Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes.

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Research Article

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Insulin Receptor Kinase in Human Skeletal Muscle from Obese Subjects with and without Noninsulin Dependent Diabetes

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Abstract

We have studied the structure and function of the insulin receptors in obese patients with and without noninsulin dependent diabetes mellitus (NIDDM) and in nonobese controls using partially purified receptors from muscle biopsies.

Insulin binding was decreased in obesity due to reduced number of binding sites but no differences were observed in insulin binding between obese subjects with or without NIDDM. The structural characteristics of the receptors, as determined by affinity labeling methods and electrophoretic mobility of the β-subunit, were not altered in obese or NIDDM compared to normal weight subjects. Furthermore, the ability of insulin to stimulate the autophosphorylation of the β-subunit and the phosphoamino acid composition of the phosphorylated receptor were the same in all groups. However, insulin receptor kinase activity was decreased in obesity using Glu4:Tyr1 as exogenous phosphoacceptor without any appreciable additional defect when obesity was associated with NIDDM.

Thus, our data are supportive of the hypothesis that in muscle of obese humans, insulin resistance is partially due to decreased insulin receptors and insulin receptor kinase activity. In NIDDM the defect(s) in muscle is probably distal to the insulin receptor kinase.

Introduction

Noninsulin dependent diabetes mellitus (NIDDM)[1] or Type II diabetes is a heterogeneous disorder characterized by defects in insulin secretion and insulin action (1–5). The overall insulin resistance in NIDDM, with or without obesity, has been well demonstrated with the use of several in vivo techniques (6–12). Quantitatively, the most important site of insulin action resides in the muscle, which plays the predominant role in insulin-mediated glucose utilization (13). However, the mechanism of insulin resistance in muscle from obese patients with or without NIDDM is unknown. Thus, the goal of this study was to ascertain if defect(s) in insulin receptors' structure or function in muscle could explain the insulin-resistant state.

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1. *Abbreviations used in this paper:* NIDDM, noninsulin-dependent diabetes mellitus; PMSF, phenylmethylsulfonyl fluoride.

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Methods

Materials. Carrier-free Na ^{125}I and [γ-^{32}P]-ATP (tetratriethyl ammonium salt; 1,000–3,000 Ci/mmol) were purchased from New England Nuclear, Boston, MA, wheat germ agglutinin Sepharose was purchased from Miles Laboratories, Elkhart, IN, and the reagents for polyacrylamide gel electrophoresis from Bio-Rad Laboratories, Richmond, CA. Crystalline porcine insulin was kindly provided by Dr. Ronald Chance of Lilly Research Laboratories, Indianapolis, IN. All other reagents and chemicals were from Sigma Chemical Co., St. Louis, MO, unless otherwise specified.

Human subjects. 10 morbidly obese patients with NIDDM and an equal number of nondiabetic morbidly obese patients (obese control) were studied. The clinical and biochemical data of these patients are presented in Table I. All subjects had maintained constant body weight during the months preceding admission. The morbidly obese patients were admitted into the hospital 4 d before gastric bypass surgery. During these 4 d they remained active at approximately their prehospital exercise level. They received a weight maintaining diet providing 50% of the calories as carbohydrates, 30% as fat (polyunsaturated/saturated fat ratio of 0.4, cholesterol content of 600 mg), and 20% as protein. In two patients who were receiving glyburide and in two who were receiving insulin, the therapy was discontinued during these 4 d. Every morbidly obese patient, without known diabetes mellitus had a 75-g oral glucose tolerance test, as recommended by the National Diabetes Data Group (14), to classify them as nondiabetics. The morbidly obese patients with or without NIDDM had a 25-g intravenous glucose tolerance test to determine the rate of glucose disappearance (K_g rate) and insulin concentrations (15).

We also studied a nonobese nondiabetic group composed of eight females and one male who were admitted into the hospital for elective cholecystectomy or hysterectomy. The characteristics of this group were as follows: age 34±4 yr, weight 67±3 kg, height 169±5 cm, fasting plasma glucose 108±7 mg/dl.

None of the subjects studied had any disease or had taken any medications known to alter carbohydrate metabolism, with the exception of the obesity and NIDDM. Written consent was obtained from all patients after they were informed about the nature and potential risks of the study.

The subjects underwent surgery after an overnight fast. General anesthesia was induced with a short-acting barbiturate and maintained by phentanyl and nitrous oxide-oxygen mixture. Only saline was given intravenously before the muscle biopsy. Immediately after exploring the abdomen, 1–2 g of abdominal wall muscle was removed through the incision, usually at a point where the muscle had already been exposed during incision. The muscle specimens were immediately frozen between aluminum tongs cooled in dry ice. Samples were stored frozen at −70°C until analyzed.

