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HETEROGENEITY OF HUMAN LIVER, MUSCLE, AND ADIPOSE TISSUE INSULIN RECEPTOR

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SUMMARY: We have studied the structure and function of the human insulin receptor in liver, skeletal muscle and adipose tissue. The α-subunit of the insulin receptor for liver, muscle and adipose tissue migrated on SDS-PAGE with Mrs 137632 ±216, 134034 ±1080, and 133575 ±165, respectively ($p < 0.05$). Treatment of these receptors with neuraminidase decreased their molecule sizes and eliminated the relative size differences between the receptors. Three monoclonal antibodies ($5A_1$, $10D_9$, and $20H_3$), directed towards different epitopes of the human insulin receptor α-subunit were used to probe immunological differences among the receptors. Antibodies $5A_1$ and $20H_3$ recognized all the receptors, whereas $10D_9$ recognized muscle and adipose tissue receptors but not liver receptors. The mobility of insulin receptor β-subunit in the absence of insulin was the same in all tissues with a similar phosphorylation-induced decrease in mobility in SDS-PAGE in the presence of insulin. However, insulin stimulated autophosphorylation per receptor was different being greatest ($p < 0.05$) in muscle (334±104 ^{32}P cpm) and similar in adipose tissue (114±10) and liver (183±68). These studies indicate, therefore, that the human insulin receptor is heterogeneous among the major target tissues for insulin, and raise the possibility that this heterogeneity may account for tissues' specific differences in insulin's biological messages.

The insulin receptor is a tetrameric glycoprotein located on the surface of target cells which serves both to concentrate insulin onto the cell and to transmit the insulin signal. The insulin receptor consists of two identical α-subunits, which are extracellular and bind the hormone, and two identical β-subunits, which are transmembrane and contain tyrosine kinase activity in their cytoplasmic domain. When insulin binds to the α-subunit of the receptor, tyrosine kinase activity is activated on the β-subunit. This activation of tyrosine kinase activity is believed to mediate many if not all the actions of the hormone.

Because of the pivotal role of the insulin receptor in mediating insulin action, we have recently studied the structure and function of the insulin receptor in liver (1), muscle (2), and adipose tissue (3) from normal and obese patients with and without Type II diabetes. Insulin receptor kinase activity expressed per insulin receptor content was significantly decreased in the liver and adipose tissue, but

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was unchanged in muscle from obese diabetics when compared with obese non-diabetics. These tissue-specific differences suggested heterogeneity of the human insulin receptor.

In our previous studies we did not observe major differences in the electrophoretic mobility of the α- and β-subunits of the insulin receptors either among different patients or among different tissues (1,2,3). However, we did not study in parallel different tissues from the same patients and, therefore, differences among tissues were not apparent. The goal of the present study, therefore, was to search for structural and functional differences among insulin receptors from liver, muscle, and adipose tissue in normal man. Such information should be useful to understand normal tissue-specific functions of insulin pathophysiology in man.

MATERIALS AND METHODS

Materials: Carrier-free Na ^{125}I and [γ-^{32}P]-ATP (tetra-trielthyl ammonium salt; 1000-3000 Ci/mmol) were purchased from New England Nuclear, Boston , MA. Wheat germ agglutinin (WGA)-sepharose was purchased from Miles Laboratories, Elkhart, IN, and the reagents for polyacrylamide gel electrophoresis from Bio-Rad, 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.

Insulin Receptor Preparation: Intraoperative liver (1-2 gm), muscle (1-3 gm), and adipose tissue (5-10 gm) biopsies were obtained from 3 patients undergoing elective cholecystectomy and 3 brain-dead organ donors who did not have any liver disease or any known metabolic diseases. Written consent was obtained from all patients after they were informed about the nature and potential risk of the study. The tissue specimens were immediately frozen between aluminum tongs precooled in dry ice. Samples were stored frozen at -70° until analyzed. Insulin receptors were solubilized and partially purified by WGA affinity chromatography as previously described for liver (1), muscle (2), and adipose tissue (3).

Studies on the Insulin Receptors Preparation: All the experiments were performed in parallel using receptor preparations from the same patients. Experiments were normalized either by the same amount of protein or the same amount of receptor binding activity as previously described (2) and specified in each experiment. ^{125}I insulin binding and competition experiments were performed with unlabeled insulin and monoclonal antibodies to the insulin receptor (4). Cross-linking of ^{125}I insulin to the α-subunit of the receptor with disuccinimidyl suberate, and autophosphorylation of the β-subunit of the receptors are performed as we have previously described (1-3). Glycosidase digestion of the α-subunit of the receptors was performed with neuraminidase (20 mUnit/ml) as described by Burant *et al.* (5). The apparent Mrs of the labeled bands were calculated by regression analysis of log molecular weight versus the relative mobility of the standard proteins with the understanding that glycoproteins do not migrate according to their true Mrs on SDS-PAGE (5).

