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Research Article

Insulin-stimulated kinase activity of adipocyte-derived insulin receptors is reduced in subjects with non-insulin-dependent diabetes mellitus (NIDDM) but normal in obese nondiabetics. To assess the reversibility of the kinase defect in NIDDM, insulin receptor kinase activity was measured before and after weight loss in 10 NIDDM and 5 obese nondiabetic subjects. Peripheral insulin action was also assessed in vivo by glucose disposal rates (GDR) measured during a hyperinsulinemic (300 mU/M2 per min) euglycemic clamp. In the NIDDMs, insulin receptor kinase activity was reduced by 50-80% and rose to approximately 65-90% (P less than 0.01) of normal after 13.2 +/- 2.0 kg (P less than 0.01) weight loss; comparable weight loss (18.2 +/- 1.5 kg, P less than 0.01) in the nondiabetics resulted in no significant change in insulin receptor kinase activity. Relative to GDR measured in lean nondiabetics, GDR in the NIDDMs was 35% of normal initially and 67% (P less than 0.01) of normal after diet therapy; weight loss in the nondiabetics resulted in an increase in GDR from 53 to 76% of normal (P less than 0.05). These results indicate that the insulin receptor kinase defect that is present in NIDDM is largely reversible after weight reduction. In contrast, the improvement in GDR, in the absence of any change in insulin receptor kinase activity in the nondiabetics, suggests that […]

Reversibility of Defective Adipocyte Insulin Receptor Kinase Activity in Non-insulin-dependent Diabetes Mellitus

Effect of Weight Loss

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Abstract

Insulin-stimulated kinase activity of adipocyte-derived insulin receptors is reduced in subjects with non-insulin-dependent diabetes mellitus (NIDDM) but normal in obese nondiabetics. To assess the reversibility of the kinase defect in NIDDM, insulin receptor kinase activity was measured before and after weight loss in 10 NIDDM and 5 obese nondiabetic subjects. Peripheral insulin action was also assessed in vivo by glucose disposal rates (GDR) measured during a hyperinsulinemic (300 mU/M² per min) euglycemic clamp. In the NIDDMs, insulin receptor kinase activity was reduced by 50–80% and rose to ~ 65–90% ($P < 0.01$) of normal after 13.2±2.0 kg ($P < 0.01$) weight loss; comparable weight loss (18.2±1.5 kg, $P < 0.01$) in the nondiabetics resulted in no significant change in insulin receptor kinase activity. Relative to GDR measured in lean nondiabetics, GDR in the NIDDMs was 35% of normal initially and 67% ($P < 0.01$) of normal after diet therapy; weight loss in the nondiabetics resulted in an increase in GDR from 53 to 76% of normal ($P < 0.05$). These results indicate that the insulin receptor kinase defect that is present in NIDDM is largly reversible after weight reduction. In contrast, the improvement in GDR, in the absence of any change in insulin receptor kinase activity in the nondiabetics, suggests that the main cause of insulin resistance in obesity lies distal to the kinase.

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM)[1] is a heterogeneous disorder characterized by defects in insulin secretion and defects in insulin action at the level of insulin-sensitive target tissues (1–11). To date, a host of cellular abnormalities have been described in NIDDM, including decreased binding of insulin to its receptor (1, 2, 5, 7–11), diminished rate of insulin receptor internalization (12), defects in the glucose transport system (1, 5, 9–11, 13, 14), and defects in the action of intracellular enzymes (15). Whereas these alterations are part of the disordered carbohydrate and insulin metabolism observed in NIDDM, it is not clear that any of these defects represent a primary alteration. We (16) and others (17–19) have recently described another cellular abnormality in tissues from NIDDM subjects that could be important in the genesis of the disordered carbohydrate metabolism. The insulin receptor is a tyrosine kinase whose activity is enhanced by insulin-stimulated, tyrosine autophosphorylation (20–22). This activation process is rapid and is the earliest known event to follow insulin binding (23, 24). A growing number of studies now suggest that the kinase activity of the insulin receptor may be critical for many, if not all, of insulin's biologic actions (16–19, 25–35). Insofar as this is true, a defect in the kinase activity of the receptor could explain many of the postbinding aspects of insulin resistance seen in NIDDM. In this regard, we and others have recently demonstrated that insulin receptors from adipocytes (16, 18), hepatocytes (17), and erythrocytes (19) of NIDDM subjects display a ~ 50–80% decrease in kinase activity.

The goal of this study was to determine whether the kinase defect in NIDDM is reversible. To accomplish this goal, insulin receptor kinase activity was studied before and after a period of weight reduction, which has been shown to be an effective means of improving glucose tolerance and peripheral glucose disposal (36–39). Weight loss, sufficient to improve glucose disposal in NIDDM subjects, thus was used to test the reversibility of the kinase defect in NIDDM. Reversibility would suggest that the functional kinase defect observed in receptors from NIDDM subjects is acquired and in some way regulated along with the other metabolic derangements characteristic of the diabetic state. Irreversibility, on the other hand, would suggest a fundamental alteration in the primary or posttranslational structure of the receptor. Along with receptor kinase activity, in vivo glucose disposal was determined before and after weight loss to permit comparisons of changes in kinase activity with changes in overall insulin action.

Methods

Materials. Monocomponent porcine insulin and [^{125}I-Tyr A^{14}]-monoiodoinsulin were generous gifts from Dr. Bruce Frank and Dr. Ronald Chance of the Eli Lilly and Company, Indianapolis, IN. Serum from a patient with anti-insulin receptor antibodies was kindly supplied by Dr. Lawrence Mandarino, Pittsburgh, PA. [γ-^{32}P]ATP (3,000 Ci/mmol) was purchased from Amersham Corp., Amersham, England. Collagenase was obtained from Worthington Biochemical Corp., Freehold, NJ; BSA fraction V from Armour Pharmaceutical Co., Chicago, IL; Protein A from Bethesda Research Laboratories, Gaithersburg, MD; wheat germ agarose beads from Vector Laboratories, Burlingame, CA; and aprotinin from Farbenfabriken Beyer Ag Pharmaceuticals, New York, NY. All other reagents were purchased from Sigma Chemical Co., St. Louis, MO, except Flurosol, which was obtained from National Diagnostics, Somerville, NJ. All materials for

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1. *Abbreviations used in this paper:* GDR, glucose disposal rate; NIDDM, non-insulin-dependent diabetes mellitus.

