Postreceptor Defects Causing Insulin Resistance in Normoinsulinemic Non-insulin-dependent Diabetes Mellitus

JAN BOLINDER, JAN ÖSTMAN, AND PETER ARNER

SUMMARY

The mechanisms of the diminished hypoglycemic response to insulin in non-insulin-dependent diabetes mellitus (NIDDM) with normal levels of circulating plasma insulin were investigated. Specific binding of mono-^{125}I (Tyr A_{14})-insulin to isolated adipocytes and effects of insulin (5–10,000 μU/ml) on glucose oxidation and lipolysis were determined simultaneously in subcutaneous adipose tissue of seven healthy subjects of normal weight and seven untreated NIDDM patients with normal plasma insulin levels. The two groups were matched for age, sex, and body weight. Insulin binding, measured in terms of receptor number and affinity, was normal in NIDDM, the total number of receptors averaging 350,000 per cell. Neither sensitivity nor the maximum antilipolytic effect of insulin was altered in NIDDM patients as compared with control subjects; the insulin concentration producing half the maximum effect (ED_{50}) was 10 μU/ml. As regards the effect of insulin on glucose oxidation, for the control subjects ED_{50} was 30 μU/ml, whereas in NIDDM patients, insulin exerted no stimulatory effect. The results obtained suggest that the effect of insulin on glucose utilization in normoinsulinemic NIDDM may be diminished in spite of normal insulin binding to receptors. The resistance may be due solely to postreceptor defects, and does not involve antilipolysis.

DIABETES *31*:911–916, October 1982.

Insulin resistance, as reflected in attenuation of the hypoglycemic response to exogenous and endogenous insulin, is a recognized feature of non-insulin-dependent diabetes mellitus (NIDDM).[1] Since the initial stage in the action of insulin consists in its binding to cell-surface receptors,[2] insulin resistance in NIDDM is possibly due to decreased insulin binding. However, the cause of insulin resistance is known to reside at the receptor level only when NIDDM is accompanied by hyperinsulinemia;[3] when the fasting insulin level is normal or low, NIDDM patients are insulin resistant in spite of normal insulin binding.[4] The mechanisms of insulin resistance in the latter form of NIDDM are not known.

In the present study, the mechanisms of insulin resistance in NIDDM patients with normal insulin levels were investigated in vitro on human adipose tissue. Insulin binding to fat cell receptors and the effect of insulin on glucose oxidation and lipolysis were simultaneously determined in adipose tissue obtained from seven untreated patients in whom NIDDM had recently been diagnosed and who had normal fasting plasma insulin levels, but who were resistant to the hypoglycemia-inducing effect of insulin in vivo. Comparison was made with a control group of seven healthy nonobese subjects. The two groups were matched for age, sex, and body weight.

MATERIALS AND METHODS

Subjects. The study was performed on seven healthy nonobese subjects and seven patients with recently diagnosed, untreated NIDDM with fasting plasma levels of insulin within two standard deviations of the mean for the control group. The two groups were matched for sex, age, and body weight (Table 1). The patients and control subjects consumed a diet consisting of about 55% carbohydrate, 25% fat, 20% protein, and about 7–8.5 MJ, according to a 24-h recall. All the diabetics had glucosuria but none had ketosis. There was an initial weight loss at the onset of the disease, but the body weight of the diabetics was stable during the last month preceding the investigation. There were no signs of inactivity secondary to the illness. After the investigation, the diabetes was treated with diet alone or in combination with sulfonylurea.

After an overnight fast, gluteal specimens of subcutaneous adipose tissue was obtained surgically. Local anesthesia was induced with prilocaine chloride in such a way that it did not affect the metabolism of adipose tissue.[5] After the biopsy, venous blood samples were taken for determination of glucose[6] and plasma immunoreactive insulin.[7] For methodologic studies, abdominal subcutaneous adipose tissue

From the Department of Medicine and the Research Center, Huddinge Hospital, Karolinska Institute, Huddinge, Sweden.
Address reprint requests to Dr. Peter Arner, Department of Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.
Received for publication 4 December 1981 and in revised form 7 May 1982.

TABLE 1
Clinical and metabolic characteristics of the study groups

	N	Sex (M/F)	Age (yr)	Body weight (% of average)	Fasting blood glucose level (mmol/L)	Fasting serum insulin level (μU/ml)	K_{itt} (% min^{-1})	Fat cell volume ($mm^3 \times 10^{-6}$)
Control	7	3/4	45 ± 4	108 ± 4	4.8 ± 0.2	8.9 ± 2.0	4.5 ± 0.3	971 ± 123
NIDDM	7	4/3	48 ± 3	110 ± 17	12.7 ± 0.7	10.0 ± 1.6	1.3 ± 0.3	860 ± 72
P		NS	NS	NS	<0.001	NS	<0.001	NS

The values are mean ± SE. The average body weight was obtained from Documenta Geigy.[34] NS = not significant. N = number of subjects.

was also obtained from nine nonobese patients undergoing elective abdominal surgery. These patients had fasted overnight and only saline was given intravenously until fat tissue had been taken.

