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PRELIMINARY REPORT

# Expression of the Two Insulin Receptor Isoforms Is Not Altered in the Skeletal Muscle and Liver of Diabetic Rats

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**Alternative splicing of the 36–base pair exon 11 of the human insulin receptor (IR) gene and of the corresponding domain of the rat IR gene results in the synthesis of two IR isoforms with distinct functional characteristics. Altered expression of these IR isoforms has been previously demonstrated in the skeletal muscle of patients with non–insulin-dependent diabetes mellitus (NIDDM); however, this observation was not confirmed by other studies and is still a matter of debate. To assess whether the reported altered isoform expression is due to the secondary metabolic derangement of diabetes, we examined alternative splicing of IR mRNAs (IR36$^+$ and IR36$^-$, corresponding to human Ex11$^+$ and Ex11$^-$) in the skeletal muscle and liver of 6-hour fasting 90% pancreatectomized insulin-resistant diabetic and control Sprague-Dawley rats, using the reverse transcriptase–polymerase chain reaction (PCR) technique. Both diabetic and control rats showed the same pattern of IR mRNA expression: the liver exclusively expressed IR36$^+$ mRNA, whereas only IR36$^-$ mRNA was detected in muscle. In conclusion, diabetes mellitus per se does not alter the expression of IR isoforms in the liver and skeletal muscle, and therefore, at least in this animal model of NIDDM, impaired insulin action develops independently from a relative increase in IR36$^+$ mRNA expression in skeletal muscle.**



THE HUMAN INSULIN RECEPTOR (IR) is an $\alpha_2\beta_2$-disulfide–linked tetrameric glycoprotein located in the plasma membrane of target cells.[1-4] It exists in two isoforms that either contain (isoform Ex11$^+$, or B) or lack (isoform Ex11$^-$, or A) a 12–amino acid sequence at the carboxy terminus of the α-subunit. These two receptor isoforms are generated by tissue-specific alternative splicing of the 36–base pair exon 11.[5-8] Recently, the rat liver IR cDNA was cloned and sequenced, and its deduced amino acid sequence had 95.2% identity with the human IR precursor[9]; in addition, the same splicing pattern of sequence homologs to exon 11 was reported to be highly conserved.[9,10] These data, together with the reported functional differences between the two receptor isoforms,[8,11-13] suggest that IR mRNA alternative splicing may play an important role in the maintenance of glucose homeostasis, and that its alteration, especially in skeletal muscle, may contribute to the development of insulin resistance. In this regard, it has been recently demonstrated at both the mRNA and the protein level that the skeletal muscle[14-16] and adipocytes[17] of patients with non–insulin-dependent diabetes mellitus (NIDDM) express an increased amount of the Ex11$^+$ isoform compared with nondiabetic control levels. However, these observations were not confirmed by other studies[7,18,19] and are still a matter of debate. A possible explanation for this discrepancy may be related to differences in plasma glucose and/or insulin and in general to the metabolic status of NIDDM patients in these studies. Furthermore, it is not known whether the reported alternative splicing alteration represents a genetic trait or is inducible by the metabolic consequences of diabetes mellitus. To test these two hypotheses, we studied skeletal muscle and liver expression of the two IR isoforms in a peculiar rodent

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model of NIDDM (90% pancreatectomized diabetic rats) characterized by chronic hyperglycemia, insulin resistance, and reduced β-cell response to a glucose load, but with normal fasting plasma insulin and normal growth.[20-28]

## MATERIALS AND METHODS

### *Animals*

Two groups of male Sprague-Dawley rats (Charles River, Wilmington, MA) were studied: 90% partially panceatectomized diabetic rats (n = 5) and control rats (n = 5). At 3 to 4 weeks of age, the first group (80 to 100 g) underwent 90% pancreatectomy as previously described.[20] Within 2 weeks after removal of 90% of the pancreas, these rats usually develop fasting hyperglycemia and moderately severe glucose intolerance; both conditions induce a state of "glucose toxicity"[25] with both impaired first-phase insulin secretion of the remaining pancreatic tissue and impaired insulin sensitivity,[21] thus closely resembling human NIDDM. Insulin resistance in these animals is characterized by reduced glycogen synthase activity,[22] reduced glucose transport/phosphorylation,[23] and reduced GLUT4 protein levels and translocation.[24]