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protein determination and SDS-PAGE were from Bio-Rad Laboratories, Richmond, CA. The weight reduction diet formula was obtained from MediBase, Advanced Healthcare, Pacific Grove, CA.

Subjects. The study was conducted on 15 obese paid volunteers recruited on the basis of body weight and results of 4-h oral glucose tolerance tests (OGTT). 10 subjects, classified as having NIDDM and 5 classified as nondiabetic, based upon criteria established by the National Diabetes Data Group (40), were admitted to the Special Diagnostic and Treatment Center of the San Diego Veterans Administration Medical Center, and informed consent was obtained. The characteristics of the subjects are listed in Table I. All subjects were screened to ensure that except for obesity and/or diabetes, they were otherwise healthy and weight stable for at least 2 mo before entry into the study. During diet therapy, physical activity was maintained at prediet levels. Of the diabetics, five subjects had been previously treated with oral hypoglycemic medication and five were on diet therapy alone; none had ever been treated with insulin. Oral hypoglycemic agents were discontinued with consent 3–4 wk before entry into the study. No subject was on medication known to alter carbohydrate metabolism.

Diets. To ensure adequate and uniform baseline caloric intake, all subjects initially consumed a weight-maintenance solid diet, containing 55% carbohydrate, 30% fat, and 15% protein (30 kcal/kg body wt) given in three feedings as one fifth, two fifths, and two fifths of the total daily calories at 0800, 1200, and 1700 h, respectively. After 4–7 d of this dietary regimen, the initial studies were conducted. Thereafter, all subjects were placed on a very low-calorie diet of 420–600 kcal/d supplemented with 200–300 kcal/d of high fiber fruits and vegetables. The formula diet was reconstituted from a powder with 8 oz of water per serving, and contained 55% carbohydrate, mainly from lactose and fructose, 42% protein of soy and milk origin, and 3% milk fat, with the essential fatty acids, linoleic and linolenic acid, supplemented with adequate nutrients to supply the daily requirements advised by the Food and Nutrition Board of the National Research Council. The weight loss period ranged from 46–120 d and was conducted entirely as inpatients on a metabolic ward. After weight loss, but before post-weight-loss studies, subjects were fed solid mixed meals of the same composition as the initial preweight-loss diet. The period of refeeding lasted 2 wk, during which time caloric intake was adjusted as necessary to ensure weight maintenance in all subjects.

Euglycemic clamp studies. In vivo insulin sensitivity was assessed by a modification of the euglycemic glucose-clamp technique as previously described (10, 38). After a priming dose of insulin, serum insulin was maintained at 1,000–1,500 μU/ml for the duration of the study by a continuous insulin infusion at a rate of 300 mU/M^2/min. Plasma glucose was maintained between 80 and 90 mg/dl. Three measurements were carried out 60 min after achieving stable rates of glucose infusion and euglycemia. The results were averaged for a particular study. After weight loss plus a 2-wk period of isocaloric refeeding, the glucose-clamp studies were repeated. At insulin infusion rates of 300 mU/M^2/min, hepatic glucose output is completely suppressed in NIDDM and obese, nondiabetic subjects (10, 11, 38). The rate of glucose disposal at steady state is therefore calculated as the rate of glucose infusion used to maintain steady state euglycemia.

Analytical methods. Insulin was assayed by a double-antibody RIA according to the method of Desbuquois and Aurbach (41). Glucose levels were measured by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Total glycosylated hemoglobin was determined in heparinized blood by the Fast Hemoglobin Test System Kit (Isolab, Akron, OH).

Partial purification of insulin receptors from isolated human adipocytes. Isolated adipocytes were prepared from adipose tissue obtained from the lower abdominal region after an overnight fast, as previously described (16) at least 1 d before or 1 d after the measurements of glucose disposal. A small aliquot of cells was taken for determination of adipocyte cell size as previously described (13). The remainder of the freshly prepared adipocytes were solubilized and partially purified by wheat germ chromatography, as previously detailed (16), and then frozen at −70°C for use in subsequent phosphorylation and insulin binding studies. Control studies showed that lectin-purified receptors stored at −70°C, have stable insulin binding and insulin-stimulated receptor kinase activities for at least 6 mo (data not shown) from the time of preparation. The protein content of the frozen aliquots was measured by the Bradford dye method (42) using the Bio-Rad Laboratories reagent.

Insulin binding assays. All insulin binding studies and subsequent kinase measurements were done simultaneously on receptors prepared from each subject's biopsies taken before and after weight reduction. To measure binding, aliquots of the frozen receptor preparations were diluted approximately threefold to a protein concentration of 0.09–0.13 mg/ml and incubated in triplicate for 18 h at 4°C with [^{125}I-Tyr A^{14}]insulin and various concentrations of unlabeled insulin. The amount of receptor-bound hormone was determined by the polyethylene glycol precipitation method (26). As a measure of the number of insulin receptors in each frozen receptor preparation, the insulin-binding capacity was determined by estimating the x intercept of the Scatchard plots of the insulin binding data (43).

Insulin receptor autophosphorylation and receptor immunoprecipitation. To minimize any contribution of interassay variability, kinase activity from each subject's pre- and postweight reduction receptor preparations was measured in the same assay. Based upon insulin binding studies, lectin-purified receptor preparations were diluted to 75 fmol/50 μl so that kinase activity was measured using equal amounts of insulin binding activity for the pre- and postweight loss samples of all subjects. 50-μl aliquots of the adjusted receptor preparations were preincubated in singleton or duplicate at 0, 2.5, 10, and 500 ng/ml (final concentrations) of unlabeled insulin in a total volume of 60 μl. After 18 h at 4°C, the phosphorylation reaction was conducted in the presence of (final concentrations) 5 mM $MnCl_2$, 4 mM $MgCl_2$, 500 μM CTP, and 50 μM [γ-^{32}P]ATP (~ 11 μCi/nmol). After incubation at 4°C for 30 min, the reaction was terminated as previously detailed (16) and the phosphorylated insulin receptor was immunoprecipitated with Protein A after a 12-h incubation at 4°C with a 1:50 dilution of a polyclonal antiserum, containing antibodies directed against the insulin receptor. The washed immunoprecipitated, phosphorylated receptors were analyzed by SDS-PAGE under reducing conditions followed by autoradiography as outlined in an earlier publication (26). The bands of the dried gels, corresponding to the β-subunits of the insulin receptor, were excised, incubated in 5 ml Flurosol at 37°C overnight, and counted by scintillation for quantitative purposes.