The study was approved by the Ethical Committee of the Karolinska Institute. Each subject was given a detailed oral and written description of the study, and his or her consent was obtained.

Insulin tolerance. After the fat biopsy, crystalline porcine insulin (0.1 U/kg body wt) was rapidly injected intravenously. After 2.5, 5, 7, 7.5, 10, 15, 20, 25, and 30 min, blood glucose was determined. A rate-constant (K_{itt}) was derived from a semilogarithmic plot of the fall in blood glucose level after the insulin injection.[8]

Insulin binding. Isolated fat cells were prepared by the method of Rodbell.[9] The cells were incubated in 0.5 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing dialyzed bovine serum albumin (40 mg/ml), glucose (1 mg/ml), ^{125}I-insulin (0.05 pmol/ml) and unlabeled insulin (0–50 pmol/ml). The final cell concentration was 8% (vol/vol). After incubation for 60 min at 24°C in triplicate or quadruplicate, 10 ml of ice-cold saline was added and the cells were centrifuged through 1.2 ml of silicone oil as described by Gliemann and Sonne,[10] and the cells were removed for radioactivity determination. Nonspecific binding was measured as the amount of ^{125}I-insulin remaining in the cell layer in the presence of 20 μmol/ml of unlabeled insulin. All the binding values presented were corrected for nonspecific binding. The results of the binding studies were calculated on the basis of fat cell number and fat cell surface area and were presented in three ways: (1) the percentage of the specifically bound total radioactivity versus the total insulin concentration; (2) the ratio of specifically cell-bound hormone to that of the concentration of free hormone versus the specifically bound insulin. From this Scatchard curve[11] the total molar binding capacity was obtained as the intercept of the curve on the horizontal axis and multiplied with 6.03×10^{23} to obtain total receptor number; and (3) the average receptor affinity profile for insulin as a function of receptor occupancy was obtained from the Scatchard curve as described by DeMeyts and Roth.[12] $K_{empty\ sites}$ and $K_{filled\ sites}$ denote the affinity states obtained at zero and maximum levels of receptor occupancy, respectively. The individual curves and affinity values were obtained by processing the binding data on a Wang 2200 T computer system using a least squares parameter-fitting method.[13] The coefficient of variance within one subject for insulin binding was 8%.

Insulin degradation. After the bound and free insulin had been separated as described above, to 0.05-ml buffer containing albumin (40 mg/ml) were added 200-μl aliquots of the buffer containing radioactive material and 0.5 ml of buffer containing 15% (vol/vol) trichloroacetic acid (TCA). The precipitate was washed once with 15% of TCA. Insulin degradation was measured as soluble radioactivity and expressed as the percentage of total radioactivity in buffer.

Lipolysis and glucose oxidation. Subcutaneous adipose tissue was preincubated for 30 min at 37°C in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing dialyzed bovine serum albumin (40 mg/ml) and glucose (2 mg/ml). Tissue segments (about 100 mg) were incubated in triplicate or quadruplicate for 2 h at 37°C in 1 ml of an albumin buffer of the above composition to which glucose (1 mg/ml), [U-^{14}C]glucose (2×10^6 cpm/ml), and insulin (0–2500 μU/ml) had been added. The incubation was performed with CO_2:O_2 (5:95) as gas phase in siliconized glass vessels capped with rubber membranes and fitted with a glass beaker containing a filter paper (Munktell, Grycksbo AB, Sweden). After incubation, 0.3 ml of 0.5-N sulfuric acid was injected into the medium and 0.3 ml of Hyamin-X was injected into the filter. Thereafter, the vessels were placed on ice for 60 min and opened; the filter papers were removed for the determination of radioactivity in CO_2.[9] Quenching was negligible. Aliquots of the medium were removed for duplicate estimation of glycerol.[14] Glycerol release and $^{14}CO_2$ production remained linear for at least 4 h. Insulin responsiveness was defined as inhibition of lipolysis and stimulation of $^{14}CO_2$ production at the maximum effective insulin concentration. The dose-response curves were linearized by using a log-logit plot for determining the insulin sensitivity.[15,16] Log insulin concentration was plotted versus log (Y/100 − Y), where Y denotes the insulin effect as a percentage of the maximum effect. The concentration producing half the maximum effect (ED_{50})is obtained from where the curve intersects the abscissa at 0. The coefficient of variance within one subject for the metabolic studies was 8%.

