Mechanism of Defective Insulin-Receptor Kinase Activity in NIDDM
Evidence for Two Receptor Populations
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We used anti-insulin-receptor and anti-phosphotyrosine antibodies to elucidate the mechanism of decreased insulin-receptor tyrosine kinase activity observed in subjects with non-insulin-dependent diabetes mellitus (NIDDM). Lectin-purified insulin receptors were labeled with $^{125}$I-labeled NAPA-DP-insulin and autophosphorylated in the presence of 500 $\mu$M unlabeled ATP. Immunoprecipitation occurred in $43 \pm 8\%$ of the autophosphorylated, $^{125}$I-labeled receptors from nondiabetic subjects with anti-phosphotyrosine antibodies in contrast to 100% immunoprecipitation with anti-insulin-receptor antibodies. Anti-phosphotyrosine antibodies immunoprecipitated only $14 \pm 6\%$ of NIDDM receptors ($P < .05$ vs. nondiabetic receptors). A significant correlation existed between maximal insulin-stimulated receptor tyrosine kinase activity and the proportion of receptors immunoprecipitated by anti-phosphotyrosine antibodies ($r = .76$, $P < .01$). These results suggest that human adipocytes contain two distinct receptor populations, both of which bind insulin but only one of which is capable of insulin-stimulated tyrosine phosphorylation. In nondiabetic subjects, 40–50% of the receptors that bind insulin are capable of insulin-stimulated tyrosine autophosphorylation. The proportion of receptors that bind insulin but are incapable of insulin-stimulated tyrosine autophosphorylation is increased in NIDDM; the magnitude of this increase correlated with the magnitude of the decrease in kinase activity. Diabetes 38:397–403, 1989

Insulin-receptor binding leads to activation of a tyrosine kinase intrinsic to the insulin-receptor $\beta$-subunit (1–3). This insulin-receptor activity catalyzes both insulin-receptor autophosphorylation and phosphorylation of exogenous and endogenous protein substrates (2,3). It has been postulated that insulin's biological effects could be mediated through phosphorylation and dephosphorylation of pertinent cellular proteins and that the receptor kinase may be important in transducing these effects (4–6).

There have been numerous studies investigating this enzyme activity in conditions of altered insulin action. Insulin resistance is a characteristic feature of obesity and non-insulin-dependent diabetes mellitus (NIDDM), and both in vivo and in vitro studies have shown that the predominant cellular mechanisms underlying these insulin-resistant states involve postbinding defects in insulin action (7–15). We recently have studied insulin-receptor kinase activity in obese nondiabetic, obese diabetic, and lean nondiabetic control subjects. Both autophosphorylation of the insulin receptor and phosphorylation of an exogenous substrate were comparable in adipocyte insulin receptors derived from control and obese nondiabetic subjects, whereas both measurements of kinase activity were strikingly decreased in NIDDM subjects (16). Similar results have been demonstrated by Comi et al. (17) in erythrocyte-derived insulin receptors and Caro et al. (18) in adipocyte and hepatocyte insulin receptors. In this study, we attempted to further elucidate the mechanisms of decreased kinase activity found in NIDDM subjects by assessing the absolute proportion of insulin receptors that undergo tyrosine phosphorylation in control and NIDDM subjects.

MATERIALS AND METHODS
Materials. B. Frank and R. Chance (Lilly, Indianapolis, IN) kindly provided monocomponent porcine insulin and A14-Tyr-$^{125}$I-monoiiodoinsulin. Serum from a patient with anti-insulin-receptor antibodies was a gift from S. Jacobs (Wellcome, Research Triangle Park, NC). Anti-phosphotyrosine antibodies were raised in rabbits by immunization with phosphotyrosine-containing peptides. Glucose 1 mM = 18 mg/dl Insulin 1 pM = 0.139 $\mu$U/ml

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antibody was generously supplied by M. Kamp (Salk Institute, La Jolla, CA). [\gamma\textsuperscript{32P}]ATP (3000 Ci/mmol) and Na\textsuperscript{25I} were purchased from Amersham (Buckinghamshire, UK). Collagenase was obtained from Washington Biochemical (Freehold, NJ), wheat-germ–agarose beads from Vector (Burlingame, CA), Protein A from Bethesda Research (Gaithersburg, MD), and bovine serum albumin from Armour (New York). All materials for protein determinations and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad (Richmond, CA). All other chemicals were purchased from Sigma (St. Louis, MO).

