Tissue-Related Changes in Insulin Receptor Number and Autophosphorylation Induced by Starvation and Diabetes in Rats

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Insulin action is subject to regulation at the level of the insulin receptor and at postreceptor levels. Starvation and diabetes are often associated with insulin resistance for glucose metabolism in various tissues. In muscle, fat, and liver, we examined whether changes in the functionality of the insulin receptor correlated with changes in insulin action in the starved and diabetic state. Insulin-stimulated receptor autophosphorylation reflects an early physiologic step in transmission of the insulin signal, and for that reason, changes in autophosphorylation activity of the insulin receptor were used as a marker to determine the functionality of the insulin receptor. Glycoprotein fractions prepared from skeletal muscle, diaphragm, epididymal fat, and liver of control, 3-day starved, short-term 3-day (S) diabetic (streptozotocin, 70 mg/kg intravenously), and long-term 6-month (L) diabetic (neonatal streptozotocin 100 μg/g intraperitoneally) rats were used in this study. Receptor activity was monitored by measuring insulin-stimulated [γ-^{32}P]adenosine triphosphate (ATP) receptor autophosphorylation. In addition, to obtain information about whether changes in receptor autophosphorylation are related to changes in receptor number, relative numbers of high-affinity insulin receptors were determined by affinity cross-linking of [^{125}I]insulin to the receptor α-chain and quantitation of the yield of labeled receptor α-chain. Control, starved, S diabetic, and L diabetic rats had plasma insulin and glucose levels of 294 ± 42, 90 ± 24, 48 ± 12, and 216 ± 30 pmol/L and 6.7 ± 0.2, 4.1 ± 0.2, 23.3 ± 0.7, and 21.6 ± 2.9 mmol/L, respectively. In all tissues, insulin-stimulated receptor autophosphorylation was normal to increased (skeletal muscle > liver > diaphragm > fat), and these changes in receptor functionality did not correlate with changes in insulin action on glucose metabolism in muscle, fat, and liver of starved and diabetic rats. This indicates that insulin resistance for glucose metabolism, when present in starved and diabetic rats, is due to postreceptor defects rather than to a decreased functionality of the receptor.

INSULIN INITIATES its actions by binding to its receptor on the plasma membrane. Binding occurs to the extracellular α-subunit of the receptor, which results in activation of a tyrosine kinase on the intracellular part of the β-subunit of the receptor and leads to autophosphorylation and phosphorylation of external substrates. Receptor autophosphorylation activity is an early physiologic step in insulin action and reflects the capacity of the receptor to transduce the insulin signal.[1]

The action of insulin is subject to regulation at the level of the insulin receptor and at postreceptor levels that are connected to insulin-sensitive effector systems. It has been shown that several insulinopenic conditions such as starvation and diabetes have great impact on the action of insulin.[2,3] It appears that different tissues can respond in different ways to altered metabolism, ie, with tissue-specific changes in insulin action[4,5] and tissue-specific changes in insulin receptor tyrosine kinase activity.[6] Previously, we have shown that adipose tissue of starved and diabetic rats is resistant to insulin-stimulated glucose uptake.[2] In addition, diabetic rats show insulin resistance to suppression of hepatic glucose production and stimulation of peripheral-tissue glucose uptake in vivo.[3] The resistance of peripheral tissues seems to be related to a decreased glucose uptake in muscle, since 80% to 90% of a glucose load in vivo is taken up by muscle.[7] In contrast to diabetic rats, starved rats show normal hepatic and increased peripheral-tissue sensitivity to insulin in vivo.[3] In this study, we have determined the status of the insulin receptor during starvation and diabetes in muscle, fat, and liver to see whether a correlation exists between the observed tissue-specific changes in insulin action and insulin receptor function, using the same animal models we have used previously.[2,3]

The status of the insulin receptor was compared in glycoprotein fractions prepared from skeletal muscle, diaphragm, liver, and epididymal adipocytes from rats with various forms of insulinopenia such as starvation, acute diabetes, and chronic diabetes. Variations in receptor activity were monitored by measuring insulin-stimulated β-chain autophosphorylation. In addition, we examined whether observed changes in autophosphorylation activity could contribute to changes in receptor number. The number of high-affinity insulin-binding sites was determined by cross-linking [^{125}I]insulin to the receptor and measuring the degree of α-chain labeling.