It was preferred to examine the effect of insulin on basal metabolism since, in an in vitro study on human adipose tissue, noradrenaline-induced lipolysis was found to be inhibited at low concentrations of insulin but stimulated at high concentrations.[16] Such dose-response curves cannot be interpreted in terms of sensitivity and responsiveness.

Fat cell size. Fat cell size was measured by the method of Sjöström and co-workers.[17] Mean fat cell volume, weight, and surface area were determined from previously derived formulas.[18,19] The number of fat cells incubated was determined as the quotient of the lipid weight of the incubated sample by the mean fat cell weight.

Chemicals. Crystalline, glucagon-free porcine insulin was from Vitrum AB (Sweden). Mono^{125}I-(Tyr A_{14})-insulin (spe-

cific activity about 200 Ci/g) was purchased from Novo (Denmark). ^{125}I-insulin (specific activity about 25 Ci/g) was from Pharmacia AB (Sweden). This tracer had 0.1–0.2 iodine atoms/insulin molecule.[20] At this low degree of iodination more than 95% of the iodoinsulin is monoiodinated.[21] [U-^{14}C]glucose (specific activity 268 mCi/mmol) was from the Radiochemical Centre (England). Bovine serum albumin (Fraction V) was from Armour Pharmaceutical Company (England) and Hyamin-X from Packard, (United States).

Statistical analysis. Student's *t* test, linear regression analysis by the method of least squares, and the F-distribution test were performed.[22] The reported values are the mean ± SE.

RESULTS

The diabetic patients registered a 70% lower value of K_{itt} than the control subjects ($P < 0.001$), but the fasting plasma insulin levels and fat cell volumes were similar in the two groups (Table 1). In vivo insulin sensitivity was presently determined after the fat biopsy. In theory, the surgical procedure might have influenced the determination of in vivo insulin sensitivity. To answer this question, five of the control subjects were reinvestigated after 3–6 mo. Blood glucose, plasma insulin, and insulin tolerance were determined after an overnight fast, but no fat biopsy was taken. In these five subjects K_{itt} was 3.8 ± 0.5%/min at the first time and 3.8 ± 0.2%/min at the second time. The fasting blood glucose and plasma insulin levels were also identical at the time of the two investigations (data not shown). On the basis of these observations we conclude that the fat biopsy procedure did not contribute to the measurements of in vivo insulin sensitivity.

Unpurified mono-^{125}I-insulins are generally used when insulin receptor binding is determined. However, these tracers are mixtures of differently iodinated molecules, some of which exhibit attenuated receptor binding. However, it has recently been demonstrated that purified Tyr A_{14} monoiodoinsulin tracer has a significantly higher binding affinity to insulin receptors than other monoiodoinsulins.[23] In a methodologic study we compared unpurified mono-^{125}I-insulin and mono-^{125}I-(Tyr A_{14})-insulin using the same human fat cell preparation. Isolated adipocytes were incubated for 60 min at 24°C; unspecific binding (four experiments) and insulin degradation in the incubation medium (5 experiments) were determined. Unspecific binding was 3.9 ± 1.5% with the Tyr A_{14} tracer and 8.9 ± 1.7% with the unpurified tracer ($P < 0.005$). Insulin degradation was 4.2 ± 1.6% with the former and 23.2 ± 0.8% with the latter tracer ($P < 0.001$). Thus both unspecific binding and insulin degradation are markedly lower when the A_{14} tracer is used as compared with the unpurified monoiodoinsulin tracer. Pedersen and his colleagues also noted insignificant insulin degradation in the incubation medium and unspecific binding using human adipocytes incubated in the presence of mono-^{125}I-(Tyr A_{14})-insulin.[24] They observed less than 1% insulin degradation and 1–4% unspecific binding. On the basis of the present and previous results we conclude that mono-^{125}I-(Tyr A_{14})-insulin should preferably be used for the determination of insulin binding; consequently, only this isotope was used in the subsequent studies. Figure 1 shows the time course for specific insulin binding in one representative subject. Steady state of binding was obtained at 30 min and maintained for at least 90 min. In five successive experiments steady-state of binding was always reached after 20–40 min of incubation at 24°C. This is quicker than that found at 37°C by Pedersen and his colleagues, who reported that 45 min were required.[24]