Partially purified insulin receptor preparation. Partial purification of the insulin receptor from human muscle was according to the procedure described by Burant et al. (16) in rat muscle with minor modifications. Briefly, frozen muscle was powdered under liquid nitrogen, weighed, and homogenized as a frozen slurry in 50 mM Hepes, pH 7.4, containing 1% Triton X-100, 2 μM leupeptin, 2 μM pepstatin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mg/ml bacitracin, 1,000 U/ml aprotinin, and 1 mM vanadate. The homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant containing cellular membranes was solubilized by stirring at 4°C for 60 min. The mixture was then centrifuged at 100,000 *g* for 60 min, and the crude solubilized extract was partially

Table I. Clinical and Biochemical Data in Obese Patients with and without NIDDM

	Obese NIDDM	Obese control
	8 females 2 males	9 females 1 male
Age (*yr*)	40±4	35±3
Weight (*kg*)	139±6	127±6
Percent body fat	49±1	48±1
Height (*cm*)	165±2	166±3
Fasting plasma glucose (*mg/dl*)	205±23	93±4*
Fasting plasma insulin ($\mu U/ml$)	42±5	28±3‡
Peak plasma insulin during IVGTT ($\mu U/ml$)	118±22	208±38‡
K_g rate (*%/min glucose disappearance*)	0.63±0.11	1.45±0.12*
Duration of obesity (*yr*)		
Preadolescence	5	4
Postadolescence	5	6
Duration of diabetes (*yr*)	5±2	
Treatment of diabetes (*No. of patients*)	Insulin 2 Sulfonylureas 2 None 6	

* $P < 0.005$; ‡ $P < 0.05$.

purified by wheat germ agglutinin affinity chromatography, as previously reported (17). Recovery of ^{125}I-insulin binding activity in partially purified preparation was over 85% in our three experimental groups.

Insulin binding, affinity-labeling of the insulin receptor, tyrosine-specific insulin receptor kinase activity, autophosphorylation of the insulin receptor, and its phosphoamino acid composition were then studied using this partially purified receptor preparation. The extensive washing of the insulin receptor bound to the wheat germ agglutinin agarose column with over 50 times its bed volume and the inclusion of protease and phosphatase inhibitors was important to decrease the contaminating activities of phosphoprotein phosphatases, proteases, and ATPases that might complicate interpretation of the data. The insulin binding and kinase activity were stable in muscle tissue stored frozen at −70°C for at least 6 mo and in partially purified insulin receptor preparation stored frozen at −70°C for at least 3 mo.

^{125}I-Insulin binding. ^{125}I-Insulin binding in the partially purified human muscle insulin receptor preparation was measured at 4°C for 16 h, as described for human liver receptors (18). In these experiments, receptor preparation containing approximately the same amount of protein as estimated by the Bradford reaction (19) was used per assay tube (~ 5 μg). The ^{125}I-insulin binding data were generated before assessing insulin receptor kinase activity and autophosphorylation of the insulin receptor since this information was used to equalize the receptor number in these experiments. Because no change in insulin binding affinity was apparent in receptor from the three experimental groups (see Results), equal number of insulin receptor was estimated, as described by Burant et al. (20), with minor modifications as follows. ^{125}I-Insulin binding per ~ 5 μg of partially purified insulin receptor was determined in the presence of 1×10^{-10} M ^{125}I-insulin and 1×10^{-9} M, 1×10^{-8} M, 1×10^{-7} M, and 1×10^{-6} M (for the determination of nonspecific binding which was consistently less than 10% of total binding) unlabeled insulin. Ratios of the specific ^{125}I-insulin binding in each group were determined at each insulin concentration except at 1×10^{-7} M insulin, since at this high concentration the specific ^{125}I-insulin binding was low and thus the relative error was large. These ratios were averaged and the mean value was used as an estimate of relative number of insulin receptors. Individual values were within 15% of this mean. Appropriate volumes of the partially purified insulin receptors were used in subsequent experiments which were diluted in the WGA-Sepharose elution buffer (50 mM Hepes, pH 7.4, containing 10 mM $MgCl_2$, 1 mM PMSF, 0.1% Triton X-100, and 0.3 M N-acetyl glucosamine).