The same experimental approach was applied to each tissue to obtain comparable data. This was instituted because it has been suggested[8] that conflicting results between laboratories with regard to receptor kinase activity[9-17] in diabetic and starved rats might be caused not only by differences in time span and severity of diabetes and starvation but also by differences in methodologies of receptor isolation and preparation.

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MATERIALS AND METHODS

Animals

Male Wistar rats (300 to 350 g) with free access to standard laboratory chow (Muracon pellets, Trouw, The Netherlands) and water were individually housed at a constant temperature (23°C) and a fixed 12-hour light/dark cycle (lights on at 7 AM). Under ether anesthesia, mature rats were rendered severely (S) diabetic by an intravenous injection of streptozotocin 70 mg/kg (Zanosar; Upjohn, Kalamazoo, MI) diluted in 0.01 mol/L citrate buffer (pH 4.5) via the penile vein, which resulted in a 3-day period of severe insulinopenia and hyperglycemia. Long-term (L) diabetes was induced by neonatal (1 day after birth) intraperitoneal injection of streptozotocin 100 μg/g, which resulted in a 6-month period of moderate insulinopenia and hyperglycemia. These diabetic rats are often referred to as type II diabetic or non–insulin-dependent diabetic rats.[18] Severe starvation was induced by a 3-day period of total fasting, which resulted in insulinopenia and hypoglycemia. Water was allowed ad libitum.

Preparation of Tissue Glycoprotein Fractions

Rats were killed between 1 and 2 PM by decapitation, and blood was collected in heparinized tubes and immediately centrifuged at 4°C. Plasma glucose level was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Palo Alto, CA), and plasma insulin level, by a specific rat radioimmunoassay as described previously[19] using a rat standard insulin (Novo, Copenhagen, Denmark). Intraassay and interassay variations for the insulin assay are 6.8% and 8.1%, respectively. The anterior tibial muscle, diaphragm, epididymal fat, and liver were quickly removed after decapitation and immediately frozen at −70°C on dry ice. Subsequently, tissues were homogenized in lysis buffer (1% Triton X-100, 50 mmol/L Tris hydrochloride, pH 7.6, 0.1 mmol/L phenylmethylsulfonyl fluoride, and 1 kU/mL Trasylol) using a polytron (Braun, Janke, und Kunkel, Staufen, Germany). Homogenization time was 3 minutes at maximum speed. The glycoprotein fraction of tibial muscle, diaphragm, epididymal fat, and liver was isolated by chromatography over wheat-germ agglutinin–Sepharose (Pharmacia, Uppsala, Sweden) as described previously.[20] The glycoprotein fraction was obtained in wheat-germ agglutinin buffer (50 mmol/L HEPES, pH 7.4, 10 mmol/L $MgCl_2$, 1 mmol/L 2-mercaptoethanol, 0.1% Triton X-100, 1 kU/mL Trasylol, 1 μg/mL leupeptin, 0.1 mmol/L phenylmethylsulfonyl fluoride, and 0.3 mol/L *N*-acetylglucosamine). Protein concentration was determined by the Bradford method (Bio-Rad, Richmond, CA). A glycoprotein concentration of 1 mg/ml was made by dilution with wheat-germ agglutinin buffer, and 1 mg/mL bovine serum albumin (molecular biology grade; Boehringer, Mannheim, Germany) was added.

Cross-Linking of [^{125}I]insulin to the Receptor

The relative number of high-affinity insulin receptors was quantified in duplicate by cross-linking [^{125}I]insulin to the α-chain of insulin receptor present in the glycoprotein fraction. Fifty micrograms glycoprotein was incubated for 16 hours at 0°C in 100 μL wheat-germ agglutinin buffer containing 30 pmol/L Mono-A^{14}-[^{125}I]iodoinsulin (specific activity, 2,000 Ci/mmol; Amersham International, Buckinghamshire, UK). Cross-linking was induced by addition of disuccinimidyl suberate in dry dimethylsulfoxide (10 mmol/L) to a final concentration of 0.2 mmol/L. After 20 minutes at 0°C, the mixture was made 50 mmol/L in Tris hydrochloride, pH 7.8, followed by addition of 1 mL 10% trichloroacetic acid. The protein precipitate was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography on preflashed Kodak XAR-5 films (Eastman Kodak, Rochester, NY). Analysis by densitometry was performed using a Joyce Loebl (Gateshead on Tyne, England) densitometer. Specificity of α-chain labeling was tested by cross-linking in the presence of 1 μmol/L nonradioactive insulin. More than 90% of covalently bound label was displaced from the α-chain under these conditions.