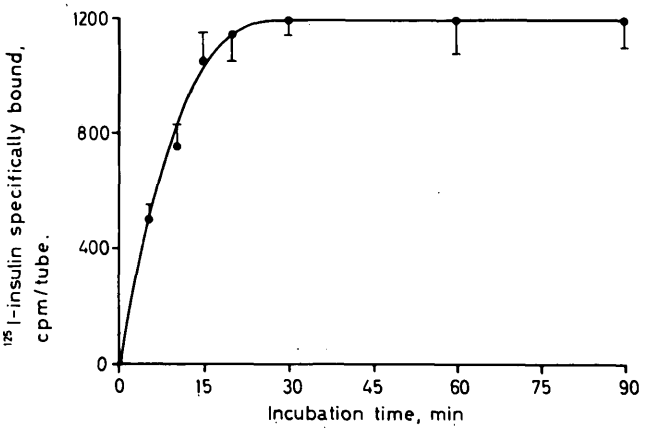


FIGURE 1. Time course for insulin binding. Isolated fat cells were prepared from one subject and incubated in quintuplicate at 24°C with mono-^{125}I-(Tyr A_{14})-insulin for 5, 10, 15, 20, 30, 60, and 90 min. Specific insulin-bound radioactivity was determined. Values are mean ± SE.

The insulin-binding capacity of adipocytes obtained from the normal subjects and NIDDM patients is summarized in Figure 2. At none of the hormone concentrations tested was there any difference between the two groups as regards specific insulin binding expressed in terms of cell surface area, and the curves in the Scatchard analysis were almost identical. Neither were there any differences in insulin binding when the binding variables were expressed in terms of fat cell number (figure not shown). The total number of receptors per unit of cell surface area as well as per cell were roughly the same in the NIDDM patients as compared with the control subjects with values of about 8/μm^2 and 350,000/cell, respectively (Table 2). The two groups did not differ significantly either as regards insulin binding affinity; the values of K_{empty} and K_{filled} sites were about 0.7 and 0.15 × 10^9 M^{-1}, respectively (Table 2). There was also no difference with regard to average affinity profiles for the two groups (data not shown). The insulin degradation was insignificant in both groups (about 3–4%).

Glucose oxidation and glycerol release in adipose tissue segments and isolated adipocytes were compared in a methodologic study (Figure 3). The basal rate of lipolysis was 20% lower in tissue segments than in isolated fat cells ($P < 0.01$). The dose-dependent insulin-induced inhibition of lipolysis, on the other hand, was almost the same for the two types of preparation. The basal rate of glucose oxidation was 80% lower in isolated adipocytes than in adipose tissue segments ($P < 0.01$); in the isolated cells there was no measurable insulin-induced stimulation of glucose oxidation, whereas in the tissue segments a dose-dependent stimulation was found. Similar differences between human adipose tissue segments and isolated adipocytes as regards lipolysis and glucose oxidation have been reported previously.[25,26] Obviously, the basal and insulin-stimulated metabolism in human adipose tissue is modified by the collagenase procedure for fat cell isolation, and for this reason subsequent metabolic determinations were performed only on adipose tissue segments.