Artificial substrate phosphorylation. 30-μl aliquots of receptor preparations, adjusted to 36.8 fmol of insulin binding activity, were incubated in triplicate at 0, 2.5, 10, and 500 ng/ml unlabeled insulin for 18 h at 4°C in a total volume of 40 μl. All assays for a given subject were performed concurrently on pre- and post-weight reduction receptor preparations. The phosphorylation assays, using the exogenous substrate Glu4:Tyr1, were performed at 4°C for 40 min as previously published (26).

Data analysis. All values are given as the mean±SEM. Analysis of variance with repeated measures and the t test for paired data were used. The Wilcoxon signed-rank test was used for analysis when the data were not normally distributed.

Results

The characteristics of the subjects studied are displayed in Table I. 10 obese diabetics (NIDDMs) were weight- and age-matched with 5 obese, nondiabetic controls. The diabetic subjects lost 13.2±2.0 kg ($P < 0.001$) over 66±7 d, and the nondiabetic controls lost 18.2±1.5 kg ($P < 0.01$) over 68±2 d. Similarly the body mass index, which correlates well with the degree of obesity, fell by 4.3±0.6 kg/M^2 ($P < 0.01$) in the diabetics and by 6.0±0.4 kg/M^2 ($P < 0.01$) in the non-diabetics. Consistent with a loss of weight and a fall in body mass

Table I. Clinical and Metabolic Patient Characteristics

	Diabetic		Nondiabetic	
	Before	After	Before	After
n	9 M/1 F	9 M/1 F	5 M	5 M
Age (*yr*)	53.0±2.4	—	58.4±2.7	—
Weight (*kg*)	112.3±8.1	99.1±6.8*	117.3±9.0	99.1±8.3*
BMI (kg/M^2)	37.1±2.7	32.8±2.3*	39.0±3.0	33.0±2.8*
Fasting glucose (*mg/dl*)	205±13	118±13*	107±5	102±3
Fasting insulin (*μU/ml*)	26.0±5.5	11.7±2.8*	19±3	8.8±0.9*
Adipocyte size (*pl*)	1,076±62	852±45*	1,021±113	765±87*

Values are expressed as means±SEM.
* $P < 0.01$; value after weight loss compared with corresponding values before weight loss.

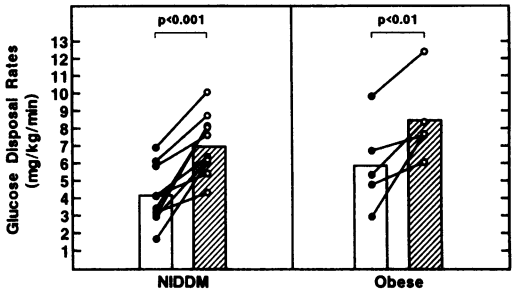

Figure 1. Effect of weight loss on insulin-stimulated glucose disposal rates. Individual and mean glucose disposal rates during 300 mU/M^2 per min insulin infusion rate euglycemic clamp done before (●) and after (○) diet therapy. Bars, means of the data points in each column upon entry (□) and after weight loss (▨). For reference, the glucose disposal rate in nonobese nondiabetics is 11±0.4 mg/kg per min (mean±SEM) under comparable euglycemic and hyperinsulinemic conditions.

index, adipocyte cell size fell significantly in both groups, with a loss of 214±48 pl ($P < 0.01$) in the NIDDM group and 256±108 pl ($P < 0.01$) in the controls. The glycosylated hemoglobin (HbAlc) fell from 11.5±0.7% before weight loss to 8.9±0.7% after weight loss ($P < 0.01$) in the diabetic subjects. This overall improvement in glycemic control was mirrored by a significant ($P < 0.01$) fall in fasting levels of plasma glucose in the NIDDM group. On entry into the study, the obese nondiabetics had normal glucose tolerance, whereas the NIDDMs had markedly abnormal glucose levels after an oral glucose load. After weight reduction, glucose tolerance improved significantly ($P < 0.01$) with 30, 60, 90, 120, and 180 min values of 270±10 mg/dl (mean±SEM), 327±23, 345±12, 306±11, and 251±7 vs. 161±13, 211±15, 222±21, 215±25, and 166±26 mg/dl before and after weight loss, respectively, in the NIDDM group. In both obese groups, fasting insulin levels were elevated initially and fell significantly ($P < 0.01$) after weight reduction. Insulin levels in the NIDDM group at 30, 60, 90, 120, and 180 min were 42±6 μU/ml (mean±SEM), 50±7, 50±4, 43±7, and 36±5 vs. 35±3, 47±6, 62±7, 56±7, and 37±8 before and after weight loss, respectively.

Effect of weight loss on glucose disposal rates (GDR). The individual and mean steady state rates of total glucose disposal attained during insulin infusion rates of 300 mU/M^2/min are illustrated in Fig. 1. This rate of insulin infusion produced a steady state insulin concentration of 1,223±108 μU/ml in the NIDDM group and 1,122±100 μU/ml in the nondiabetic group; these steady state insulin levels did not vary significantly after weight loss in either group. We have previously demonstrated that this degree of insulinemia produces 85–100% of the maximal effect on glucose disposal in both obese subgroups (10, 11, 38). As depicted in Fig. 1, the GDR of all subjects increased after weight loss. In the nondiabetics, the mean GDR increased from 5.90±1.0 mg/kg per min before to 8.41±0.95 ($P < 0.05$) after weight loss; in the NIDDM group, the GDR rose from 4.12±0.50 to 7.07±0.16 ($P < 0.01$) with weight reduction. For comparative purposes, the mean GDR measured at similar levels of insulinemia and glycemia in nondiabetic, nonobese subjects is 11.0±0.4 mg/kg per min in our laboratory. Thus, mean GDR was ~ 35% of normal in the NIDDMs before weight loss and 67% of normal afterwards; comparable calculations in nondiabetics yielded 53% before and 76% after diet therapy.