Cross-linkage of solubilized insulin receptors with ^{125}I-insulin. Following optimal ^{125}I-insulin binding in the presence and absence of unlabeled insulin (1×10^{-6} M) to equal amounts of protein (~ 5 μg), the ^{125}I-insulin receptor complex was covalently cross-linked with 0.5 mM disuccinimidyl suberate for 15 min at 4°C by the method of Pilch and Czech (21). The reaction was stopped by adding 50 mM Tris-HCl, pH 7.0, containing 1% sodium dodecyl sulfate (SDS), 0.005% bromophenol blue, 2% glycerol in the presence and absence of 50 mM dithiothreitol and boiling for 5 min. Polyacrylamide gel electrophoresis was performed according to the method of Laemmli (22) in 5–15% gradient gel for nonreduced conditions and 7.5% gel for reduced conditions. The gels were fixed, stained with 0.25% Coomassie Blue, dried, and autoradiographed for 24 h at −70°C with Kodak X-Omat film using lightening plus screen.

Tyrosine-specific protein kinase activity, autophosphorylation, and phosphoamino acids analysis of the insulin receptors. Equal amounts of human muscle insulin receptors from the three experimental groups were incubated in the presence and absence of different concentrations of insulin at 4°C for 16 h. Following this incubation, the tyrosine-specific protein kinase activity was determined at room temperature by the method of Grunberger et al. (23), with minor modifications, as reported for the human liver (18). [^{32}P]ATP (2 μCi, 100 μM) was added in the presence of 2.5 mg/ml exogenous substrate, Glu^4-Tyr^1, 10 mM $MgCl_2$, and 0.5 mM $MnCl_2$ in 50 mM Hepes buffer, pH 7.4. The reaction was stopped 30 min later with 10% TCA containing 10 mM pyrophosphate and 3 mg/ml bovine serum albumin (BSA), and processed as described before (18). The reaction was linear for at least 90 min, and at 30 min the reaction was linear over 1.5 to 23.3 μg protein concentrations. Thus, the time and range of protein concentration used in the three experimental groups were optimal.

The autophosphorylation of the insulin receptors was initiated by adding [^{32}P]ATP (20 μCi, 5 μM) in the presence of 5 mM $MgCl_2$ and 10 mM $MnCl_2$ in 50 mM Hepes buffer, pH 7.4, and continued for 60 min at 4°C since the reaction was linear for at least 120 min. The reaction was then stopped with 50 mM Hepes buffer containing 10 mM EDTA, 100 mM NaF, 20 mM pyrophosphate, and 4 mM ATP. This mixture was incubated with 1/200 dilutions of our polyclonal insulin receptor antibodies raised in rabbits against rat liver insulin receptors. After 6–10 h incubation at 4°C, a 10% (wt/vol) suspension of Pansorbin, prepared as described by Kasuga et al. (24), was added to the immunoprecipitation reaction mixture and the incubation was continued for at least 1 h at 4°C. After this incubation, the Pansorbin was sedimented by centrifugation at 500 *g*. The pellet was washed once with 50 mM Hepes buffer, pH 7.4, containing 0.1% Triton X-100, again with 50 mM Hepes buffer, pH 7.4, containing 0.5 M NaCl, and finally with 50 mM Hepes buffer, pH 7.4, containing 0.01% SDS. Then, the pellet was suspended in Laemmli buffer and boiled for 5 min. Finally, the Pansorbin was sedimented by centrifugation and the supernatant subjected to SDS-polyacrylamide slab gel (7.5%) electrophoresis. The β-subunit of the insulin receptor was localized by autoradiography, excised, and counted in a liquid scintillation counter. Equal areas from each lane judged free of radioactivity by autoradiography were cut, counted, and substracted as background. Then, the dried polyacrylamide gel fragments were reswollen in 5 mM NH_4HCO_3, 0.1% SDS, 5% β-mercaptoethanol. The gel pieces were pulverized and the suspension was heated at 100°C for 5 min and then incubated overnight at 35°C. The extraction procedure was repeated and the extracts were combined and precipitated with five volumes of acetone/NH_4OH (18:1 vol/vol). The material was centrifuged at 2,000 *g* for 15 min and the pellets were washed with 100 μl of ethanol/ether (1:1 vol/vol) and centrifuged again. The pellets were dissolved in 6 N HCl by heating at 100°C for 1 min, and were hydrolyzed for 1 h at

110°C in tubes sealed under vacuum. The hydrolysates were dried in a Speedvac and analyzed on cellulose thin-layer plates (100 μm), as described by Hunter and Sefton (25) with some minor modifications, by electrophoresis at pH 1.9 for 2 h at 1.5 kV in glacial acetic acid/formic acid/H_2O, (78:25:897 vol/vol) and at pH 3.5 for 45 min at kV in glacial acetic acid/pyridine/H_2O (50:5:945 vol/vol). The markers were detected by staining with ninhydridine and the radioactive phosphoaminoacids by autoradiography.

Statistical analysis. Descriptive statistics (mean±SEM) and comparative statistics (analysis of variance and *t* tests) were performed using a computerized program (StatView, the graphic statistics for the Macintosh).