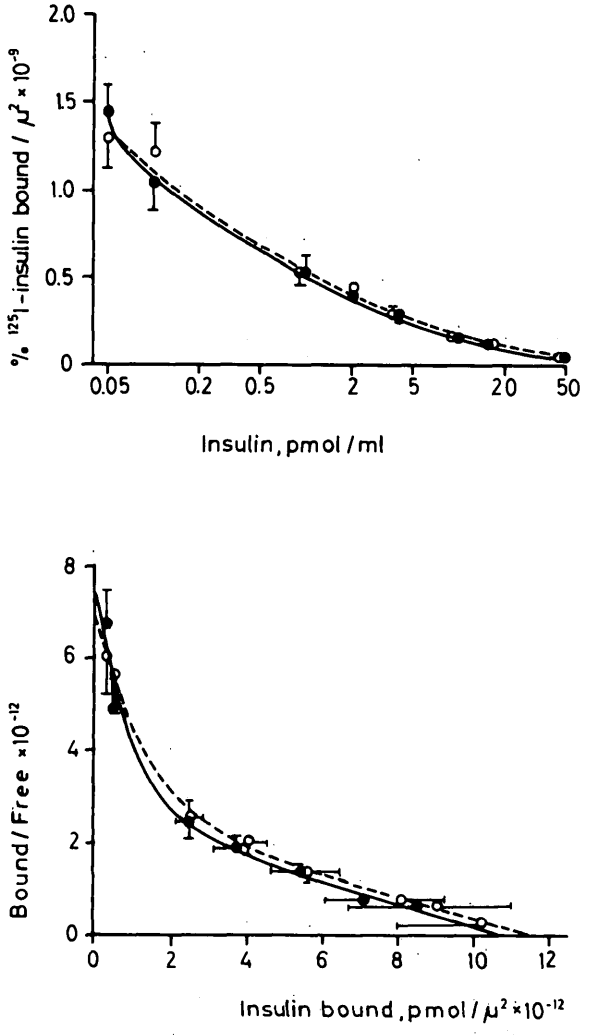


FIGURE 2. Insulin binding to isolated fat cells of seven subjects with untreated normoinsulinemic non-insulin-dependent diabetes mellitus (NIDDM, ●) and seven control subjects (○). Data are expressed as the percentage of ^{125}I-insulin specifically bound at various concentrations of insulin (*upper curve*). The mean ratio of bound to free hormone and the amount of bound insulin at a particular insulin concentration were determined from the binding values relating to each subject, and a Scatchard curve[11] was drawn using a computerized curve-fitting technique[13] (lower curve). All the values are corrected for nonspecific binding and expressed in terms of cell surface area. For further details see legend to Figure 1.

The rate of basal lipolysis was more rapid in the NIDDM group, as in the controls, with values of 11.6 ± 1.5 and 7.0 ± 2.1 μmol/10^7 cells/2 h, but the difference was not significant. Comparison of the insulin dose-response curves for antilipolysis relating to the two groups showed no difference in the level of sensitivity (Figure 4); ED_{50} was 10 μU/ml. The responsiveness to the antilipolytic effect of insulin was also not different between the two groups, about 3.5 μmol/10^7 cells/2 h (Figure 4).

The basal rate of [U-^{14}C]glucose oxidation in the NIDDM group was significantly lower than in the controls with values of 0.32 ± 0.06 and 0.90 ± 0.15 μmol/10^7 cells/2 h, respectively ($P < 0.001$). In the control group there was a dose-dependent insulin-induced stimulation of glucose oxidation (Figure 5), but at a higher insulin concentration than that at which there was an effect of insulin on lipolysis. ED_{50} for $^{14}CO_2$ production was 30 μU/ml. Similar differences in ED_{50} reflecting the effect of insulin on lipolysis and glucose

TABLE 2
Receptor number and receptor affinity in the study groups

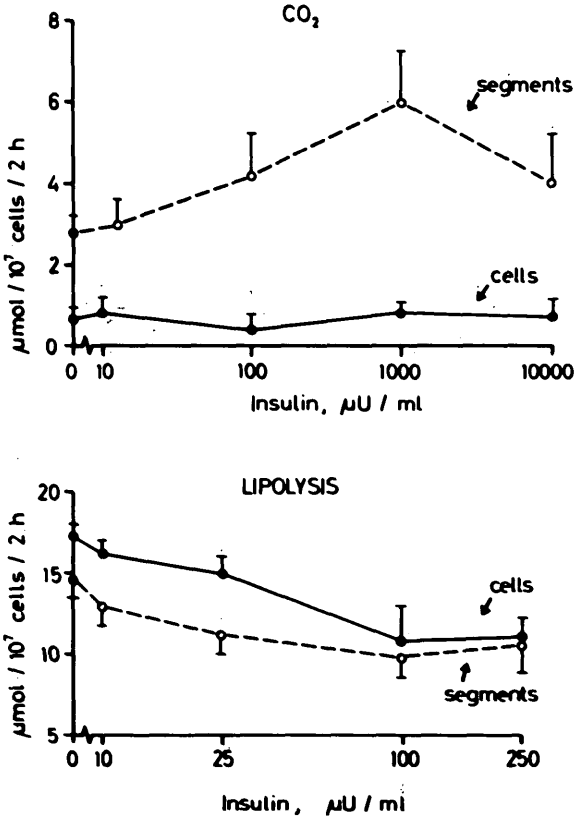
	Total number of receptors		Receptor affinity, $M^{-1} \times 10^{-9}$	
	Per μm^2	Per cell $\times 10^3$	Empty sites	Filled sites
Control	7.5 ± 1.6	323 ± 61	0.69 ± 0.21	0.19 ± 0.03
NIDDM	8.2 ± 1.4	352 ± 67	0.73 ± 0.14	0.13 ± 0.02
P	NS	NS	NS	NS

The total number of receptors was calculated from the individual Scatchard[11] curves obtained from seven control subjects and seven NIDDM patients as described in the MATERIALS AND METHODS section. See footnotes to Table 1 for further details.

utilization have previously been observed in human adipose tissue.[27] In the diabetic group there was no significant increase in glucose oxidation at insulin concentrations of up to 2500 μU/ml (Figure 5). With 5000 and 10,000 μU/ml used in some uncharted experiments there was no further stimulation of $^{14}CO_2$ production in the NIDDM patients. Responsiveness to the effect of insulin on $^{14}CO_2$ was seven times greater in the control state than in NIDDM ($P < 0.01$).

The differences between the NIDDM and the control group in respect to the rates of lipolysis and $^{14}CO_2$ production, expressed in terms of fat cell number, were similar when expressed in terms of lipid weight.