Insulin-Stimulated Autophosphorylation of the Receptor

Insulin receptor transduction of the insulin-binding signal was measured in duplicate by quantifying insulin-stimulated receptor β-chain autophosphorylation. The same glycoprotein fractions as used for the insulin cross-linking assay, were used. Fifty micrograms glycoprotein was incubated without or with 10 nmol/L insulin (human monocomponent; Novo) for 1 hour at 22°C. Autophosphorylation was initiated by addition of $MnCl_2$ (final concentration, 6 mmol/L) and [γ-^{32}P]adenosine triphosphate ([ATP] final concentration, 25 μmol/L; 200 Ci/mmol). Incubation time was 6 minutes at 22°C. The reaction was terminated by addition of 1 mL 10% trichloroacetic acid. In glycoprotein fractions, we have never observed a marked ATPase activity that is able to hydrolyze a substantial fraction of the ATP, which is present in high concentrations (25 μmol/L), as illustrated by the following experiment: Using the same protocol described by us previously,[21,22] we searched for proteins binding guanosine diphosphate and guanosine triphosphate (GTP) in glycoprotein fractions before and after insulin stimulation. In this protocol, we analyze the ratio of guanosine diphosphate and GTP directly by thin-layer chromatography. In those experiments, we never detected marked GTPase activity.

The protein pellet was washed with 1 mL ethanol and dissolved in 50 μL 50-mmol/L $NaHCO_3$/0.1% SDS. The protein precipitate was analyzed by SDS-PAGE and autoradiography on preflashed Kodak XAR-5 films. Analysis by densitometry was performed using a Joyce Loebl densitometer. Initially, insulin receptor β-chain labeling was analyzed after immunoprecipitation of the insulin receptor, as described previously.[23] Since the tissues used express high levels of insulin receptors, we found that immunoprecipitation was not essential, and this step was subsequently omitted when a complete series of experiments was repeated.

Statistical Analyses

ANOVA was used for multiple-comparison. When ANOVA showed a significant difference among several groups, Fisher's least-significant difference test was used for between-group comparisons. The criterion for significance was set at $P < .05$. All data are presented as the means ± SEM.

RESULTS

Animals

Table 1 shows characteristics of experimental rats at the time of decapitation. Three-day–starved and S diabetic rats showed a comparable catabolic state. Both groups of rats lost 10% to 15% of body weight within a 3-day period. L diabetic rats were also catabolic, since control rats reached 300 to 350 g body weight within 3 months, whereas L diabetic rats reached that body weight after 6 months. For comparison, body weight of 6-month-old control rats was 505 ± 21 g. Plasma glucose was increased fourfold in S diabetic and L diabetic rats. In starved rats, plasma glucose levels were ±30% reduced. On the other hand, plasma insulin levels were significantly reduced in S diabetic and

Table 1. Characteristics of Animals Studied

	Body Weight			Plasma Insulin (pmol/L)	Plasma Glucose (mmol/L)	Liver Wet Weight (g)
	Start (g)	After 3 Days (g)	Change (%)			
Control	317 ± 5	335 ± 4	5.6 ± 0.5	294 ± 42	6.7 ± 0.2	15.4 ± 0.9
Starved	337 ± 10	285 ± 9	−15.5 ± 0.4*	90 ± 24*	4.1 ± 0.2*	8.0 ± 0.4*
S diabetic	330 ± 5	293 ± 3	−11.0 ± 2.9*	48 ± 12*	23.3 ± 0.7*	13.2 ± 0.7
L diabetic	342 ± 6	NA	NA	216 ± 30	21.6 ± 2.9*	21.3 ± 0.9*