Results

^{125}I-Insulin binding. Fig. 1 shows the Scatchard plots of ^{125}I-insulin binding from partially purified muscle insulin receptors of morbidly obese patients with and without NIDDM and nonobese nondiabetic patients. ^{125}I-Insulin binding was identical in the two weight, age, and sex-matched obese groups with or without NIDDM. However, ^{125}I-insulin binding in the nonobese group was higher than in the two obese groups at every insulin concentration used ($P < 0.05$–0.005). The increase in ^{125}I-insulin binding appears to be due to an increase in the number of insulin binding sites without a change in the apparent affinity of these sites for insulin. Furthermore, these changes in insulin binding were not a reflection of differences in nonspecific binding, which was consistently < 10% of total ^{125}I-insulin binding at tracer concentrations of insulin. Likewise, the differences in insulin binding could not be attributed to differences in insulin degradation, which was < 10% under the experimental conditions used, or to differences in association time of insulin binding, since apparent steady state of insulin binding at 4°C was reached after 8 h and maintained for at least 24 h.

The diminished ^{125}I-insulin binding observed in the human muscle could provide an explanation to the decreased insulin sensitivity of obesity, but fails to explain the decreased insulin responsiveness of NIDDM. Thus, we studied the structure of the insulin receptor by affinity labeling and one of the newly recognized postinsulin binding functions, i.e., insulin receptor kinase activity, in an attempt to understand the mechanism of postinsulin binding resistance in NIDDM.

Affinity labeling of the insulin receptor. Fig. 2 demonstrates that incubation of the insulin receptor with ^{125}I-insulin and subsequent addition of disuccinimidyl suberate under reduced conditions specifically labeled a protein of an approximate molecular mass of 125,000 D, which corresponds to the molecular mass of the α-subunit of the insulin receptor. The radioactivity associated with the α-subunit was totally displaced by a large excess of unlabeled insulin supporting the specificity of insulin binding of this band. This figure also demonstrates that no apparent differences were observed in the electrophoretic migration of the cross-linked α-subunit of the insulin receptor among nonobese controls, obese controls, and obese diabetics. Furthermore, labeling efficiency of the insulin receptor in this group of patients was the same. Identical experimental conditions to those for ^{125}I-insulin binding (Fig. 1) were used with regard to incubation time, temperature, and amount of protein (~ 5 μg) chromatographed, and the ratios of the intensity of the specific radiolabeled band in each group (Fig. 2) were similar to those from the ^{125}I-insulin binding (Fig. 1). Similar results were obtained when the

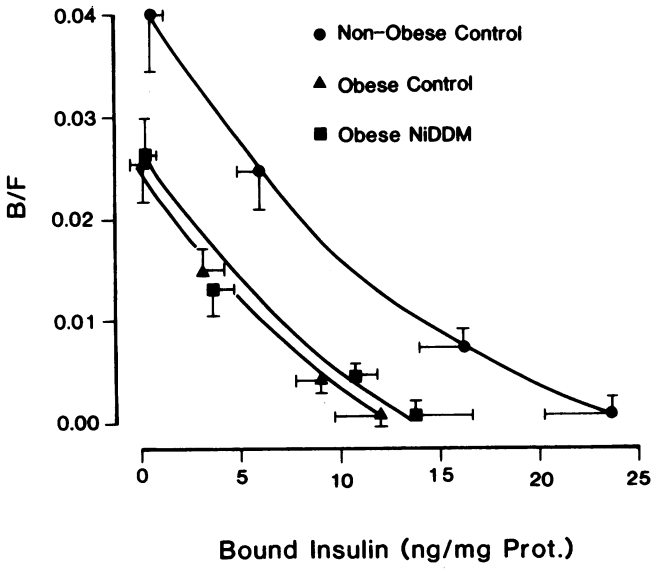

Figure 1. Insulin-binding to solubilized insulin receptors from human muscle. Wheat germ agglutinin purified solubilized insulin receptors (~ 5 μg of protein) were incubated with ^{125}I-insulin (1×10^{-10} M) and increasing concentrations of unlabeled insulin ($0 - 10^{-6}$ M) in a total volume of 250 μl of 50 mM Hepes buffer, pH 7.4, containing 0.1% Triton X-100. After incubation for 16 h at 4°C, the receptor-bound insulin was precipitated with polyethylene glycol (12.5% final concentration) using bovine γ-globulin as carrier protein. The figure shows the Scatchard plots of the specific insulin binding data (mean±SEM) from 8 obese patients with NIDDM (●), 8 obese controls (△), and 8 nonobese controls (□).