Rats were weighed twice weekly, and blood was drawn simultaneously from the tail vein for determination of fed plasma glucose concentrations. Fasting plasma glucose was also determined weekly on tail vein blood. Diabetic animals were included in the study only with a fed plasma glucose greater than 15 mmol/L on three consecutive occasions. These animals, which represent a well-characterized model of NIDDM,[20-28] grow normally and therefore can be compared with control rats matched both by age and by weight. The animals were fed ad libitum and kept in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals.*

Six weeks after pancreatectomy was performed, vein blood was collected in 6-hour fasting diabetic and matched control rats for determination of plasma glucose and insulin concentrations. Later, the rats were anesthetized with an injection of phenobarbital (60 mg/kg body weight intravenously), the leg muscles were quickly exposed and the abdomen was opened, and the quadriceps muscles and liver were freeze-clamped between aluminium tongs precooled in liquid nitrogen and then stored at −80°C before mRNA purification. The heart was also removed for a separate study protocol. The plasma glucose level was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA), and plasma insulin by radioimmunoassay using rat insulin standard (Novo Nordisk, Bagsvaerd, Denmark).

### *RNA Preparation*

Quadriceps muscle and liver specimens (~200 mg each) were pulverized in liquid nitrogen. Poly(A)$^+$ RNA was isolated using the Micro-Fast Track mRNA isolation kit (Invitrogen, San Diego, CA) according to the manufacturer's protocol.

### *cDNA Synthesis and Polymerase Chain Reaction Amplification*

First-strand cDNA synthesis was performed on 2 to 4 μg Poly(A)$^+$ RNA using M-MLV reverse transcriptase and random hexamer primers in a total volume of 20 μL of 50 mmol/L KCl, 10 mmol/L Tris hydrochloride, pH 8.3, 5 mmol/L MgCl, and 1 mmol/L deoxynucleoside triphosphates.

The entire 20-μL cDNA-synthesis reaction volume was combined in a 100-μL final reaction volume for polymerase chain reaction (PCR) amplification containing 0.2 μmol/L oligonucleotide primers spanning nucleotides 2181 to 2202 (5′-TTCATTCAGGAAGACCTTCGA-3′) and 2417 to 2438 (5′-AGGCCAGAGATGACAAGTGAC-3′) of the rat liver IR cDNA sequence[9] and 2.5 U Taq DNA polymerase. PCR amplification was performed for 25 cycles of 20 seconds at 96°C, 30 seconds at 58°C, and 1.5 minutes at 72°C using a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). Products of PCR amplification were resolved by electrophoresis on 20% polyacrylamide gels. The gels were silver-stained, and band density was quantified by laser-scanning densitometry (Hoefer, San Francisco, CA). Alternatively spliced IR mRNA transcripts were measured twice in each muscle and liver sample.

### *Reagents*

M-MLV reverse transcriptase was purchased from GIBCO-BRL (Gaithersburg, MD), random hexamer primers were from Pharmacia (Uppsala, Sweden), and Taq DNA polymerase (AmpliTaq) and deoxynucleoside triphosphates were from Perkin-Elmer Cetus (Norwalk, CT). All other reagents were from Sigma (St Louis, MO).

## RESULTS AND DISCUSSION

Clinical characteristics of the animals studied are presented in Table 1. Six-hour fasting plasma glucose was significantly higher in diabetic compared with control rats, whereas 6-hour fasting plasma insulin was similar (Table 1).

Figure 1 shows the electrophoretic analysis of the fragments representing isoforms IR36$^+$ (258 base pairs) and IR36$^-$ (222 base pairs) obtained by PCR amplification of cDNA derived from skeletal muscle and liver mRNAs of both groups using oligonucleotide primers flanking the corresponding rat domain of the exon 11 splice site of the IR sequence. Both diabetic and control rats showed the same pattern of IR mRNA expression: the liver exclusively expressed IR36$^+$ mRNA, whereas the muscle expressed only IR36$^-$ mRNA.

Since insulinopenic diabetes, such as streptozotocin (STZ)-induced diabetes, causes profound alterations in IR function[29-32] and gene transcription,[33] we selected a well-studied[20-28] rodent model of diabetes mellitus characterized by severe hyperglycemia but with normal fasting plasma insulin and normal growth (the two groups of rats studied were age- and weight-matched). Therefore, we avoided the confounding effects of either insulinopenia (eg, STZ-treated rats) or obesity (eg, genetic rat models of NIDDM).