Effect of weight loss on insulin-binding activity. Insulin-binding capacity, estimated by Scatchard analysis, was determined in the lectin-purified adipocyte insulin receptor preparations from each subject. The results, expressed as the amount of insulin bound per microgram of recovered glycoprotein, are seen in Fig. 2. The insulin-binding capacity increased after weight loss in every subject, both NIDDMs and nondiabetics. The mean binding capacity in the nondiabetics before weight loss was 8.9±0.8 fmol insulin bound/μg glycoprotein and increased by 41% to 12.5±1.1 ($P < 0.01$) after weight reduction. Comparable weight loss in the NIDDM group resulted in a 26% increase in binding capacity from 6.9±0.4 to 8.7±0.5 ($P < 0.01$). Whereas insulin-binding capacity uniformly increased with weight loss in all subjects, insulin-binding affinity was unaltered (data not shown).

Effect of weight loss on insulin receptor autophosphorylation. The effect of weight reduction on insulin receptor autophosphorylation was assessed simultaneously in receptors prepared from adipocytes before and after weight loss. We have

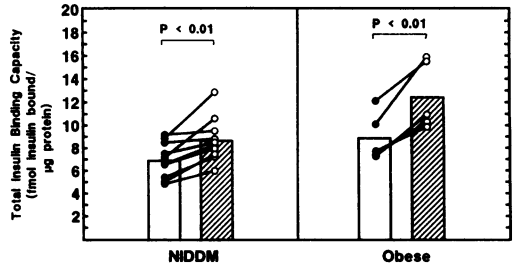

Figure 2. Effect of weight loss on insulin receptor number in lectin-purified preparations. Individual and mean insulin binding capacities before (●) and after (○) weight loss in lectin-purified insulin receptor preparations. For each data point, the binding capacity represents the mean of triplicate determinations of *x* intercepts estimated from Scatchard plots and expressed in terms of micrograms of recovered glycoprotein. Bars, means of the data points in each column before (□) and after (▨) weight reduction.

previously shown that before weight loss, receptors from NIDDMs have ~ 50% less insulin-stimulated autophosphorylation compared with either obese or lean nondiabetics (16). In these experiments, receptors were allowed to autophosphorylate in vitro and then analyzed on SDS-PAGE; the bands corresponding to the β-subunit, at each insulin concentration, were excised from the gels and counted. The results of this analysis are shown in Fig. 3 *A*. Similar to our previous results (16), insulin-stimulated autophosphorylation was decreased in the NIDDM group compared with nondiabetic obese subjects before weight loss. Basal autophosphorylation was unchanged by weight loss in both the NIDDMs (0.40±0.1 fmol phosphate (P)/30 min before weight loss and 0.60±0.14 afterwards) and nondiabetics (0.64±0.09 preweight loss vs. 0.54±0.10 postweight loss). In contrast to basal autophosphorylation, insulin-stimulated autophosphorylation changed significantly at all insulin concentrations in NIDDMs after weight loss ($P < 0.01$). As shown in Fig. 3 *B*, maximal insulin-stimulated autophosphorylation increased by 55% from 2.17±0.24 fmol P/30 min to 3.14±0.38 after weight loss ($P < 0.05$) in the NIDDMs. The nondiabetic obese subjects showed no significant change in insulin-stimulated autophosphorylation with weight loss; maximal autophosphorylation was 3.38±0.29 fmol P/30 min before and 3.61±0.25 after weight loss. Despite the wide variation in kinase activity from one subject to another among the NIDDMs (Fig. 3 *B*), we noted a < 12% interassay coefficient of variation on aliquots from the same receptor preparation. Furthermore, our baseline measurements in this study included new adipose tissue biopsies on eight of the same subjects used in our previously published work on human adipocytes (16). The values for insulin-stimulated receptor kinase activity were remarkably similar in receptor preparations from these two biopsies, with a coefficient of variation of 15%.

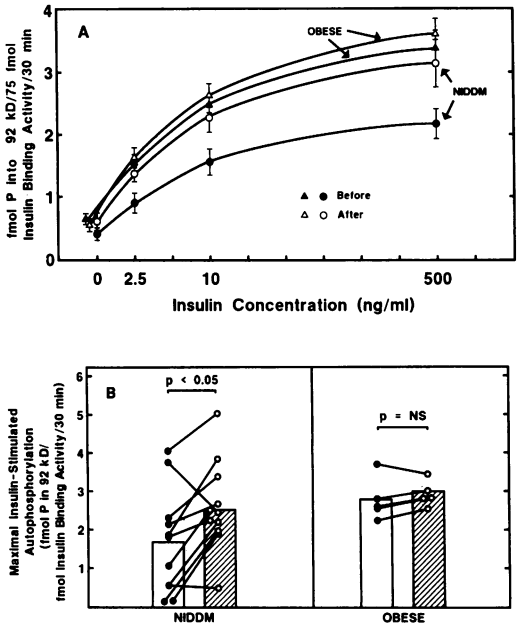

Figure 3. Effect of weight loss on insulin receptor autophosphorylation in diabetic and nondiabetic, obese subjects. (*A*) 75-fmol aliquots of insulin-binding activity were preincubated in singleton or duplicate in the absence or presence of 2.5, 10, and 500 ng/ml insulin before the initiation of autophosphorylation as described in Methods. The phosphorylated insulin receptor was immunoprecipitated with anti-insulin receptor antiserum and subjected to SDS-PAGE. The bands corresponding to the β-subunit of the insulin receptor were excised and counted. For each subject, autophosphorylation reactions were conducted concurrently on frozen receptors prepared during baseline (● and ▲) and post-weight loss (○ and △) studies. The results are expressed as means±SEM for 10 NIDDMs (● and ○) and 5 obese, nondiabetic subjects (▲ and △). (*B*) Maximal (500 ng/ml) insulin-stimulated autophosphorylation above basal levels for each subject before (●) and after (○) weight loss. Bars, means of the data points in each column.