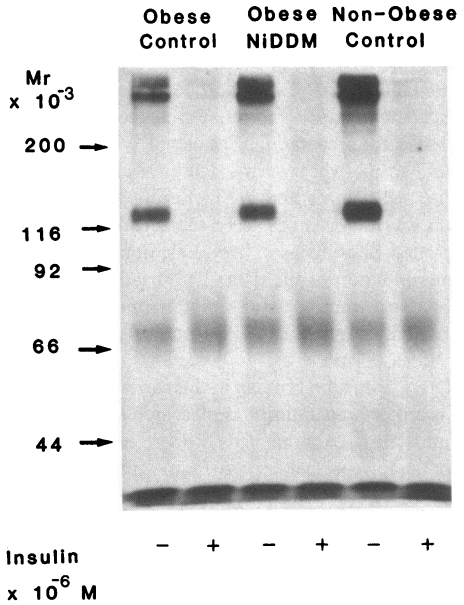

Figure 2. Autoradiography of SDS-PAGE of solubilized insulin receptors from human muscle. ^{125}I-insulin (5×10^{-10} M) binding was performed in wheat germ agglutinin purified solubilized insulin receptor, as described in the legend of Fig. 1, in the presence and absence of 1×10^{-6} M unlabeled insulin. The ^{125}I-insulin receptor complex was cross-linked with 0.5 mM disuccinamidyl suberate, reduced with 50 mM dithiothreitol, and subjected to SDS electrophoresis in 7.5% acrylamide resolving gel. The fixed stained and dried gels were autoradiographed on Kodak X-OMAT film with intensifying screen at −70°C for 24 h.

cross-linked insulin receptors were subjected to gel electrophoresis studied under nonreducing conditions.

Autophosphorylation and tyrosine-specific protein kinase activity of the insulin receptor. Fig. 3 demonstrates that in the nonobese control, obese diabetic, and obese control groups, respectively, insulin increases the incorporation of ^{32}P from [$\gamma^{32}P$]ATP into a protein of a molecular mass between 90,000 and 95,000 D, which is specifically immunoprecipitated by insulin receptor antibodies. These findings identify this protein as the β-subunit of the insulin receptor. There were no reproducible changes in the electrophoretic mobility of this protein among the three groups. Furthermore, Fig. 4, which shows the amount of radioactivity associated with the β-subunit of the receptor from eight patients in each group demonstrates no statistical differences among the groups in basal or insulin-stimulated phosphorylation of the β-subunit. Also, as shown in Fig. 5, insulin stimulation of ^{32}P incorporation into the β-subunit of the receptor occurred exclusively on tyrosine residues in all three groups.

In addition to this comparison of basal and maximal insulin-stimulated ^{32}P incorporation into the β-subunit of the receptor in the entire group of patients studied (Fig. 4), we performed additional experiments in three patients from each group from whom we had the largest muscle biopsies. In two patients from each group the experiments were performed using equal amounts of insulin receptor, and the third one using equal amounts of protein. Autophosphorylation was measured in these patients over a range of four or five insulin concentrations (1×10^{-11} M to 1×10^{-7} M). The insulin dose-response curves were similar in this small group of patients. Half-maximal insulin stimulation occurred at $\sim 1 \times 10^{-9}$ M insulin and maximal insulin stimulation at $\sim 1 \times 10^{-8}$ M insulin (data not shown). Thus, muscle from patients with different degrees of in vivo insulin resistance failed to demonstrate any abnormality of the autophosphorylation of the insulin receptor when examined using the currently available methodologies.

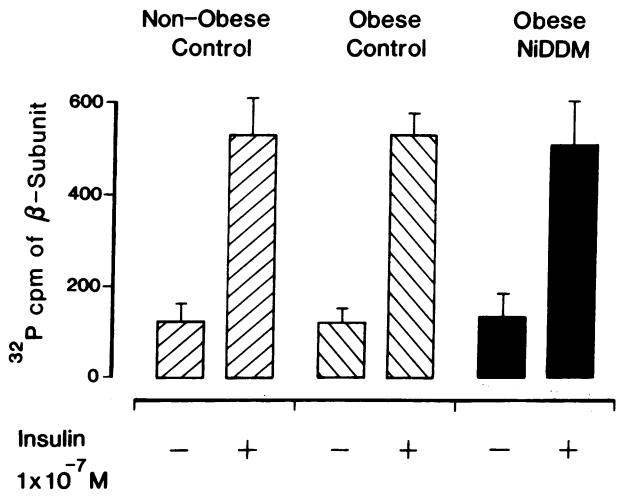

Figure 4. Autophosphorylation of the β-subunit of insulin receptors from human muscle. The β-subunit of insulin receptors phosphorylated in the presence and absence of insulin as described in the legend of Fig. 3 was localized by autoradiography, excised, and counted as described in Methods. The values are the mean±SEM from eight different patients in each group.