**Subjects.** Clinical and metabolic characteristics of the NIDDM and control subjects are presented in Table 1. All subjects were studied while inpatients on the Special Diagnostic and Treatment Unit of the San Diego Veterans Administration Medical Center. All protocols were approved by the University of California (San Diego) School of Medicine Human Subjects Committee, and informed consent was obtained from each subject. Subjects were classified as NIDDM or nondiabetic based on a standard 75-g oral glucose tolerance test according to the criteria of the National Diabetes Data Group (19). Two of the NIDDM subjects studied initially were restudied after weight loss (6 and 11 kg) and 3 wk of isocaloric refeeding.

**Analytical methods.** Insulin was assayed by a double-antibody radioimmunoassay technique (20), and glucose levels were measured by glucose oxidase method with a glucose analyzer (YSI, Yellow Springs, OH).

**Preparation of insulin receptors from human adipose tissue.** Adipose tissue (7–24 g) was obtained from open biopsy of the lower abdominal region after an overnight fast (21). Adipose tissue was digested in collagenase (6 mg/g fat) as previously described (22), and isolated adipocytes were solubilized for 30 min in an ice-cold mixture containing 1.5% Triton X-100, 7.5 mg/ml bacitracin, 15 mM benzamidine, 500 KIU/ml aprotinin, 2.5 mM phenylmethylsulfonyl fluoride (PMSF), and 25 mM HEPES at pH 7.4. The cellular extract was then centrifuged at 10,000 × g for 15 min at 4°C. The soluble protein extract was then partially purified by wheat-germ agglutinin chromatography as previously detailed. Partially purified receptors were frozen at −70°C for use in all subsequent studies.

**TABLE 1**

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Body mass index (kg/m²)</th>
<th>Age (yr)</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Fasting insulin (μU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>25.9</td>
<td>38</td>
<td>96</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>25.3</td>
<td>33</td>
<td>71</td>
<td>26</td>
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<td>3</td>
<td>24.8</td>
<td>34</td>
<td>97</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>24.3</td>
<td>50</td>
<td>93</td>
<td>6</td>
</tr>
<tr>
<td>NIDDM 1</td>
<td>57.5</td>
<td>37</td>
<td>391</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>37.4</td>
<td>67</td>
<td>200</td>
<td>29</td>
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<td>3</td>
<td>37.4</td>
<td>47</td>
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<td>4</td>
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<td>47</td>
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<td>37</td>
</tr>
<tr>
<td>6</td>
<td>37.4</td>
<td>54</td>
<td>196</td>
<td>29</td>
</tr>
</tbody>
</table>

**Insulin-binding assays.** Aliquots of partially purified insulin-receptor preparations were incubated in triplicate with A14-Tyr-\[^32P\]iodoinsulin (final concn 0.5 ng/ml) and varying concentrations of unlabeled insulin (0–1000 ng/ml) for 18 h at 4°C as previously described (16,23). Nonspecific binding was assayed by incubation in the presence of 5000 ng/ml unlabeled insulin and accounted for <3% of the total radioactivity added to each assay tube. The insulin-receptor content of each preparation was then determined by Scatchard analysis (24).

**Artificial-substrate phosphorylation.** Receptor aliquots were adjusted based on the measured insulin-receptor content to equal amounts of receptors (1.25 FM insulin receptors/μl) and then preincubated in the presence or absence of unlabeled insulin for 18 h at 4°C. Phosphorylation was initiated at 4°C by the addition of 10 μl of the exogenous substrate 4Glu:1Tyr (final concn 2 mg/ml) and 10 μl of (final concn) 5 mM MnCl₂, 12 mM MgCl₂, 500 μM CTP, and 50 μM [\gamma\textsuperscript{32P}]ATP (~5 μCi/μmol). The reaction was terminated after 40 min by the addition of 50 mM ATP. Incorporation of \[^32P\] into 4Glu:1Tyr was assayed by the filter-paper method as previously described (25). Background radioactivity, measured in the absence of exogenous substrate, was <10% of the basal activity measured in the presence of 4Glu:1Tyr.