FIGURE 3. Effect of insulin on glucose oxidation and glycerol release for adipose tissue segments and isolated adipocytes. Segments and isolated adipocytes obtained from four subjects during abdominal surgery were incubated in medium containing [U-^{14}C]glucose in the absence and presence of various concentrations of insulin, as indicated in the figures. Glycerol release into the medium and the effect of insulin on incorporation on ^{14}C into CO_2 were determined. For further details see legend to Figure 1.



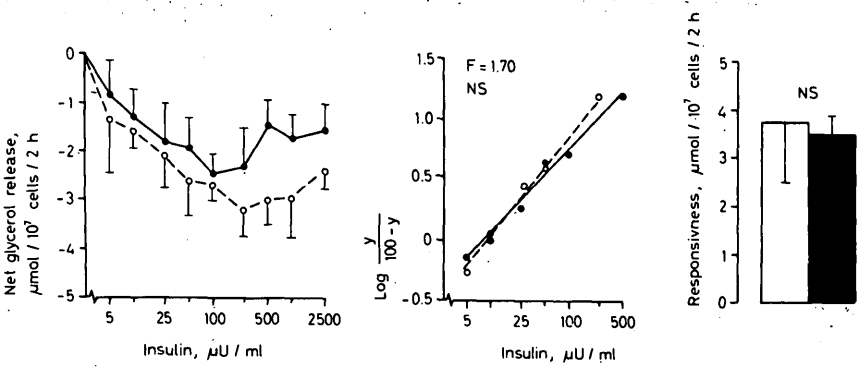


FIGURE 4. Antilipolytic effect of insulin. Subcutaneous adipose tissue segments from seven NIDDM patients and seven control subjects were incubated with and without insulin (5–2500 μU/ml). Basal minus insulin-induced glycerol release was calculated. Insulin sensitivity and insulin responsiveness were calculated as described in MATERIALS AND METHODS. The positions of the regression lines were compared by the F-distribution test.[22] NS = not significant. For further details see legends to Figures 1 and 2.

DISCUSSION

Since human fat cells exhibit their maximum response to insulin when only a minority of insulin receptors are occupied,[28] the functional consequences of alterations at the receptor and postreceptor level must differ.[29] In the former case, changes in insulin binding and sensitivity are observed, whereas in the latter case there is an alteration of the maximum insulin response.[29]

The present study of insulin action in adipose tissue of untreated NIDDM patients, characterized by a decreased in vivo hypoglycemic effect of insulin and normal fasting plasma insulin levels, demonstrated an almost completely blunted effect of insulin on glucose oxidation in spite of normal insulin binding to receptors and normal sensitivity to the antilipolytic effect of insulin. These results indicate that the resistance to the effect of insulin on glucose utilization in normoinsulinemic NIDDM is due to defects in the postreceptor pathways, rather than at the receptor level. However, it must be born in mind that only a minute part of orally ingested glucose is used by human adipose tissue.[30] Therefore, it seems unlikely that the diminished effect of insulin on the utilization of glucose by adipose tissue is the sole cause of the overall insulin resistance; resistance located in other major target tissues (i.e., liver and skeletal muscle) may also be of importance. The number of insulin receptor sites per human fat cell was presently calculated to be 325,000–350,000. This is similar to that previously reported by Olefsky,[31] who observed 300,000 sites per cell in subcutaneous adipose tissue obtained from nonobese, nondiabetic subjects undergoing elective surgery.

A decreased basal rate of glucose oxidation was demonstrated in diabetic adipose tissue. This result indicates further that postreceptor abnormalities in the regulation of glucose metabolism occur in NIDDM patients with normal circulating insulin levels.

Age, sex, body weight, and fat cell size are factors that may influence insulin receptor binding and insulin action.[2,3] It is improbable that these factors had any bearing on the present findings since, first, the control and NIDDM groups were matched for age, sex, and body weight and, second, fat cell size was similar in the two groups. The fact that, for methodologic reasons, fat segments were used for measuring the metabolic effects of insulin may account for variability of the penetration of insulin to the cell membranes. This variability, however, has no bearing on the interpretation of the results, since the dose-response curves that represented the antilipolytic effect of insulin were similar for isolated fat cells and tissue segments. Moreover, the intrasubject variability of the metabolic determinations was small, and the incubation period was as long as 2 h.