In the present study, we demonstrated that chronic hyperglycemia associated with normal fasting plasma insulin concentrations does not alter the relative mRNA expression of IR isoforms in the liver and skeletal muscle. Interestingly, similar results were also obtained in STZ-diabetic rats[34]; however, it is likely that the full effect of insulinopenia had not yet been established in that study, as suggested by the reported decrease of skeletal muscle IR mRNA, which contradicts the well-documented IR upregulation in STZ-rats.[32] Unfortunately, neither the insulin concentration nor the duration of diabetes were reported.[34]

For insulinoma patients, we recently reported a positive correlation between the Ex11$^+$/Ex11$^-$ (IR36$^+$/IR36$^-$) mRNA

**Table 1. General Characteristics of the Control and Diabetic Rats**

| Characteristic | Control | Diabetic |
|---|---|---|
| No. of animals | 5 | 5 |
| Body weight (g) | 271 ± 6 | 276 ± 8 |
| Age (d) | 55 ± 3 | 58 ± 2 |
| 6-h fasting plasma glucose (mmol/L) | 7.3 ± 1.4 | 19.2 ± 1.8* |
| 6-h fasting plasma insulin (pmol/L) | 188 ± 23 | 204 ± 34 |

NOTE. Values are the mean ± SEM.
*$P < .01$ *v* control rats (2-tailed).

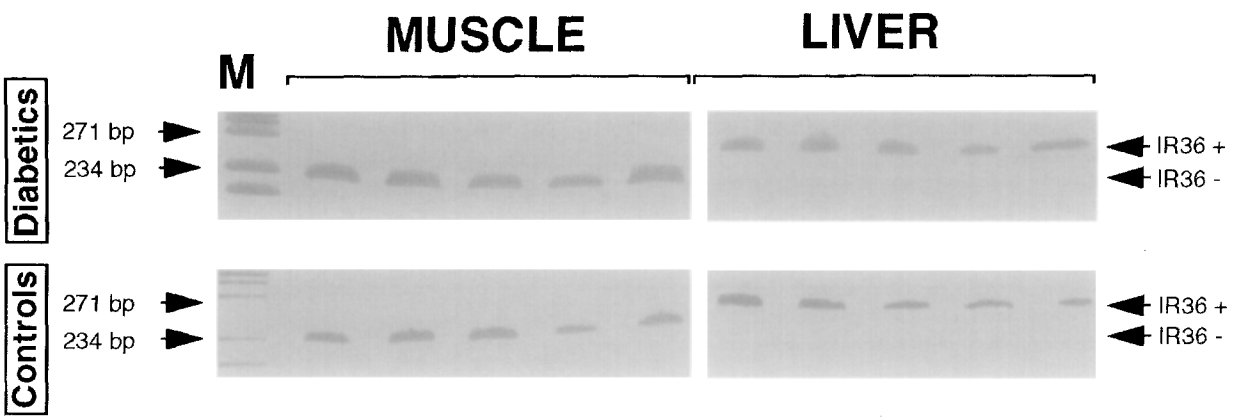


**Fig 1. Reverse transcriptase–PCR amplification of alternatively spliced IR mRNA transcripts in skeletal muscle and liver of control and diabetic rats. Silver-stained nondenaturing 20% polyacrylamide gel shows the 258– and 222–base pair (bp) DNA fragments generated by PCR amplification of the 2 (IR36$^+$ and IR36$^-$) IR cDNA species. *Hae*III-digested ΦX174 DNA molecular size markers (M) are shown in the first lane.**

isoform ratio in skeletal muscle and the plasma insulin concentration.[35] Furthermore, an enhanced relative expression of the Ex11$^+$ mRNA isoform was recently reported in FAO rat hepatoma cells after incubation with insulin,[36] as well as in skeletal muscle of a diabetic patient in poor metabolic control after initiation of insulin treatment.[37] We therefore believe that while insulin may regulate its own receptor differential isoform expression, plasma glucose levels are completely devoid of any regulatory effect. Taken together, these findings may explain the discordant data reported by different groups examining receptor isoform distribution in diabetic patients.[7,14-19] In fact, overtly diabetic patients with fasting hyperglycemia and low plasma insulin levels could have normal IR isoform expression, whereas patients with mild diabetes and compensatory hyperinsulinemia could display an altered ratio of the two IR isoforms.

In conclusion, our data demonstrate that diabetes mellitus per se does not alter IR mRNA isoform expression in the liver and skeletal muscle of diabetic rats. Therefore, at least in this animal model of NIDDM, impaired insulin action develops independently from a relative increase of IR36$^+$ mRNA expression in skeletal muscle.

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