Since Freidenberg et al. (26) have demonstrated that the relative insulin receptor kinase activity towards exogenous substrates may be different from that observed with autophosphorylation, however, we also measured receptor kinase activity using the exogenous substrate Glu4:Tyr1 as the phosphoacceptor. When equal amounts of receptors from the three groups of patients phosphorylated Glu4:Tyr1 in the absence of insulin, basal values were similar (nonobese control 1,497±310; obese control 1,491±342; obese NIDDM 1302±321; mean±SEM ^{32}P counts per minute $n = 8$ in each group). Fig. 6 demonstrates the ability of submaximal (1×10^{-9} M) and maximal (1×10^{-7} M) concentrations of insulin to stimulate exogenous substrate phosphorylation. At both insulin concentrations the phosphorylation of Glu4:Tyr1 was greater in the nonobese control than in the obese control or in the obese NIDDM group ($P < 0.05$ at 1×10^{-9} M; $P < 0.005$ at 1×10^{-7} M insulin). However, there

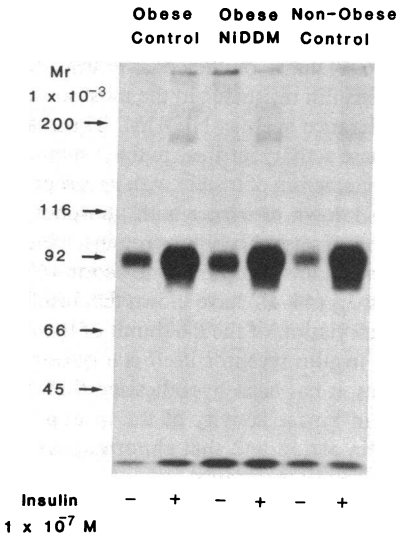

Figure 3. Autoradiogram of insulin-stimulated autophosphorylation of the β-subunit of solubilized insulin receptors from human muscle. Wheat germ agglutinin purified solubilized insulin receptors from nonobese controls and equal insulin binding activities from obese controls and obese subjects with NIDDM were incubated in the absence and presence of insulin 1×10^{-7} M under identical conditions to that of ^{125}I-insulin binding described in Fig. 1. Then 5 μM [^{32}P]ATP (20 μCi/tube) was added in the presence of 5 mM $MgCl_2$ and 10 mM $MnCl_2$ in 50 mM Hepes. After 60 min at 4°C, the reaction was terminated with 50 mM Hepes buffer, pH 7.4, containing 10 mM EDTA, 100 mM NaF, 20 mM pyrophosphate and 4 mM ATP. The insulin receptors were immunoprecipitated with insulin receptor antibodies reduced with 50 mM DTT and subjected to SDS-PAGE, as described in Fig. 2.

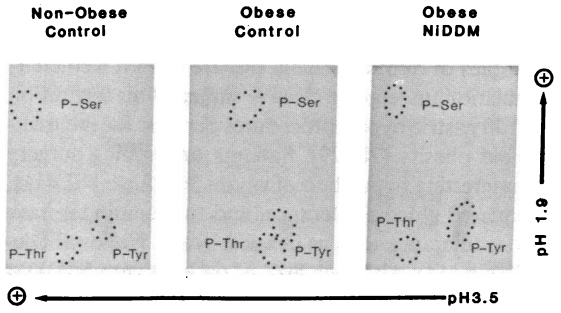

Figure 5. Phosphoamino acid analysis of the β-subunit of insulin receptors from human muscle. The β-subunit of insulin receptors was phosphorylated in the presence of insulin 1×10^{-7} as described in the legend of Fig. 3, localized by autoradiography, extracted and hydrolyzed as described in Methods. Then, the phosphoaminoacids were analyzed as described by Hunter and Sefton (25).