Descriptive statistics (mean ± SEM) and comparative statistics (analyses of variance and t tests) were performed using a computerized program (Stat View, the graphic statistics for the McIntosh).

RESULTS

The protein yield per gram of starting frozen tissue was 0.24 ± 0.04 mg, 0.18 ± 0.05 mg, and 0.02 ± 0.001 mg of partially purified insulin receptors for the liver (n = 6), muscle (n = 6), and adipose

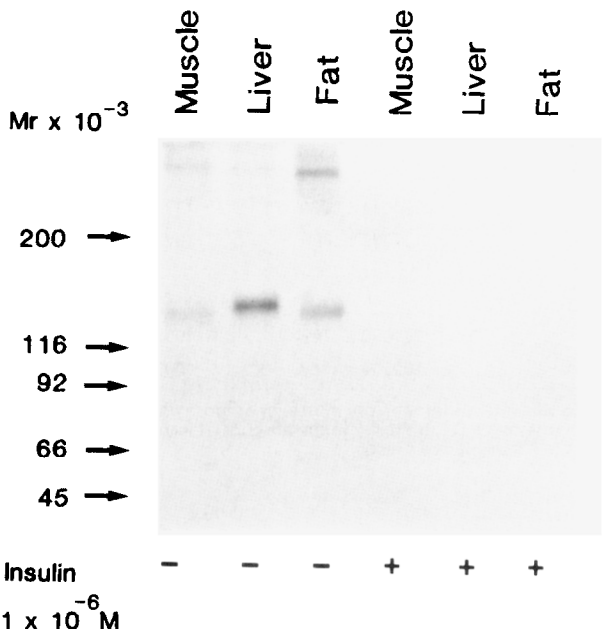

Figure 1. Autoradiography of SDS-PAGE of solubilized insulin receptors from human liver, muscle and adipose tissue. Wheat germ agglutinin purified solubilized insulin receptors were incubated with ^{125}I-insulin (5×10^{-10}M) in the presence and absence of unlabeled insulin (1×10^{-6}M) in a total volume 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 ^{125}I-insulin receptor complex was cross-linked with 0.5 mM disuccinimidyl suberate, immunoprecipitated with polyclonal insulin receptor antibodies, 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.

tissue (n=6), respectively. The specific ^{125}I insulin binding at tracer concentration (1×10^{-10}M) assessed at 4°C at apparent steady state was 8.05 ± 0.75 ng, 8.05 ± 1.62 ng, and 11.20 ± 2.12 ng per mg protein of partially purified receptor for liver, muscle, and adipose tissue, respectively. The apparent affinity of the receptors from the different tissues was similar since increasing concentrations of unlabeled insulin displaced ^{125}I insulin (1×10^{-10}M) binding to a similar degree.

Figure 1 demonstrates the α-subunit of the insulin receptor from muscle, liver, and adipose tissue. The α-subunit of the receptor was identified by covalent coupling to ^{125}I insulin using disuccinimidyl suberate and examination of the reaction product under reduced conditions by SDS-PAGE followed by autoradiography. The radioactivity associated with the α-subunit was immunoprecipitated by polyclonal insulin receptor antibodies (2), and was totally displaced by a large excess of unlabeled insulin supporting the specificity of the band in the three tissues. The apparent molecular weight of the cross-linked α-subunit was significantly greater ($p < 0.05$) in liver receptors (~138 kDa) than in the muscle (~134 kDa) and adipose tissue receptors (~134 kDa). This finding was reproduced in four different experiments. The approximate Mr of α-subunit from these experiments calculated by regression analyses of log molecular weight versus relative mobility of the standard is

Table I - Liver, Muscle and Adipose Tissue Mrs of α- and β-Subunits of Insulin Receptors

Insulin Receptor Subunits	Muscle	Liver	Adipose Tissue
		Mr Daltons	
α-Subunit (n = 4)			
Untreated	134034 ± 1080	137632 ± 216[a]	133575 ± 165
Neuraminidase	133293 ± 690	132875 ± 148	132536 ± 288
β-Subunit (n = 6)			
No Insulin	101930 ± 789[b]	103286 ± 1046[b]	102870 ± 1041[b]
1x10-7M Insulin	103534 ± 1163	104262 ± 1356	103952 ± 1112

[a]Liver α-subunit Mr significantly different from muscle or adipose tissue Mr ($p<0.05$ unpaired t test).
[b]Liver, muscle and adipose tissue β-subunit Mr in the absence of insulin significantly different than in the presence of insulin ($p<0.05$, paired t test).

shown in Table 1. This table also shows that treatment of the insulin receptors with neuraminidase increased the mobility of the α-subunit of the receptor from the three tissues and eliminated the relative size differences among tissues. This finding suggested that differences in sialic acid content are responsible for the changes in molecular weight described.