The findings relating to insulin binding to isolated adipocytes are consistent with the normal insulin binding to monocytes observed in NIDDM patients with normal insulin levels.[4]

Regarding the antilipolytic effect of insulin, the observed similarity of both sensitivity and responsiveness to the hormone in the NIDDM and control groups indicates that this property of the hormone was unimpaired at either the receptor or the postreceptor level. This finding, which accords with the results obtained in a recent study of lipolysis in human adipose tissue in NIDDM,[16] indicates that antilipolysis is not involved in the insulin resistance observed in normoinsulinemic NIDDM patients. While insulin resistance is usually discussed in terms of the entire organism, the present results relating to lipolysis and glucose utilization suggest that, at least in some forms of human diabetes mellitus, the mechanism of the resistance may be confined to a single metabolic pathway of the hormone action.

Evidence of heterogeneity among patients with NIDDM has recently been presented.[32] One form of the disease

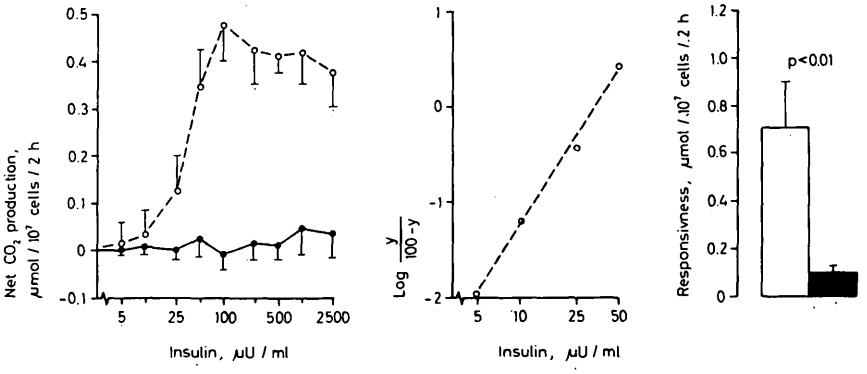


FIGURE 5. Insulin-induced stimulation of glucose oxidation in human adipose tissue in NIDDM and the control state. The incorporation of radioactive glucose into CO_2 was determined in the experiments described in Figure 4, and insulin-induced $^{14}CO_2$ production minus basal $^{14}CO_2$ production was calculated. For further details see legends to Figures 1–3.

would appear to be essentially due to insulin deficiency, while in another form resistance to the action of the hormone would seem to be the underlying cause. The cause of insulin resistance may vary as well. For example, insulin resistance in hyperinsulinemic NIDDM is believed to be due to decreased insulin binding to receptors.[3] It has also been inferred[33] that in patients with mild carbohydrate intolerance the resistance is due to receptor defects, whereas in patients with more severe intolerance and hyperglycemia the attenuated response to the hormone is due to a combination of receptor and postreceptor defects. The results of the present study provide experimental evidence that patients with fasting hyperglycemia and normal fasting insulin levels constitute another NIDDM subgroup; in these patients, receptor binding is normal and postreceptor defects are the cause of the resistance to the action of the hormone.

ACKNOWLEDGMENTS

Grants were received from the Swedish Medical Research Council, the Swedish Diabetes Association, the Karolinska Institute, the Groschinsky Foundation, the Osterman Foundation, the Tore Nilsson Foundation, the Folksam Foundation, and the Swedish Nutritional Research Foundation.

REFERENCES

[1] Reaven, G. M.: Insulin-independent diabetes mellitus: metabolic characteristics. Metabolism *29*:445–54, 1980.

[2] Roth, J.: Peptide hormone binding to receptors: a review of direct studies in vitro. Metabolism *8*:1059–73, 1973.

[3] Olefsky, J. M.: Insulin resistance and insulin action. An in vitro and in vivo perspective. Diabetes *30*:148–62, 1981.

[4] Olefsky, J. M., and Reaven, G. M.: Insulin binding in diabetes: relationships and plasma insulin levels and insulin sensitivity. Diabetes *26*:680–88, 1977.

[5] Arner, P., Arner, O., and Östman, J.: The effect of local anesthetic agents on lipolysis by human adipose tissue. Life Sci. *13*:161–69, 1973.

[6] Coburn, H. J., and Carrol, J. J.: Improved manual and automated calorimetric determination of serum glucose with use of hexokinase and glucose-6-phosphate dehydrogenase. Clin. Chem. *19*:127–30, 1973.

[7] Wide, L., Axen, R., and Porath, J.: Radioimmunosorbent assay for proteins. Chemical couplings of antibodies to insoluble dextran. Immunochemistry *4*:381–86, 1967.

[8] Harrison, L. C., and King-Roach, A. P.: Insulin sensitivity of adipose tissue in vitro and the response to exogenous insulin in obese human subjects. Metabolism *25*:1095–1101, 1976.

[9] Rodbell, M.: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. *239*:375–80, 1964.

[10] Gliemann, J., and Sonne, O.: Binding and receptor-mediated degradation of insulin in adipocytes. J. Biol. Chem. *253*:7857–63, 1978.