NOTE. Blood samples were obtained at time of decapitation. Data are the mean ± SEM of 3 to 4 rats per group.
Abbreviation: NA, not applicable.
*$P < .05$, significant difference from control value.

starved rats, whereas L diabetic rats showed no difference in 5-hour fasting insulin levels as compared with control rats. However, in L diabetic rats this can be considered a relative state of hypoinsulinemia, since these low plasma insulin levels are observed in the face of severe hyperglycemia. Wet liver weight in starved, S diabetic, and L diabetic rats was decreased, normal, and increased, respectively. This indicates that these various forms of insulinopenia are characterized by their own specific metabolic perturbations.

Analysis of Changes in Receptor Number

Changes in the relative number of high-affinity insulin receptors were determined by affinity cross-linking of [^{125}I]insulin to the α-chain of the insulin receptor. α-Chain labeling reflects the relative number of insulin receptors in the glycoprotein fraction. Four tissues (skeletal muscle, diaphragm, liver, and fat) from four groups of rats were examined. A typical example of muscle and liver is shown in Fig 1. The labeled α-bands were analyzed by densitometry, and results are listed in Table 2. The amount of α-chain labeling in control rats was set at a relative value of 1. From the binding data, it is clear that all four tissues of starved, S diabetic, and L diabetic rats show a normal to increased number of high-affinity insulin receptors. Starvation induces the most prominent increase in receptor number. However, binding varies considerably among the four different tissues and among the four groups of rats. Comparing the four different tissues, it appears that starvation and diabetes induce the largest increase in receptor number in liver, followed by muscle and diaphragm, and then fat.

Insulin Binding

Cross-linking experiments were performed at an insulin concentration of 30 pmol/L and are thought to reflect the relative number of high-affinity insulin receptors. We have tested whether changes in high-affinity receptor number as detected by this method parallel true changes in the number of insulin receptors on the cell surface. This was accomplished by measuring insulin binding to intact epididymal adipocytes isolated by collagenase treatment from control, starved, and S diabetic rats, as previously described.[19] Insulin binding was now performed at three concentrations, ie, at 30 pmol/L as in the cross-linking experiments, at 10 nmol/L as used in the autophosphorylation assays, and at 1.5 μmol/L to determine nonspecific binding. To this extent, 10% adipocytes vol/vol (cell concentration, ±5 × 10^5 cells/mL) were incubated in duplicate in Krebs-Ringer buffer supplemented with 30 pmol/L [^{125}I]insulin and 0, 10 nmol/L, or 1.5 μmol/L unlabeled insulin. The data were corrected for nonspecific binding and are presented as the specific cell-bound fraction (Table 3). From the control to the starved and diabetic state, the same change in insulin binding occurs at both insulin concentrations (30 pmol/L and 10 nmol/L; ie, 1.40-fold *v* 1.53-fold in starved rats and 1.37-fold *v* 1.39-fold in diabetic rats), which