**Photolabeling and phosphorylation of insulin receptors.** \[^125I\]NAPA-DP-insulin (100–200 cpn/pg) was prepared as previously outlined (26) and used within 4 wk. Aliquots of adipocyte-derived insulin receptors were incubated with \[^125I\]NAPA-DP-insulin (80 ng/ml) in a total volume of 40 μl for 1 h in the dark at 4°C. The incubation mixtures were then placed under a long-wave (360 nm) UV lamp for 15 min at 4°C. Under these conditions, >90% of the receptors are occupied with the biologically active photactive insulin derivative, and ~15–20% of these receptors are covalently labeled (27,28). The insulin-occupied receptors were then allowed to self-phosphorylate for 1 h at 4°C in the presence of a high concentration (500 μM) of unlabeled ATP in a buffer containing (final concn) 5 mM MnCl₂, 500 μM ATP, and 20 μM sodium vanadate. Reactions were terminated by the addition of (final concn) 10 mM sodium pyrophosphate, 100 mM NaF, 1% Triton, 1 mM NaVO₄, 5 mM EDTA, 2 mM PMSF, and 25 mM HEPES at pH 7.6. Either monoclonal anti-receptor antibody, anti-phosphotyrosine antibody, or normal human serum (final dilutions 1:200) were added. After 16 h at 4°C, the antibody-receptor complexes were precipitated with Protein A (80 μl of 20% [wt/vol] solution) and subsequently washed 3 times in a solution containing 125 mM NaCl, 25 mM HEPES, 0.05% Triton, 10% [wt/vol] glycerol, and 2 mM orthovanate at pH 7.6. The washed receptor-antibody complexes were boiled for 5 min in Laemmli’s buffer containing (final concn) 10 mM ATP, 100 mM dithiothreitol, 10 mM EDTA, 1% SDS, 10% glycerol, 0.05% bromophenol blue, and 0.5 M Tris at pH 6.8 (29). The labeled proteins were analyzed by SDS-PAGE (7.5% resolving gel) and autoradiography. Areas of the gel containing \[^125I\]NAPA-DP-insulin–labeled α-subunits were excised and counted in a γ-counter. Equal areas of the dried gel, free of any \[^125I\] activity, were counted and subtracted as background. Labeled receptors incubated with normal human serum contained no \[^125I\] activity over background counts.
RESULTS
As seen in Table 1, NIDDM subjects were significantly heavier (P < .01) than the lean control subjects. Fasting levels of glucose and insulin were elevated in the NIDDM group compared with control subjects.

We have previously reported that for an equal amount of insulin-binding activity, autophosphorylation of the insulin receptor derived from the NIDDM subjects was ~50% reduced compared to controls at maximally stimulating concentrations of insulin (16). Similarly, the extent of insulin-stimulated tyrosine kinase activity, assessed by phosphorylation of the exogenous substrate 4Glu:1Tyr, was significantly reduced (~40%) in the NIDDM subjects (16). As a result, we postulated that the decrease in β-subunit autophosphorylation could reflect either 1) a decrease in the intrinsic kinase activity of each individual receptor within the total receptor preparation or 2) the existence of two populations of receptors that bind insulin, one subpopulation having normal kinase activity and the other subpopulation having no kinase activity, with an increase in the kinase-defective population in NIDDM.

To discriminate between these two possibilities, we immunoprecipitated phosphorylated 125I-NAPA-DP-labeled receptors with a monoclonal anti-receptor antibody and an anti-phosphotyrosine antibody. We have used 125I-NAPA-DP-insulin as a means of labeling the insulin receptor (26, 27, 30, 31). In the past, it has been demonstrated that this photoaffinity analogue of insulin has 80–90% of insulin's biological activity. In addition, covalent occupancy of the insulin receptor by 125I-NAPA-DP-insulin leads to a functionally active receptor complex (28). The monoclonal anti-receptor antibody was found to immunoprecipitate all of the 125I-NAPA-DP-labeled insulin receptors. To determine the proportion of insulin receptors that contain phosphotyrosine in the NIDDM subjects compared to those of control subjects, we took advantage of the ability of an anti-phosphotyrosine antibody to immunoprecipitate only those insulin receptors containing phosphorylated tyrosine residues. In control experiments with [γ-32P]ATP labeling of receptors, we found that the anti-phosphotyrosine antibody was able to immunoprecipitate all of the 32P incorporated in vitro into the insulin receptor. Figure 1 illustrates an experiment in which insulin receptors were autophosphorylated in vitro with [γ-32P]ATP. In these control experiments with human adipose tissue receptors, all of the 32P counts were immunoprecipitated with the anti-phosphotyrosine antibody. Immunoprecipitation of the 125I-NAPA-DP-labeled insulin receptors from this same receptor preparation by the anti-phosphotyrosine antibody was only 27% compared with immunoprecipitation by the anti-receptor antibody, thus demonstrating complete immunoprecipitation of the phosphate label despite incomplete immunoprecipitation of the 125I-NAPA-DP-insulin-labeled total receptor complement by the anti-phosphotyrosine antibody. These findings are also consistent with the view that essentially all in vitro autophosphorylation occurs on tyrosine residues. Double-labeling studies with 125I-NAPA-insulin and [γ-32P]ATP also show that all of the 125I-labeled receptors are brought down by the anti-receptor antibody, and all of the 32P-labeled receptors are brought down by the phosphotyrosine antibody. Thus, the monoclonal anti-receptor antibody quantitatively immunoprecipitates the total receptor pool, whereas the phosphotyrosine antibody quantitatively immunoprecipitates only the auto-phosphorylated receptors. Therefore, a comparison of the 32P counts immunoprecipitated by the anti-phosphotyrosine antibody indicates the percent of the total insulin receptors that are autophosphorylated and contain phosphotyrosine.