Effect of weight loss on exogenous substrate (Glu4:Tyr1) phosphorylation. The effect of weight loss on the ability of the receptor preparations to catalyze the phosphorylation of the exogenous substrate Glu4:Tyr1 was determined in all subjects. The results are presented in Fig. 4, *A* and *B*. Basal phosphorylation was similar, and not significantly altered by weight loss in either group (32.7±4.4 fmol P into Glu4:Tyr1/40 min before vs. 33.9±2.5 after weight loss in the NIDDMs; 31.4±2.0 before vs. 29.9±3.5 after in the nondiabetics). In contrast to basal activity, insulin-stimulated Glu4:Tyr1 phosphorylation was decreased in the NIDDM group before weight reduction and was significantly enhanced at all insulin concentrations after weight loss in the NIDDMs ($P < 0.01$) but not significantly altered in the nondiabetics (Fig. 4 *A*). Thus, at maximally stimulating concentrations of insulin, mean Glu4:Tyr1 kinase activity over basal rose from 17.6±2.3 fmol P/40 min to 27.6±2.6 ($P < 0.05$) in the NIDDMs but did not change in the nondiabetics (29.4±1.8 initially vs. 30.1±1.2 afterwards, P = NS), as demonstrated in Fig. 4 *B*. The concentration of insulin capable of stimulating half maximal Glu4:Tyr1 phosphorylation (EC_{50}) also changed with weight reduction in the NIDDM subjects. This effect is most easily seen when the insulin-stimulated kinase data of Fig. 4 *A* are plotted as a function of the percent of maximal effect for each subject. The results for the individual subjects of each group are shown in Fig. 4 *C* and indicate a significant decrease in the mean EC_{50} from 9.9±1.7 ng/ml to 5.2±0.8 ($P < 0.05$) in the NIDDM group as a consequence of weight loss. In contrast, a similar analysis revealed no significant change in the EC_{50} (4.0±0.5 ng/ml preweight loss vs. 2.7±0.5 after weight loss, P = NS) in the nondiabetics.

Correlation analyses. The effect of weight loss on the relationship between insulin receptor kinase activity and GDR was directly examined in a single NIDDM subject who underwent two sequential sets of studies after progressive weight loss and refeeding. The subject was studied at his initial weight of 130.6 kg and sequentially after 15.8 and 27.0 kg total weight loss. The results are depicted in Fig. 5. The initial GDR of 6.9 mg/kg per min remained unchanged (6.6 mg/kg per min) after 15.8-kg weight loss, but increased by 51% to 10.0 mg/kg per min after 27.0 kg of weight loss. In a similar manner, insulin-stimulated autophosphorylation and Glu4:Tyr1 phosphorylation were both quite low initially and showed little change after 15.8 kg weight loss. However, an additional 11.2-kg weight loss resulted in a fourfold increase in maximal insulin-stimulated autophosphorylation and a sixfold increase in maximal insulin-stimulated Glu4:Tyr1 phosphorylation.

Previously, using insulin receptors from human adipocytes, we observed a significant correlation between receptor kinase activity, measured as autophosphorylation and receptor kinase activity measured as Glu4:Tyr1 phosphorylation among lean and obese nondiabetics and obese NIDDMs (16).

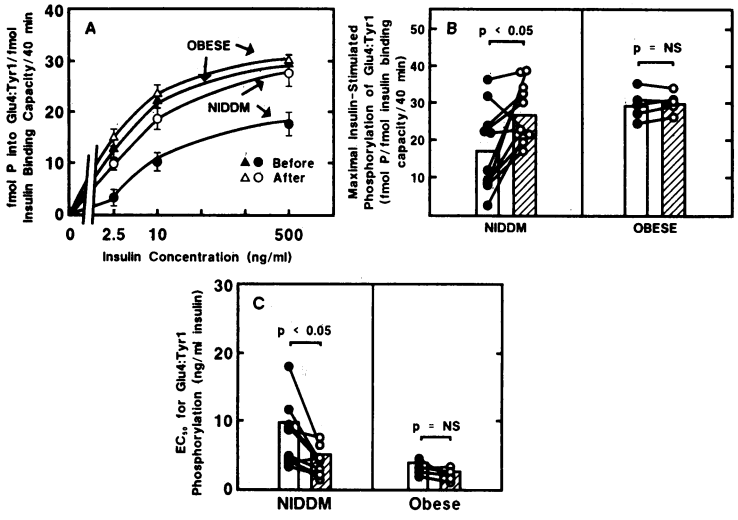

Figure 4. Effect of weight loss on Glu4:Tyrl insulin receptor kinase activity. Triplicate aliquots of insulin-binding activity (36.8 fmol) were preincubated in the absence (basal) or presence of 2.5, 10, and 500 ng/ml insulin before performing a Glu4:Tyrl phosphorylation reaction as described in Methods. For each subject, the kinase activity of receptors prepared before weight loss (● and ▲) was measured simultaneously with receptors prepared after weight loss (○ and △). (*A*) The results are expressed as means±SEM for 10 NIDDMs (● and ○) and 5 obese nondiabetics (▲ and △). Basal kinase activity in each case has been subtracted from all insulin-stimulated values and the results represent insulin stimulation above basal. (*B*) Individual and mean (*bars*) values of maximal (500 ng/ml) insulin-stimulated Glu4:Tyrl kinase activity over basal before (● and □) and after (○ and ▨) weight loss. (*C*) The concentration of insulin that produced half-maximal kinase activity (EC_{50}) was estimated from individual dose-response curves before and after weight loss. Individual (● and ○) and mean (*bars*) values for EC_{50} are graphed as nanograms per milliliter insulin.

A similar correlation was noted in this study for the obese and NIDDM subjects ($r = 0.80$, $P < 0.001$) before weight loss. In addition, when the relationship between autophosphorylation and Glu4:Tyrl kinase activity was examined using the diet-induced changes calculated for these two kinase parameters, a significant correlation also was noted (Fig. 6).