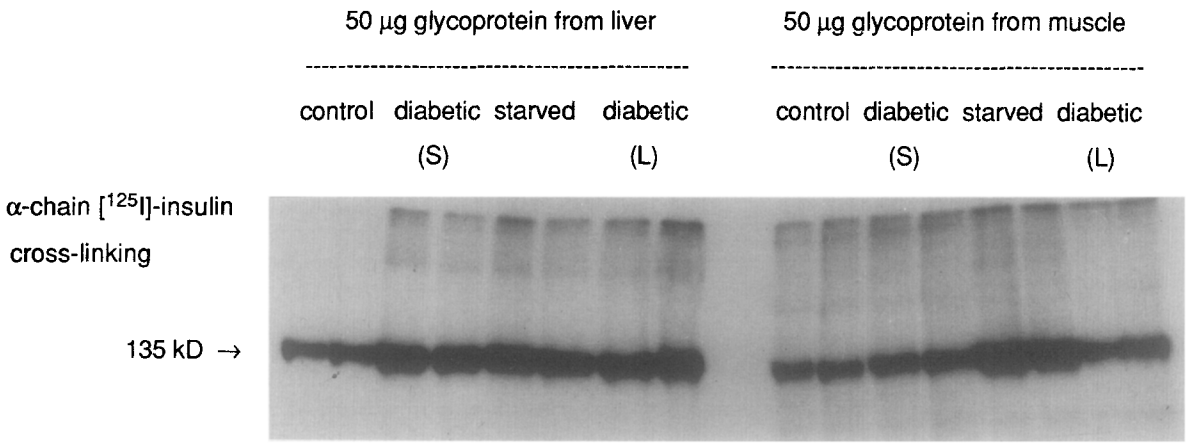

Fig 1. Changes in relative number of insulin receptors in glycoprotein from liver and anterior tibial muscle from control, S diabetic, starved, and L diabetic rats. Relative number of receptors is determined by cross-linking of [^{125}I]insulin to the receptor α-chain. α-Chain labeling is visualized by SDS-PAGE and autoradiography. Representative experiment is shown.

Table 2. Affinity Cross-Linking and Autophosphorylation Levels of Insulin Receptors Using Glycoprotein From Anterior Tibial Muscle, Diaphragm, Epididymal Fat, and Liver From Control, Starved, and Streptozotocin-Diabetic Rats

	Skeletal Muscle		Diaphragm		Epididymal Fat		Liver	
	Cross-Linking	Autophos	Cross-Linking	Autophos	Cross-Linking	Autophos	Cross-Linking	Autophos
Control	1	1	1	1	1	1	1	1
Starved	3.0 ± 0.5*	2.3 ± 0.6*	2.4 ± 0.3*	1.8 ± 0.6	2.1 ± 0.3*	1.9 ± 0.2*	3.0 ± 0.4*	2.9 ± 0.4*
S diabetic	1.7 ± 0.2	2.7 ± 0.3*	1.6 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	1.6 ± 0.2	2.4 ± 0.6*	1.8 ± 0.3
L diabetic	1.1 ± 0.1	3.6 ± 0.3*	1.8 ± 0.4	1.5 ± 0.2	1.2 ± 0.2	0.7 ± 0.2	3.2 ± 0.4*	2.9 ± 0.5*

NOTE. Data are normalized to control value of 1 and are the mean ± SEM of duplicate determinations of 3 to 4 independent experiments in control, starved, and diabetic rats.

Abbreviation: Autophos, autophosphorylation of the receptor.

*$P < .05$, significant difference from control value.

indicates that no redistribution between various affinity states in the receptor pool occurs. Besides, binding and cross-linking studies with adipocytes both show a tendency toward increased receptor number and/or affinity in the starved and S diabetic state. These findings indicate that the cross-linking approach reflects the relative number of insulin receptors.

Insulin Receptor Autophosphorylation

Using glycoprotein, autophosphorylation was determined by insulin-stimulated [γ-^{32}P]ATP incorporation in the β-chain of the insulin receptor. β-Chain labeling was visualized by SDS-PAGE and autoradiography. An example of an autoradiograph of muscle is shown in Fig 2. Autoradiographs were analyzed by densitometry, data were normalized to a relative autophosphorylation level of 1 in control rats, and resulting autophosphorylation values are listed in Table 2. Insulin-stimulated autophosphorylation in all four tissues of starved, S diabetic, and L diabetic rats is normal to increased. However, autophosphorylation varies considerably among the four different tissues and among the four groups of rats. Among the four different tissues, starvation and diabetes induce increases in autophosphorylation, which is most prominent in muscle, followed by liver, then diaphragm, and then fat.

When levels of insulin-stimulable receptor autophosphorylation were compared in identical amounts of glycoprotein from control rats, the following relative values were obtained: liver, 1; diaphragm, 5; tibial muscle, 5; and fat, 4. These data show that glycoprotein from muscle tissue and fat contains much higher levels of stimulable insulin receptors than that from liver, which is in agreement with observations reported by Burant et al.[24] They showed that although liver contains 10-fold more insulin receptors than muscle on a tissue-weight basis, the proportion of stimulable receptors (per insulin-binding site) is ±twofold less.