Figure 2 illustrates this concept in a representative SDS-PAGE autoradiogram from a single control subject. Insulin receptors are visualized as the 125I-insulin–labeled α-subunit on these reduced gels, and 125I-NAPA-DP-insulin–labeled receptors are quantitatively immunoprecipitated by the anti-insulin-receptor antibody (lane A) and, to a lesser extent, by the anti-phosphotyrosine antibody (lane B). There were no labeled receptors in the immunoprecipitates with normal human serum (lane C). Conversely, autoradiograms of the postimmunoprecipitation supernatants revealed a large amount of 125I-NAPA-DP-insulin–labeled receptor in the normal human serum lane (F), no labeled receptors in the anti-insulin-receptor lane (D), and an intermediate amount of 125I-labeled receptor in the anti-phosphotyrosine lane (E). The ratio of the amount of labeled insulin receptor recovered on SDS-PAGE gels after immunoprecipitation with an anti-insulin-receptor antibody relative to the amount detected with the anti-phosphotyrosine antibody is the proportion of insulin receptors that are tyrosine phosphorylated. Gel slices containing the receptor bands were excised and counted as described in MATERIALS AND METHODS for each subject. The determination of the proportion of tyrosine-phosphorylated
receptors was based on the mean of two separate assays for each subject; the separate assays of each receptor preparation from each subject differed with a mean coefficient of variation of 11% for the monoclonal anti-receptor–anti-body precipitation and 7% for the anti-phosphotyrosine antibody.

Figure 3 illustrates for both control and NIDDM subjects the percent of the insulin receptors that, after maximal in vitro insulin-stimulated autophosphorylation, contain phosphotyrosine. We found that 43 ± 8% of the insulin receptors derived from control subjects contained phosphotyrosine residues, whereas only 14 ± 6% of the NIDDM subjects’ insulin receptors were phosphorylated (P < .05). Thus, a much smaller fraction of the total receptors from NIDDM subjects were able to undergo insulin-stimulated autophosphorylation compared to the control subjects. Additional studies have shown that the insulin receptors not precipitated by the anti-phosphotyrosine antibody were completely devoid of kinase activity (with histone as substrate), and thus, the kinase activity found in the total receptor preparations was attributable to those receptors precipitated by the anti-phosphotyrosine antibody.

The relationship between the exogenous kinase activity of receptors and the proportion of receptors capable of autophosphorylation is given in Fig. 4. A highly significant linear relationship exists such that the greater the proportion of receptors capable of tyrosine autophosphorylation, the greater the ability of the receptors to phosphorylate the exogenous substrate 4Glu:1Tyr.

We also studied two of the NIDDM subjects before and after weight reduction. These patients lost 6 and 11 kg and then had 3 wk of isocaloric refeeding and weight maintenance. Weight loss led to a marked improvement in glycemia (fasting glucose 178 and 193 mg/dl before vs. 118 and 89 mg/dl after weight loss) and a 70% reversal of peripheral insulin resistance assessed by the glucose-clamp technique (12,32). The effects of weight loss on receptor kinase activity are seen in Fig. 4. Results before and after weight loss for substrate phosphorylation and percent immunoprecipitability by the phosphotyrosine antibody for both subjects are connected by dashed lines. Clearly, there is a marked and concurrent improvement in both aspects of receptor kinase function after weight reduction. Note that the correlation between kinase activity and percent precipitation shown in Fig. 4 is still highly significant when the data after weight loss are not included (r = .75, P < .02).