The relationship between weight loss and change in insulin-stimulated receptor kinase activity for all 10 NIDDM subjects is graphed in Fig. 7 *A* (Glu4:Tyrl) and *C* (autophosphorylation). When analyzed using Glu4:Tyrl kinase activity, a significant linear correlation ($r = 0.66$, $P < 0.05$) was observed; using autophosphorylation activity, a similar tendency was noted but was not statistically significant. The relationship between change in maximal insulin-stimulated kinase activity and improvement in overall GDR as a consequence of weight loss is depicted in Fig. 7 *B* (Glu4:Tyrl) and *D* (autophosphorylation) for the NIDDM subjects. No significant correlation between these two variables existed, regardless of whether GDR was expressed in terms of kilograms body weight ($r = -0.10$), body surface area ($r = -0.35$), or urinary creatinine excretion ($r = -0.15$). Furthermore, no significant correlation was found when change in insulin-stimulated kinase activity was examined as a function of either change in fasting blood glucose ($r = 0.29$) or change in adipocyte cell size ($r = 0.11$). In addition, neither baseline GDR nor baseline fasting glucose levels were significantly correlated with baseline insulin-stimulated kinase activity.

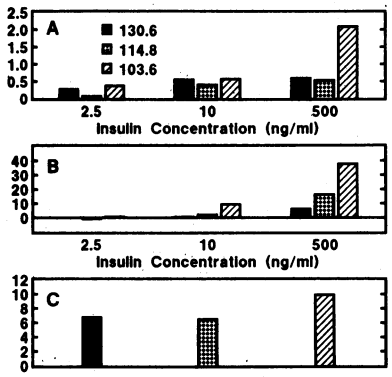

Figure 5. Effect of weight loss on glucose disposal, autophosphorylation, and Glu4:Tyrl kinase activity in a NIDDM subject. A diabetic subject was studied at baseline and successively after a total of 15.8 and 27.0 kg weight loss. Equal amounts of insulin-binding activity from the three study periods were preincubated in the absence (basal) or presence of 2.5, 10, and 500 ng/ml insulin. (*A*) Autophosphorylation and (*B*) Glu4:Tyrl phosphorylation were measured as described in the legends to Figs. 3 and 4, respectively. Basal kinase activity was subtracted in each case and the results represent insulin stimulation above basal. (*A*) Insulin-stimulated phosphorylation (fmol P in 92 kD/75 fmol insulin-binding capacity/30 min). Bars correspond to values of 0.28 fmol P, 0.12, and 0.39 at 2.5 ng/ml insulin; 0.52, 0.43, and 0.51 at 10 ng/ml insulin; and 0.55, 0.48, and 2.1 at 500 ng/ml insulin. (*B*) Insulin-stimulated phosphorylation of GLU4:TYR1 (fmol P/fmol insulin-binding capacity/40 min). Bars correspond to values of 0.2 fmol P, −1.0, 0.8 at 2.5 ng/ml insulin; 0.7, 1.5, and 11.3 at 10 ng/ml insulin; and 7.0, 17.0, and 38.5 at 500 ng/ml insulin. (*C*) Steady-state euglycemic glucose disposal rates (mg/kg per min) measured during a 300 mU/M²/min insulin infusion are shown for the three study periods (6.89 mg/kg per min, 6.64, and 10.05 at the first, second, and third study periods, respectively).

Discussion

NIDDM (1, 2, 4–6, 8–11) and nondiabetic obesity (11, 39, 44–46) are characterized by variable degrees of insulin resis-

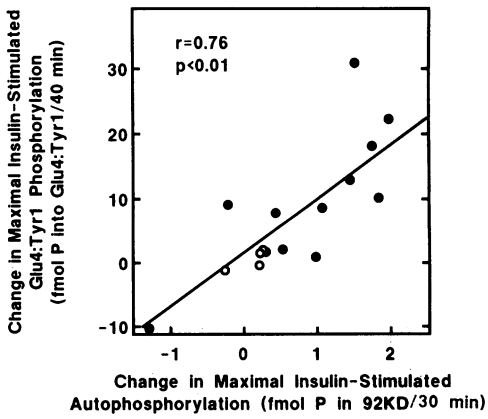

Figure 6. Correlation of diet-induced changes in insulin-stimulated autophosphorylation and Glu4:Tyrl kinase activity. Individual data representing the change with weight loss in maximally insulin-stimulated autophosphorylation (Fig. 3 *B*) is plotted relative to the diet-related changes in maximally insulin-stimulated Glu4:Tyrl (Fig. 4 *B*) phosphorylation for obese nondiabetics (○) and NIDDMs (●).