Table 3. Specific Bound Fraction (%) of [^{125}I]insulin to Intact Isolated Epididymal Adipocytes From Control, 3-Day–Starved, and 3-Day Streptozotocin-Diabetic Rats

	Control	Starved	S Diabetic
Specific binding at 30 pmol/L insulin (%)	1.63 ± 0.34	2.29 ± 0.33	2.24 ± 0.23
Increase normalized to control (-fold)	1.00	1.40	1.37
Specific binding at 10 nmol/L insulin (%)	0.38 ± 0.12	0.58 ± 0.13	0.53 ± 0.07
Increase normalized to control (-fold)	1.00	1.53	1.39

NOTE. Specific [^{125}I]insulin binding was expressed per 450,000 cells from 5 control, 5 starved, and 6 diabetic rats. Data are the mean ± SEM.

Intrinsic Insulin Receptor Autophosphorylation

Since we have shown that the cross-linking approach reflects the relative number of insulin receptors, it seems valid to normalize insulin-stimulated autophosphorylation to constant receptor number using our cross-linking data. When the level of insulin-stimulated receptor autophosphorylation is divided by the relative number of insulin receptors (see Table 2 for data to use in calculation), the intrinsic activity of the insulin receptor to transmit the insulin-binding signal can be obtained. This intrinsic autophosphorylation activity of the insulin receptor is constant between tissues of all rats. Only in muscle is a significant increase in intrinsic receptor activity observed in S diabetic (-fold, 1.6 ± 0.1, $P < .05$) and L diabetic (-fold, 3.3 ± 0.5, $P < .05$) rats. In contrast, muscle from starved rats shows no significant change. To determine whether the 3.3-fold increase in intrinsic receptor activity in muscle of L diabetic rats could be caused by the difference in age between 3-month-old control rats and 6-month-old L diabetic rats, we have also isolated glycoprotein from muscle of 6-month-old control rats (body weight, 505 ± 21 g). The relative intrinsic muscle receptor activity of 6-month-old control rats versus 3-month-old control rats was 0.9 ± 0.2, which is not significantly different.

DISCUSSION

The present study compares four different tissues from four groups of rats each characterized by a different metabolic status. Differences in the study protocol (ie, method of receptor isolation and experimental kinase conditions) were thereby avoided and possible tissue-specific changes in receptor binding and autophosphorylation could be detected.

The effect of two different catabolic conditions, ie, starvation and diabetes, on insulin receptor function was compared. Both starvation and diabetes are characterized by changes in insulin action and insulinopenia, but starvation is accompanied by hypoglycemia and diabetes by hyperglycemia. In addition, we determined the effect of