Mean counts per minute in the anti-receptor–anti-phosphotyrosine–antibody immunoprecipitates for each pa-
tient are provided in Table 2. The differences in count per minute, particularly in the anti-receptor antibody lanes, are largely reflective of differences in the specific activity of the iodinated $^{125}$I-NAPA-DP-insulin preparation used in the particular experiment.

**DISCUSSION**

Insulin resistance is a major feature of NIDDM and is an important pathophysiologic component underlying this disorder. Based on various in vivo and in vitro studies, it has been shown that the cellular mechanisms underlying this insulin-resistant state are secondary to one or more post-binding defects in insulin action (7–15). The insulin receptor contains an intrinsic kinase activity leading to autophosphorylation of the receptor β-subunit in vitro and in vivo (1–3). It has been shown that autophosphorylation further augments the kinase properties of the insulin receptor, making it a more active tyrosine kinase (33,34). Substantial evidence exists indicating that the receptor kinase activity is capable of phosphorylating endogenous substrates and that, in some way, this is an integral component of the insulin-action sequence (35–37). We have previously shown that insulin-receptor autophosphorylation and receptor-mediated phosphorylation of an exogenous protein substrate is decreased with adipocyte insulin receptors from NIDDM subjects compared with either lean or obese non-diabetic control subjects (16). The purpose of this study was to further explore the biochemical mechanisms underlying the impaired receptor kinase activity in NIDDM.

A decrease in the kinase activity of an insulin-receptor preparation could be due to impaired kinase activity of all the receptors or a decrease in the proportion of receptors capable of undergoing phosphorylation and activation. For example, Pang et al. (38) have already demonstrated that only ~40% of the total cellular insulin receptors in FaO cells, a human hepatoma cell line, display insulin-stimulated tyrosine phosphorylation, whereas the remainder of the receptors do not and are presumably inactive with respect to kinase activity. Although the proportion of phosphorylation-incompetent receptors may vary from tissue to tissue and across species, clearly, an increased proportion of kinase-incompetent receptors would result in an overall decrease in kinase activity of a preparation of total cellular insulin receptors. Furthermore, insofar as receptor kinase activity is integral to insulin's biological effects, this would also result in a state of cellular insulin resistance.

To approach this issue, we photoaffinity-labeled insulin receptors from normal and NIDDM subjects. This leads to insulin occupancy of essentially all (>90%) of the receptors and introduces a covalent radioactive label ($^{125}$I-insulin) into receptors from the entire population. Next, the receptors were allowed to autophosphorylate in the presence of a
high concentration (500 μM) of unlabeled ATP. Finally, the
$^{125}$I-labeled phosphorylated receptor preparations were
immunoprecipitated with either an anti-receptor or anti-phos-
phototyrosine antibody. Because in vitro insulin-stimulated
autophosphorylation occurs entirely on tyrosine residues, we
reasoned that all of the $^{125}$I-insulin–labeled receptors would
be immunoprecipitated by the monoclonal anti-receptor an-
tibody, whereas only the phosphorylated receptors would
be immunoprecipitated by the phosphotyrosine antibody. In
support of this line of reasoning, control studies showed that
>95% of the total NAPA-$^{125}$I-labeled receptors were precipi-
tated by the anti-receptor antibody and that >95% of the
phosphorylated receptors were precipitated with the phos-
phototyrosine antibody. From the latter findings, we conclud-
red that all tyrosine-phosphorylated receptors were precipitable
by the phosphotyrosine antibody.

Our studies demonstrated the existence of at least two
distinct populations of receptors—one containing phospho-
tyrosine residues and one incapable of insulin-stimulated
tyrosine phosphorylation. The results showed that 43% of
the receptors from nondiabetic subjects were capable of
autophosphorylation in the presence of maximally stimulat-
ing concentrations of insulin. Thus, in normal subjects, −50–
60% of the receptors do not undergo tyrosine phosphoryl-
lation and presumably do not contribute to the kinase activity
of the cell’s complement of insulin receptors. These results are
comparable with the values we obtained in normal rat
adipocytes (data not shown). Interestingly, because we have
shown that −90% of the adipocyte insulin receptors are at
the cell surface in the basal state, most of these “kinase-
negative” receptors must be at the cell surface, where they
apparently bind insulin in a physiologic manner. In the
NIDDM group, only 14% of the receptors were capable of
tyrosine autophosphorylation. This decrease in the propor-
tion of receptors that were phosphorylated was well corre-
lated to the decrease in exogenous substrate phos-
photorylation and appears to largely explain the defect in
receptor kinase activity we (16) and others (17,18) have
observed in NIDDM.