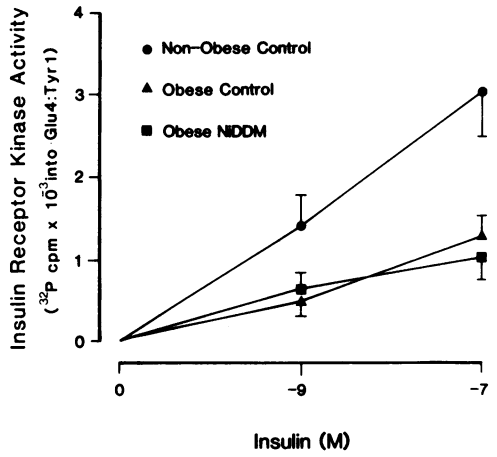

Figure 6. Glu4:Tyr1 phosphorylation by human skeletal muscle-derived insulin receptors. Wheat germ agglutinin purified solubilized insulin receptors from nonobese controls, obese controls, and obese subjects with NIDDM were incubated in the presence and absence of 1×10^{-9} M and 1×10^{-7} M insulin, as described in the legend of Fig. 1. Then 100 μM ATP (20 μCi/vial) was added in the presence of Glu4:Tyr1 (2.5 mg/ml), 10 mM $MgCl_2$ and 0.5 mM $MnCl_2$ in 50 mM Hepes. After 30 min at room temperature, the reaction was stopped with 10% TCA containing 10 mM pyrophosphate and 3% BSA. The data are the mean±SEM from 8 nonobese controls (●), 8 obese controls (△), and 8 obese patients with NIDDM (▫).

were no differences between the two obese groups with or without NIDDM.

Discussion

It is now well established that skeletal muscle in normal man plays a fundamental role in the disposal of glucose (27), and it is the site responsible for postprandial hyperglycemia in insulin resistant states such as obesity and NIDDM (12, 13). However, the mechanism(s) of these abnormalities has remained largely unexplored for several reasons: (*a*) obtaining human muscle biopsies from subjects with NIDDM is difficult; (*b*) the methodology for studying insulin receptor structure and function in muscle has only recently been developed; and (*c*) an in vitro insulin responsive human muscle cell preparation has not yet been developed.

In this study, we believe we have overcome two of these problems by applying the methodology described by Burant et al. (16), to study insulin receptor structure and function in rat muscle to samples of human skeletal muscle from a well characterized population undergoing elective surgery. Our institution performs ~ 100 gastric bypass procedures per year for the treatment of morbid obesity (28, 29). Patients undergoing surgery comprise an interesting population of whom 30% have NIDDM, 10% have impaired glucose tolerance, and the remainder have normal glucose tolerance tests as defined by the National Diabetes Data Group (14). Although 80% of patients with NIDDM are obese, we recognize that we are studying only those who are extremely obese. Thus, we do not wish to imply that the data presented here in morbidly obese patients with NIDDM can be extrapolated to the entire population with NIDDM. Furthermore, the data generated from the abdominal wall muscle might not be representative of the majority of the muscle mass with regard to insulin action, as has been demonstrated in the case of subcutaneous adipose tissue from different regions (30). This might be particularly relevant in patients with morbid obesity in whom the abdominal wall muscle must be under significant mechanical stress. In spite of these limitations, we believe this is the only large population of noncritically ill patients with NIDDM, from whom enough muscle tissue can be obtained without any significant additional risk at the same time when a therapeutic surgical procedure is performed.

We have demonstrated, as predicted by Evans et al. (31) that there is a decrease in the number of insulin receptors in skeletal muscle in human obesity. This finding in human muscle from obese patients is in agreement with that of Arner et al. (32) and ours (18) in human liver and that of most (33–35), but not all (36–37), studies in human adipocytes. We have also demonstrated that NIDDM and obesity together, in an age and sex matched population, does not result in a further decrease in the number of insulin receptors in human muscle. This is similar to most studies in human adipocytes (37, 38) and to our recent finding in freshly isolated human hepatocytes demonstrating that surface insulin receptors were similarly decreased in obese patients with or without NIDDM (18). The intracellular pool of liver insulin receptor was, however, significantly decreased in NIDDM when compared with obese or nonobese controls (18). Due to the poor understanding of the cellular distribution of the insulin receptors in muscle, and because of the lack of either an in vitro human muscle cell preparation or an adequate human muscle plasma membrane preparation, the contribution of the cell surface insulin receptors or the intracellular receptors to the total pool of detergent extractable insulin receptor studies here is totally unknown.

The structure of the human insulin receptors in adipocytes (39) and liver (18, 32) from obese subjects had been previously studied using affinity labeling techniques. No appreciable differences between obese and nonobese subjects were found. In agreement with these studies, we have failed to demonstrate any structural defect in human skeletal muscle between nonobese and obese patients with or without NIDDM.

Most in vivo studies (6–9) have predicted a postinsulin binding defect(s) to explain insulin resistance in the most severe forms of carbohydrate intolerance such as NIDDM. Thus, we have studied the tyrosine kinase activity intrinsic to the β-subunit of the insulin receptor. The interaction of insulin with its receptor in the plasma membrane is known to trigger both phosphorylation and dephosphorylation of several cellular proteins, which may be a basic regulatory mechanism of hormone action (40–42). Kasuga et al. (43) and others (44–48) have shown that insulin binding induces the phosphorylation of the β-subunit of the insulin receptor and that the insulin receptor itself is a tyrosine-specific protein kinase. Thus, it has been hypothesized that the phosphorylation and protein kinase activity of the insulin receptor might mediate insulin action and that abnormalities of this function could explain insulin resistance.