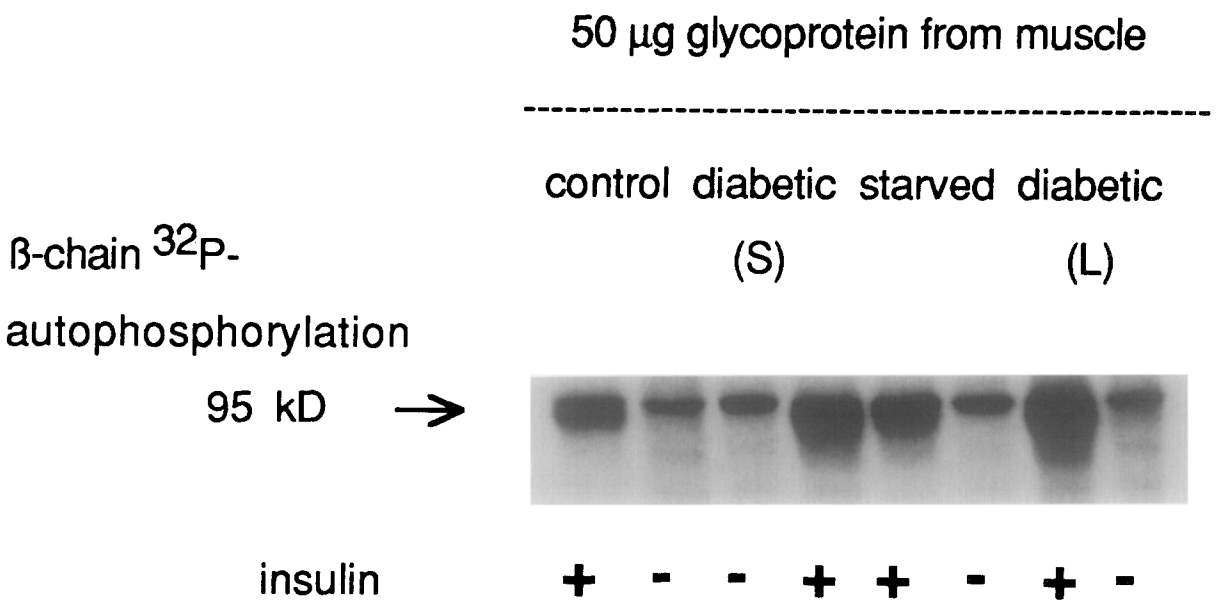

Fig 2. Functionality of the insulin receptor in glycoprotein from anterior tibial muscle from control, S diabetic, starved, and L diabetic rats. Insulin receptor functionality is reflected by insulin-stimulated autophosphorylation of the insulin receptor β-chain. 0 or 10 nmol/L insulin was used for stimulation of autophosphorylation. Receptor was immunoprecipitated, and β-chain labeling is visualized by SDS-PAGE and autoradiography. Representative experiment is shown.

acute and chronic diabetes on receptor function by using 3-day (S) and 6-month (L) diabetic rats.

Insulin receptor autophosphorylation is an early step in insulin action and reflects a process that occurs also in vivo. The magnitude of the autophosphorylation signal reflects the overall activity of the insulin receptor. Our study shows that autophosphorylation of the insulin receptor is increased or unchanged during starvation and diabetes in the various tissues.

When changes of in vivo insulin action are compared with changes of insulin receptor function observed in this study in starved and diabetic rats, it is clear that tissue-specific changes in insulin action and insulin receptor function do not correlate. Data on insulin action[2,3] and insulin receptor status (this study) during starvation and diabetes are listed in Table 4. In these previous studies,[2,3] we used the same experimental rats (ie, 3 days of starvation and diabetes) as used in the present study. From the data in Table 4 it can be concluded that the changes in insulin action are at least partially caused by postreceptor processes. This is indicated most strongly in diabetic rats, where the number and functionality of the receptor are increased but insulin action is decreased. The observation that no decrease in insulin receptor tyrosine kinase is observed in the various tissues under conditions of insulin resistance argues in favor of involvement of a postreceptor component in the development of insulin resistance. Recently, two reports have been published that also describe preserved insulin receptor function and activity in rat models of hypoinsulinemia and insulin resistance.[25,26]

Using our methodology for preparation of glycoprotein fractions, receptors on the cell surface and in intracellular sites are collectively purified. In theory, by preparing plasma membrane fractions, only insulin receptors from the cell surface (which are accessible to insulin and therefore important for signal transduction) can be isolated. We have considered this approach. However, in practice, a high enrichment of plasma membrane fractions requires long centrifugation steps under conditions where proteolytic activity is difficult to suppress fully. Since the insulin receptor β-chain is very sensitive to proteolysis as compared with the α-chain, one can lose autophosphorylation capacity without affecting insulin binding.

The conclusion of our results is that insulin resistance results from postreceptor alterations. Our experimental approach yields a wrong conclusion only if under the conditions of starvation and diabetes an increased fraction

Table 4. Comparison of Changes in Insulin Action on Glucose Metabolism and Insulin Receptor Status in Peripheral Tissues (muscle), Epididymal Fat, and Liver of 3-Day–Starved and 3-Day Diabetic Rats

	Insulin			
	Sensitivity (ED_{50})	Responsiveness ($\dot{V}max$)	Receptor Number	Receptor Autophosphorylation
Diabetic rats				
Muscle	→	↓	↑	↑
Fat	↑	↓	→	→
Liver	↓	→	↑	→
Starved rats				
Muscle	↑	→	↑	↑
Fat	↑	↓	↑	↑
Liver	→	→	↑	↑

NOTE. In muscle and fat, insulin sensitivity and responsiveness are determined for glucose uptake, and in liver, for glucose production (from Wieringa and Krans[2] and Koopmans et al[3]). Insulin receptor number and autophosphorylation are from Table 2. Only statistically significant changes are indicated by ↑ or ↓.