These results have shown that in adipocytes from normal
human subjects, 50–60% of the cell’s insulin receptors are
not capable of insulin-stimulated tyrosine autophosphoryl-
ation. In receptors from NIDDM subjects, the proportion of
receptors that do not display tyrosine phosphorylation is in-
creased, and the magnitude of this increase appears to cor-
relate with the magnitude of the decrease in kinase activity
measured. Furthermore, we found that receptors that do not
undergo autophosphorylation are also not active in mediat-
ing substrate phosphorylation and are functionally inactive
as kinases (data not shown). The exact mechanism for the
impaired tyrosine phosphorylation of this subpopulation of
insulin receptors is not known, although several possibilities
exist. First, an increase in the ATP $K_m$ could result in less
phosphate transferred to these receptors. However, this
does not seem likely in view of the high concentrations of
ATP (500 μM) used in the phosphorylation reactions. A sec-
ond possibility is that there is a structural or conformational
alteration in the β-subunit that alters the phosphoacceptor
sites. Burant and Buse (15) have demonstrated a subpop-
ulation of receptors in streptozocin-induced diabetic rat muscle
that exhibits decreased kinase activity and altered
glycosylation, but our studies provide no evidence for such
a mechanism in human NIDDM. Last, a certain percentage
of the insulin receptors may contain phosphorylated serine
residues that are carried over from the in vivo state, pre-
venting subsequent in vitro β-subunit tyrosine phosphoryl-
ation. Pang et al. (38), with $^{32}P$orthophosphate-labeled Fao
hepatoma cells, found that a subpopulation of insulin recep-
tors containing phosphoserine and phosphothreonine
but no phosphotyrosine was present in the basal state and
that these receptors underwent tyrosine phosphorylation
very slowly or not at all after the addition of insulin. Similarly,
the phorbol ester tetradecanoxyphorbol acetate has been
shown to cause serine phosphorylation of the insulin receptor
while inhibiting insulin-stimulated tyrosine autophosphoryl-
ation of the β-subunit (39).

Regardless of the biochemical mechanism leading to the
increased proportion of kinase-negative receptors, our re-
results indicate that this defect is reversible. Thus, two of the
NIDDM patients were restudied after weight reduction and
displayed a marked and concomitant improvement in both
the proportion of tyrosine kinase–competent receptors and
the overall kinase activity (with respect to exogenous sub-
strate phosphorylation) of the total receptor population. This
high degree of reversibility argues that the cause of the
kinase defect in NIDDM subjects involves some postrans-
lation modification of the receptor that is related to the
diabetic state. Furthermore, the fact that weight loss im-
proves the receptor kinase abnormality and at the same time
ameliorates the insulin resistance and hyperglycemia in
NIDDM underscores the possibility that the receptor kinase
defect may play an important role in the pathogenesis of the
cellular insulin resistance in this disorder.

In these studies, we used a phosphotyrosine antibody to
distinguish the population of receptors capable of insulin-
stimulated tyrosine phosphorylation. However, this does not
necessarily mean that the autophosphorylated receptors
represent a homogeneous population. Thus, the antibody
may recognize all or only specific phosphotyrosine residues,
and the immunoprecipitated receptors may differ in the
number or position of phosphotyrosines. In any event, in view of
the excellent correlation between percent immunoprecipi-
tation and substrate phosphorylation, it would appear that
the antibody recognizes phosphotyrosine sites that are
critical to the intrinsic kinase activity state of the receptor.
Furthermore, because all of the in vitro–phosphorylated re-
cessors were immunoprecipitable, either the antibody rec-
ognizes multiple sites or a single phosphotyrosine common
to all the phosphorylated receptors.

Our results suggest that immunoprecipitated tyrosine-
phosphorylated receptors account for all of the kinase ac-
tivity of the total receptor population. Although the increased
population of tyrosine autophosphorylation-incompetent re-
cessors largely accounts for the kinase defect in receptor
preparations from NIDDM subjects, we cannot be certain
that the phosphorylated immunoprecipitated receptors from
NIDDM subjects are entirely normal.

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