization. As seen in Figure 4, adipocytes from PWIDD were characterized by impaired non-insulin-stimulated lipogenesis ($P < 0.05$). The maximally insulin-stimulated lipogenesis above basal level was 37 ± 36% in PWIDD and 51 ± 16% in NW ($P < 0.05$). Because of the very low insulin responsiveness in the dose-response studies of lipogenesis in diabetic subjects, the individual values for insulin concentrations giving half-maximal stimulation could not be estimated. Lipogenesis in adipocytes from NPW exhibited the same trend as in PWIDD with nonsignificant reductions of non-insulin- and maximally insulin-stimulated lipogenesis (Figure 4). Insulin responsiveness above basal level was significantly reduced (27 ± 21%, $P < 0.05$). Again, individual values for changes in insulin sensitivity could not be estimated with any precision.

In studies of glucose oxidation in adipocytes from NPW and PWIDD, nonsignificantly reduced basal values were found (Figure 5), whereas maximal insulin responsiveness above basal level was significantly reduced in NPW (33 ± 29%, $P < 0.05$) and in PWIDD (23 ± 26%, $P < 0.05$) when compared with NW (86 ± 23%). Individual values for changes in the insulin sensitivity could not be estimated (Figure 5).

DISCUSSION

In the present paper we confirm previous reports that insulin receptor binding to blood cells from both normal and diabetic women in late pregnancy is similar to that found in normal nonpregnant women equally dispersed between the two phases of the menstrual cycle.[8] It was therefore surprising to find that insulin receptor binding on adipocytes from both groups of pregnant women was significantly reduced. Our

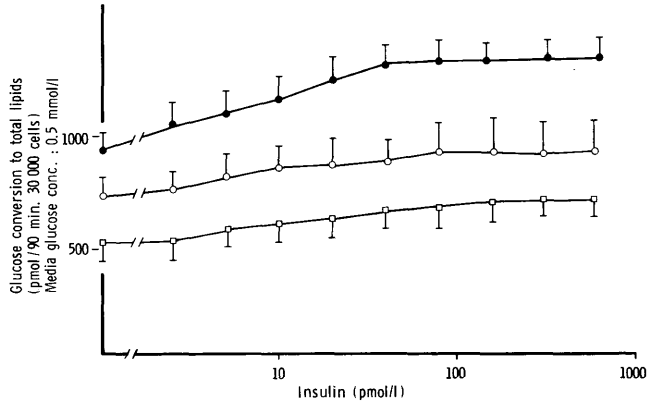

FIGURE 4. Lipogenesis at 37°C of fat cells as estimated by conversion of D-U-[^{14}C]glucose to total lipids at a glucose concentration of 0.5 mmol/L. Adipocytes from 10 nonpregnant normal women (●), 8 pregnant normal women (○), and 9 pregnant insulin-dependent diabetic women (□). Results are mean ± SEM.

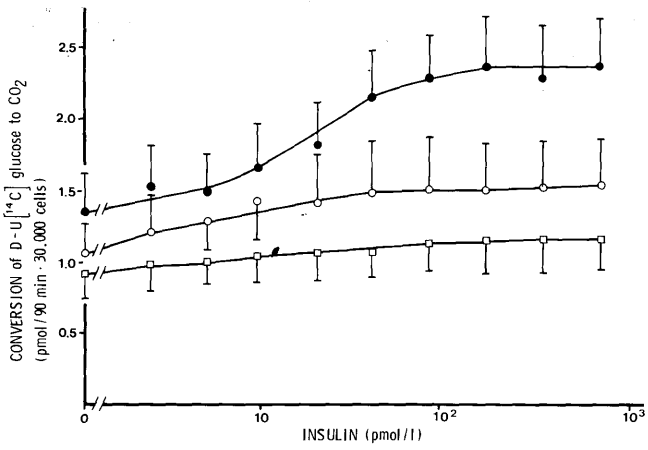

FIGURE 5. Glucose oxidation at 37°C of fat cells as estimated by conversion of D-U-[^{14}C]glucose to CO_2 at a glucose concentration of 0.5 mmol/L. Adipocytes from 10 nonpregnant normal women (●), 8 pregnant normal women (○), and 9 pregnant insulin-dependent diabetic women (□). Results are mean ± SEM.

finding in adipocytes is consistent with the previous report by Pagano et al.,[4] although not directly comparable because the latter report gave no information about the time during the menstrual cycle in which the control women were studied. However, it might be added that we have not been able to find, in the present work or previously,[15] any significant difference in insulin binding to adipocytes between women in the secretory and the proliferative phases of the cycle. Hence, the effect of menstrual cycle on insulin binding may be most pronounced in blood cells.[20]

In the present work, insulin binding to monocytes and erythrocytes measured at 15°C and insulin binding to fat cells measured at both 15 and 37°C showed no significant relationships when compared on an individual basis. These observations add to the accumulating mass of evidence that insulin binding to one type of cell does not necessarily reflect the receptor status of other types of cells.[17]

The major objective of our study was to identify possible postbinding defects of adipocyte insulin action in normal and diabetic pregnancy. Examinations of insulin dose-response characteristics showed rightward shifts of insulin-stimulated glucose transport of adipocytes from both groups of pregnant women. Theoretically, this reduction of insulin sensitivity may be the functional consequence of the impaired adipocyte insulin binding.[21] No significant correlations could, however, be demonstrated between adipocyte insulin binding at tracer insulin concentration and the insulin concentrations giving half-maximal responses of glucose transport in either of the groups. Hence, it cannot be ruled out that the rightward shifts of the insulin dose-response curves might have been caused by modulation of the cellular insulin action at postbinding steps.[21]

In pregnant women, non-receptor-controlled postbinding defects, as manifested by impaired maximal responses, were identified at both the transport and the metabolism steps of adipocyte glucose processing. Another prominent alteration of glucose metabolism in adipocytes from pregnant women was the depressed non-insulin-stimulated glucose metabolism rates.

Our study cannot answer whether pregnancy-associated factors are inducing the changes of non-insulin- and insulin-stimulated adipocyte glucose processing. In future studies of human pregnancy it might be relevant to examine the possible relationships between the ambient concentrations of cortisol, estrogens, progesterone, human placenta lactogen, prolactin, and relaxin and the values of non-insulin- and insulin-enhanced adipocyte glucose utilization.[22,23] Under certain conditions, more of these gestational hormones possess regulatory effects on cellular insulin action.

In our study the three groups were not comparable with regard to the type of anesthetic procedure. However, as the fat tissue was removed in the beginning of the surgery, it is unlikely that the differences introduced in this short time had any significant influence on the results. The different diets eaten by the diabetic pregnant women might also have influenced the insulin binding and action. However, in a previous study, we found that a diet rich in starch and low in fat improved the insulin binding and action in fat cells.[24] Hence, the present results in pregnant diabetic women cannot be explained by the dietary difference.

The similarities in results between the two pregnant groups should be emphasized. In fact, no significant differences were observed between the nondiabetic and diabetic pregnant women. In nonpregnant women with insulin-dependent diabetes mellitus, we have found impaired adipocyte insulin binding and a concomitant decrease in basal and maximally insulin-stimulated glucose metabolism.[25] From the findings in the present study of no further deterioration of insulin binding and action in the diabetic pregnant women compared with the nondiabetic pregnant women, it may be surmised that the in vitro insulin resistance observed in these two clinically insulin-resistant states are not additive.

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