Abbreviations: ED_{50}, medial effective dose; $\dot{V}max$, maximum rate.

of the receptor population is internalized as compared with the control. Since receptor internalization is stimulated by high insulin concentrations, which is not the situation in diabetic and starved states, we believe that an increased receptor internalization in diabetic and starved states is unlikely. This assumption is corroborated by our data on direct insulin binding to adipocytes.

In this study, observed changes in autophosphorylation activity can result from changes in receptor number or changes in intrinsic activity. By determining the relative number of insulin receptors by affinity cross-linking and in the case of adipose cells by insulin binding, we observed that increases in autophosphorylation mostly paralleled increases in receptor number. This observation indicates that the intrinsic autophosphorylation activity remains constant. So even if we normalize the data on autophosphorylation for receptor number, the changes in insulin action[2,3] do not correlate with receptor function. In addition, insulin resistance cannot be explained by reduced receptor functionality. Even if a slight decrease in receptor autophosphorylation per receptor were to exist, this would be of limited biological significance for the development of insulin resistance, since receptor number is in general increased in our rat models. Therefore, at physiologic insulin concentrations, the increase in receptor number would increase insulin sensitivity and this would most likely offset the effect of a slight reduction in receptor autophosphorylation, when present. Only at high insulin concentrations would a decrease in receptor autophosphorylation become evident and have biological relevance. Similar conclusions have been reached by Saad et al,[27] who studied the regulation of insulin receptor substrate-1 in liver and muscle of animal models of insulin resistance.

Our study shows that in the case of skeletal muscle, insulin-stimulated intrinsic receptor autophosphorylation is increased during acute diabetes and chronic diabetes, whereas it is unchanged during starvation. From this it is concluded that diabetes-induced changes in muscle receptor function are established after a short period and do not change when diabetes exists for a longer period. In addition, similar catabolic conditions like starvation and diabetes each can have a different impact on insulin receptor function. There was no change in levels of counterregulatory hormones during starvation and diabetes.[3] These hormones therefore seem not to be responsible for this discrepancy in receptor function between starvation and diabetes. It is tempting to speculate that the level of glycemia might influence autophosphorylation activity of the receptor. Recently, it has been shown that hyperglycemia increased skeletal muscle insulin receptor kinase activity.[28]

Our results show that body tissues undergo specific changes during starvation and diabetes according to their distinctive morphologic, metabolic, and functional characteristics. Although the hormonal and metabolic environment within an experimental animal is in general similar and comparable for all the tissues (with the exception of the liver, which is in general exposed to higher hormonal and substrate levels), each tissue has different reactive patterns to these hormonal and metabolic changes. The previously reported differences in receptor function between muscles[29-31] may explain the differences we have observed in receptor function between diaphragm and tibial muscle during starvation and diabetes. Tibial muscle consists of one third fast glycolytic fibers and two thirds fast glycolytic-oxidative fibers, whereas diaphragm consists of a mixture of fast glycolytic, fast glycolytic-oxidative, and slow oxidative fibers.[30] Furthermore, the function of both muscles is different. Diaphragm is a constantly working muscle, and the activity of tibial muscle depends on physical exercise.

In our study, both insulin receptor number and autophosphorylation are upregulated when expressed per microgram glycoprotein prepared from the different tissues. Since starved, S diabetic, and L diabetic rats are characterized by a (relative) hypoinsulinemia, the upregulation of insulin receptors in several tissues is well described[32,33] when they are exposed to low insulin concentrations.[34] However, for receptor autophosphorylation this is a rather new phenomenon. Recently, it was shown that chronic hyperinsulinemia leads to downregulation of receptor autophosphorylation.[35] This would fit with the idea that a long-term change in insulinemia is inversely correlated with insulin-stimulated receptor autophosphorylation.

In summary, in all the studied tissues of starved and diabetic rats, insulin-stimulated receptor autophosphorylation is normal to increased (skeletal muscle > liver > diaphragm > fat), and these changes in receptor functionality do not correlate with changes in insulin action on glucose metabolism in muscle, fat, and liver of starved and diabetic rats. This indicates that insulin resistance for glucose metabolism, when present in starved and diabetic rats, is due to postreceptor defects rather than to decreased functionality of the receptor.

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