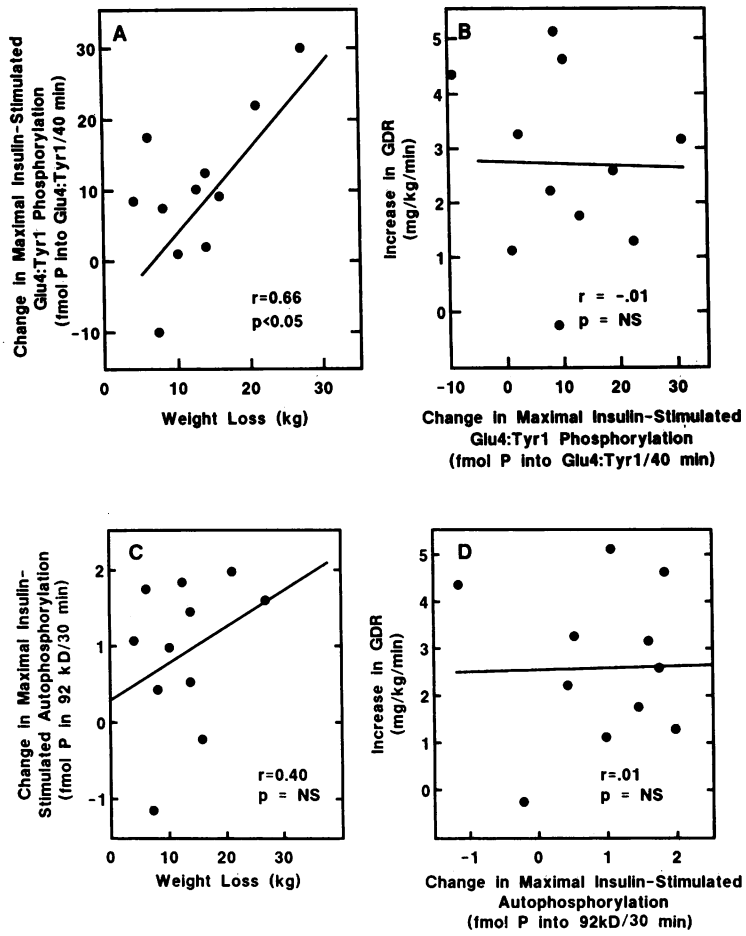

Figure 7. Correlation of changes in receptor kinase activity with changes in weight and overall glucose disposal in NIDDM subjects. (*A*) Weight loss is plotted relative to the diet-induced change in maximal (500 ng/ml insulin) insulin-stimulated Glu4:Tyr1 kinase activity measured in 11 baseline and 11 postweight loss biopsies from 10 diabetic subjects. The subject in Fig. 5 is included twice because he had one baseline and two postweight loss biopsies. (*B*) The increase in overall glucose disposal rate (GDR) measured in vivo, is plotted against the change in maximal insulin-stimulated kinase activity measured in vitro. (*C*) The change in maximal insulin-stimulated autophosphorylation as a function of weight loss. (*D*) The increase in GDR is graphed relative to the change in maximal insulin-stimulated autophosphorylation.

tance. The preponderance of in vivo and in vitro data indicate that postinsulin binding defects are responsible for most of the insulin resistance observed in both of these disorders. The earliest known postinsulin-binding event is activation of the tyrosine kinase intrinsic to the β-subunit of the receptor (23, 24). The interaction of insulin with its receptor initiates a process of autocatalyzed phosphorylation of tyrosine residues within the β-subunit (20–22). This process, termed autophosphorylation, greatly enhances the ability of the insulin receptor to catalyze the phosphorylation of tyrosine-containing exogenous substrates in vitro (21–23). In intact cells, insulin stimulates tyrosine phosphorylation of several endogenous protein substrates, raising the possibility that the insulin receptor kinase plays a major role in insulin action (47–50). Defects in receptor kinase activity might therefore be causally related to the insulin resistance observed in NIDDM and obesity.

This study demonstrates that, before weight loss, insulin receptors isolated from adipocytes of NIDDM subjects display a substantial reduction in kinase activity compared with receptors from obese, nondiabetic controls. The reduction was ~ 50% at maximally stimulating concentrations of insulin and close to 80% at submaximal concentrations of insulin. The reduction was seen for both autophosphorylation and phosphorylation of the exogenous substrate Glu4:Tyr1. This finding is comparable to results we reported in an earlier study (16) designed to compare the kinase activity of insulin receptors from lean nondiabetics, obese nondiabetics, and obese diabetics (NIDDMs). In that report, we not only noted a 50–80% reduction in kinase activity of insulin receptors from NIDDMs relative to obese controls but also observed that the kinase activity of receptors from obese nondiabetics was similar to that measured in lean nondiabetics. Similar findings in NIDDMs have also been reported by Comi et al. in erythrocytes (19), and by Sinha et al. in adipocyte-derived (18) and by Caro et al. in hepatocyte-derived (17) insulin receptors from diabetic and nondiabetic obese humans.

We have now extended our initial observations to include receptor kinase measurements conducted after a period of weight loss in both NIDDM and nondiabetic obese subjects. We have found that the kinase defect observed at baseline in NIDDM was at least partially, and perhaps completely, reversible after weight reduction. At maximally stimulating concentrations of insulin, the mean kinase activity after weight loss in the NIDDM group reached 90% of the mean kinase activity of the obese nondiabetics. At submaximal concentrations of insulin, the kinase defect in the NIDDMs was reversed by ~ 66% after weight loss. As shown in Fig. 4 *C*, before weight loss, the kinase defect observed at submaximal concentrations of insulin was more severe than that measured at maximally stimulating concentrations of insulin as reflected by an increase in the EC_{50} for Glu4:Tyr1 phosphorylation relative to the nondiabetic controls. After weight loss the EC_{50} values reverted to near normal levels. As observed in our original report (16), we did not find a right shift in the EC_{50} for autophosphorylation in the NIDDM's compared with the nondiabetics; furthermore, no change in the autophosphorylation

EC_{50} occurred after weight loss in either group. The reason for the differences between autophosphorylation and Glu4:Tyr1 phosphorylation with regard to the shift in the EC_{50} is unknown.

In contrast to the substantial changes observed in kinase activity in the NIDDMs after weight loss, the kinase activity of receptors from the obese nondiabetics was not significantly different before and after weight reduction. Since we (16) have already established that the kinase activity of insulin receptors from obese nondiabetics is comparable to that of lean nondiabetics in adipocytes, it is clear from this study that adipocyte receptor kinase activity of the nondiabetics is normal at baseline and remained normal after weight loss.

The mechanism(s) underlying the defect in kinase activity in NIDDM are not fully established. However, the reduction in maximal kinase activity in the NIDDMs before weight loss is consistent with two possible interpretations. Either all the NIDDM receptors are equally defective and have diminished kinase activity, or subpopulations of receptors exist such that of the receptors that bind insulin, some have completely normal insulin-stimulable kinase activity (kinase competent) and some lack insulin-stimulable kinase activity altogether (kinase incompetent). An increase in the relative size of the pool of kinase incompetent receptors in NIDDMs could account for the observed reduction in overall kinase activity. We have recently provided evidence that strongly supports the second interpretation (51). Using anti–receptor and anti–phosphotyrosine antibodies, we found that in lean and obese nondiabetics, 43% of the receptors which bound insulin were capable of tyrosine phosphorylation and kinase competent. In contrast, only 14% of the receptors were tyrosine phosphorylated and kinase competent in the NIDDMs. After weight reduction, the percent of kinase-competent receptors rose substantially in the NIDDMs. Overall, in lean and obese nondiabetics, as well as NIDDMs, the percent of receptors capable of insulin-stimulated tyrosine autophosphorylation was well correlated with the amount of insulin-stimulated Glu4:Tyr1 kinase activity. These findings strongly suggest that a large part, and perhaps all, of the reduced kinase activity in NIDDMs can be attributed to a reduction in the relative pool of kinase competent receptors; moreover, this alteration appears to be reversible.