Insulin receptor kinase activity has been demonstrated to be decreased in muscle (20) and liver (49) of streptozotocin-induced diabetes in rat, in the muscle of insulin-resistant obese mice (50), and in the liver from 3 d fasted rats (26). However, others have found no defect in the rat liver of streptozotocin-induced diabetes (51, 52). Earlier studies in humans with insulin resistance due to rare genetic defects, using peripheral blood cells or fibroblasts (53, 54), have demonstrated insulin receptor kinase defects in

some patients, but not all (55, 56). We have recently shown that in human liver (18) and adipose tissue (35) the insulin receptor kinase is significantly decreased in obese patients with NIDDM. This observation has been recently confirmed by Freidenberg et al. (57) in adipocytes and by Comi et al. (58) in red blood cells from patients with NIDDM.

In the present study, we have demonstrated in human skeletal muscle that the ability of insulin to stimulate the phosphorylation of the artificial exogenous substrate Glu4:Tyr1 was equally decreased in obesity with or without NIDDM. It is likely that this defect of the insulin receptor kinase, in association with the decreased receptor number, plays a role in the mechanism of insulin resistance in the muscle of obese subjects. However, our studies fail to explain the mechanism of the more severe insulin resistance in muscle from patients with NIDDM, since we have found no additional defect when obesity is associated with NIDDM. This study, however, helps to illustrate the important concept that a cellular defect in one tissue cannot be extrapolated to the entire body. The same population of obese patients with NIDDM have decreased insulin receptor kinase in the liver (18) and the adipose tissue (35). In the muscle, the defect in insulin receptor kinase appears to be related to obesity per se rather than to NIDDM. A step(s) distal to the insulin receptor kinase or a primary defect(s) in the glucose transporters are then the possible candidates to explain insulin resistance of muscle in NIDDM. This is further supported by our demonstration that the ability of insulin to stimulate the autophosphorylation of the β-subunit of the receptor is identical in the three groups of subjects studied.

The dissociation between the decreased insulin receptor kinase activity toward an artificial exogenous substrate and normal autophosphorylation of the receptor in muscle of obese patients with or without NIDDM described here has been demonstrated in other insulin resistant states. Freidenberg et al. (26), studying rat liver during prolonged fasting, showed a marked decrease in the phosphorylation of Glu4:Tyr1 but normal insulin receptor autophosphorylation. Similarly, Burant et al. (20), studying the skeletal muscle of streptozotocin-induced diabetes in the rat, demonstrated diminished kinase activity using histone H_2b and angiotensin II as exogenous phosphoacceptors. In contrast, the decreased autophosphorylation of the insulin receptor was only due to diminished basal activity since no differences in insulin-stimulated autophosphorylation above basal were observed between the muscle insulin receptors of the diabetic rat and control rats.

The relationship between the insulin receptor-associated tyrosine kinase activity and the autophosphorylation of the receptor is largely unknown. It has been demonstrated that the insulin receptor kinase activity is markedly activated by tyrosine phosphorylation of its β-subunit, which may be an important regulatory mechanism in intact cells (47, 48, 59). This relationship might be abnormal in some states of insulin resistance. Also, our data and those of others (20, 26) suggest that the insulin receptor demonstrates substrate specificity since no differences are noted with the receptor as substrate for the insulin receptor kinase, but large differences are seen with exogenous substrates in the rat liver (26), rat muscle (20), and human muscle from insulin-resistant states. It is possible that these abnormal states result in conformational or structural changes in the receptor such as that some, but not all substrates' recognition sites on the receptor are accessible to the catalytic site (26).

In spite of the effort of several laboratories, including our own, to relate insulin resistance to insulin receptor kinase activity, we believe the accumulated data, although exciting, should be analyzed with great caution. First, it is not apparent from these studies if the defect(s) in kinase activity is primary and involved in the mechanism of the disease or it is only one of the many metabolic derangements secondary to the disease. Second, the biological significance of these studies performed in cell-free systems is not clear at this time. These studies would be on more solid ground if the defects of even putative endogenous substrates (60, 61) of the insulin receptor kinase were demonstrated in a cell-free system or, even more importantly, in an intact cell system, since there may be fundamental differences in these processes if they are examined in vitro or in vivo (62). Our data thus are only supportive of the hypothesis that in muscle of obese human, the insulin resistance is at least partially due to a decrease in the number of insulin receptors and defects in its protein kinase activity. In NIDDM the defect(s) in muscle is probably distal to the insulin receptor kinase.

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