[11] Scatchard, G.: The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. *51*:660–72, 1949.

[12] DeMeyts, P., and Roth, J.: Cooperativity in ligand binding: a new graphic analysis. Biochem. Biophys. Res. Commun. *66*:1118–26, 1975.

[13] Rosenthal, H. E.: A graphic method for the determination and presentation of binding parameters in a complex system. Anal. Biochem. *20*: 525–32, 1967.

[14] Chernick, S. S.: Determination of glycerol in acylglycerols. Methods Enzymol. *4*:627, 1969.

[15] Goldstein, A., Aronow, L., and Kalman, S. M.: Principles of Drug Action: The Basis of Pharmacology, 2d edit. New York, John Wiley & Sons, 1974, pp. 101–104.

[16] Arner, P., Bolinder, J., Engfeldt, P., and Östman, J.: The antilipolytic effect of insulin in human adipose tissue in obesity, diabetes mellitus, hyperinsulinemia and starvation. Metabolism *30*:753–60, 1981.

[17] Sjöström, L., Björntorp, P., and Vrana, J.: Microscopic fat-cell size measurements of frozen-cut adipose tissue comparison with automatic determinations of osmium-fixed fat cells. J. Lipid Res. *12*:521–30, 1971.

[18] Hirsch, J., and Gallian, E.: Methods for the determinations of adipose cell size and cell number in man and animals. J. Lipid Res. *9*:110–19, 1968.

[19] Zinder, O., and Shapiro, B.: Effect of cell size on epinephrine- and ACTH-induced fatty acid release from isolated fat cells. J. Lipid Res. *12*:91–95, 1971.

[20] Jørgensen, K. H., and Binder, C.: ^{125}I-insulin as a tracer of insulin in different chemical processes. *In* Labelled Proteins in Tracer studies. Donodo, L., Ed. Brussels, Euratome, 1966, pp. 329–33.

[21] Freychet, P., Roth, J., and Neville, D. M., Jr.: Monoiodoinsulin: demonstration of its biological activity and binding to fat cells and liver membranes. Biochem. Biophys. Res. Commun. *43*:400–408, 1966.

[22] Neter, J., and Wasserman, W.: Comparison of two regression lines. *In* Applied Linear Statistical Models. Irwin, R. D., Ed. Illinois, 1974, p. 160.

[23] Gliemann, J., Sonne, O., Linde, S., and Hansen, B.: Biological potency and binding affinity of monoiodoinsulin with iodine in tyrosine A 14 or tyrosine A 19. Biochem. Biophys. Res. Commun. *87*:1183–90, 1979.

[24] Pedersen, O., Hjøllund, E., Beck-Nielsen, H., Lindskov, H. O., Sonne, O., and Gliemann, J.: Insulin receptor binding and receptor-mediated insulin degradation in human adipocytes. Diabetologia *20*:636–41, 1981.

[25] Engfeldt, P., Arner, P., and Östman, J.: Influence of adipocyte isolation by collagenase on phosphodiesterase activity and lipolysis in man. J. Lipid Res. *21*:443–48, 1980.

[26] Gries, F. A., and Steinke, J.: Comparative effects of insulin on adipose tissue segments and isolated fat cells of rat and man. J. Clin. Invest. *46*:1413–21, 1967.

[27] Jacobsson, B., Holm, G., Björntorp, P., and Smith, U.: Influence of cell size on the effects of insulin and noradrenaline on human adipose tissue. Diabetologia *12*:69–72, 1976.

[28] Pedersen, O., and Gliemann, J.: Hexose transport in human adipocytes: factors influencing the response to insulin and kinetics of methylglucose and glucose transport. Diabetologia *20*:630–35, 1981.

[29] Kahn, C. R.: Insulin resistance, insulin sensitivity and insulin unresponsiveness: a necessary distinction. Metabolism *27*:1893–1902, 1978.

[30] Björntorp, P., and Sjöström, L.: Carbohydrate storage in man: speculations and some quantitative considerations. Metabolism *27*:1853–65, 1978.

[31] Olefsky, J. M.: Insulin binding to isolated human adipocytes. Diabetes *23*:565–71, 1974.

[32] Ginsberg, H., and Rayfield, E. J.: Effect of insulin therapy on insulin resistance in Type II diabetic subjects. Evidence for heterogeneity. Diabetes *30*:739–45, 1981.

[33] Olefsky, J. M., and Kolterman, O. G.: Mechanisms of insulin resistance in obesity and noninsulin-dependent (type II) diabetes. Am. J. Med. *70*:151–68, 1981.

[34] Dien, K., Ed.: Documenta Geigy. Scientific Tables, 7th edit. Basel, Ciba-Geigy, 1970, p. 711.