The mechanism responsible for the reduction in the relative pool of kinase-competent receptors in NIDDM is unknown. One possibility may involve serine phosphorylation of the receptor in NIDDM since serine phosphorylation is known to inhibit subsequent insulin-stimulated tyrosine phosphorylation (24, 52). An increase in serine phosphorylation in NIDDM could thereby result in an increase in the number of receptors capable of binding insulin but incapable of kinase activity. Another possibility relates to hyperglycemia. Although the role, if any, of hyperglycemia in the modulation of kinase activity is not known, most of the kinase defects described to date have occurred in insulin-resistant humans or animals with glucose intolerance. Decreased insulin-stimulated kinase activity thus has been noted in liver (17), adipose (16, 18), muscle (53, 54), and red blood cells (19) of humans with NIDDM; in some subjects with the type A syndrome of insulin resistance (29–31); in liver (28) and muscle (27) of rats made diabetic with streptozotocin; and in muscle from mice made obese and diabetic with gold thioglucose (25). On the other hand, insulin-stimulated kinase defects have been absent in most, but not all (53, 54), situations characterized by insulin resistance and normal glucose tolerance, such as obesity in humans (16, 19) and mice (25), and glucocorticoid treatment (48) or aging in rats (55). Moreover, the kinase defect noted in rats made diabetic with streptozotocin was largely reversed when hyperglycemia was corrected with insulin therapy (28). In a similar manner, we observed that only hyperglycemic subjects had reduced kinase activity; the obese subjects with normal glucose tolerance had normal kinase activity (16). With weight loss, the hyperglycemia diminished and the kinase defect improved in the NIDDM subjects but did not change in the obese nondiabetics. Note that several reports show that insulin receptor kinase activity in nondiabetic obesity is normal in human adipocytes (16), human erythrocytes (19), and murine skeletal muscle (25). Using hepatic insulin receptors, it has been found that kinase activity is decreased in obese NIDDM subjects relative to nondiabetic obese subjects (17), but whether kinase activity was entirely normal in the obese subjects is not clear, as lean controls were not studied (17). Regarding human skeletal muscle, the results are somewhat confusing, since normal autophosphorylation (53) but reduced kinase activity for exogenous substrate have been found in obesity (53, 54). It thus is possible that kinase activity may not be normal in all tissues from obese nondiabetic humans. Apart from these exceptions, the preponderance of data suggests that the insulin receptor kinase is usually normal in conditions characterized by insulin resistance and normal glucose tolerance.

Reversibility of the kinase defect in NIDDM strongly suggests that a posttranslational alteration in the insulin receptor is responsible for this abnormality in the first place. However, reversibility does not completely exclude the possibility of a genetically altered receptor in some NIDDM subjects; such an abnormal receptor could predispose to the secondary development of a reversible, functional kinase defect.

In parallel with the measurements of kinase activity in vitro, insulin-mediated glucose disposal and glucose tolerance were studied in vivo in all subjects before and after weight loss. Euglycemic clamps were done at near maximally stimulating concentrations of insulin; at these high insulin levels, decreases in glucose disposal reflect postbinding defects in insulin action rather than changes in insulin binding. Before diet therapy, both obese groups were insulin resistant with a 50–70% decrease in insulin mediated glucose disposal compared with normal-weight, nondiabetic controls. After weight loss, all subjects showed improvement in insulin action with augmentation of insulin-stimulated glucose disposal. This finding is consistent with previous reports of improvement in maximally insulin-stimulated glucose disposal after weight reduction in NIDDMs (37, 38) and obese nondiabetics (39). Weight reduction in the NIDDMs resulted in an increase of mean in vivo glucose disposal to 67% of normal and a concurrent increase of in vitro determined receptor kinase activity to 65–90% of normal. Despite the overall similar magnitude and direction of change in both of these variables these same data, taken individually, revealed no correlation between kinase activity and glucose disposal, either at baseline or in response to weight reduction (Fig. 7 *B* and *D*). These results appear to differ from those of Takayama et al., who recently described a significant correlation between in vitro autophosphorylation and glucose transport measured in isolated adipocytes from obese Pima Indians with varying degrees of glucose tolerance (56). The

explanation for the lack of correlation among individuals in this study is not clear. However, in vivo glucose disposal is the net result not only of glucose transport but a variety of complex cellular events, some of which might not involve the insulin receptor kinase. For example, the data in the nondiabetics showed that after weight loss, glucose disposal improved in the absence of any change in kinase activity. To the extent that weight reduction in the NIDDMs resulted in improvement in insulin receptor kinase activity as well as concomitant improvement in other steps important to glucose disposal, a correlation between kinase activity and overall in vivo glucose disposal could be obscured. Furthermore, NIDDM is a heterogeneous disorder and may have multiple causes. Conclusions pertaining to a select group like Pima Indians, might not apply universally to all NIDDMs. For example, we noted an apparent correlation between improvement in kinase activity and glucose disposal in some but not all NIDDMs. Lastly, dose-response studies indicate that insulin-stimulated glucose transport is maximal at insulin levels which submaximally stimulate receptor kinase. This raises the possibility that a correlation between kinase activity and in vivo glucose disposal might be better seen at an insulin level lower than those used in our glucose clamp studies.

In summary, our current results confirm that insulin receptors from subjects with NIDDM have reduced insulin-stimulated kinase activity compared with receptors from obese nondiabetics, whose kinase activity is comparable to that of lean nondiabetics. We now extend these observations and show that progressive weight loss reverses the kinase defect in NIDDM subjects but has no effect on kinase activity in obese nondiabetics. The high degree of reversibility strongly suggests that the cause of the receptor kinase defect in NIDDM involves some posttranslational modification specific to the diabetic state. Along with an improvement in insulin-stimulated kinase activity in the NIDDM group, weight loss also resulted in improvements in glucose disposal and overall amelioration of insulin resistance; however, no clear relationship linked the improvements in these parameters. In contrast to the situation in NIDDM, conclusions in obesity were more straightforward. The normal kinase activity in obesity, in conjunction with an improvement in glucose disposal despite no change in kinase activity, indicates that the cause of insulin resistance in obesity lies distal to the receptor kinase step.

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