In figure 2, monoclonal antibodies to the α-subunit of the human insulin receptor were studied. The left upper panel of this figure shows that increasing concentrations of unlabeled insulin inhibited

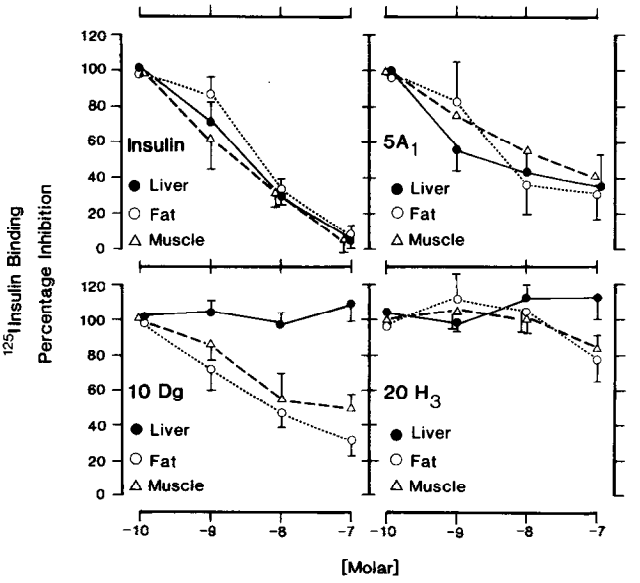

Figure 2. Effect of monoclonal antibodies ($5A_1$, $10D_9$ and $20H_3$) and insulin to competitively inhibit ^{125}I-insulin binding. Wheat germ agglutinin purified solubilized insulin receptors from human liver, muscle and adipose tissue were incubated with ^{125}I insulin ($1x10^{-10}$M) as described in the legend of Figure 1 in the absence and presence of increasing concentrations of insulin or monoclonal antibodies to the human insulin receptor. After incubation for 16 h at 4°C, the receptor and receptor-bound insulin were precipitated with polyethylene glycol (12.5% final concentration) using bovine γ-globulin as carrier protein. the data represent the mean ± SEM from 5 different experiments.

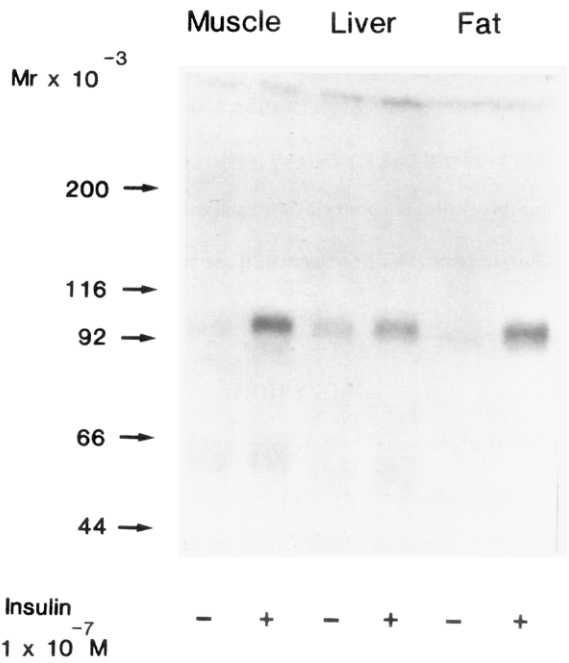

Figure 3. Autoradiogram of insulin-stimulated autophosphorylation of the β-subunit of insulin receptors. Wheat germ agglutinin purified insulin receptors with equal bound insulin from human skeletal muscle, liver and adipose tissue were incubated in the presence and absence of insulin 1×10^{-7}M as described in the legend of Figure 1. Then 100 μM [^{32}P]ATP (20 μCi/tube) was added in the presence of 5 mM $MgCl_2$ and 10 mM $MnCl_2$. After 60 min at 4°C, the reaction was terminated with equal volume of 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 Figure 2.

^{125}I insulin binding equally in all three tissues. The right upper panel also shows that monoclonal antibody $5A_1$ to the human insulin receptor inhibit ^{125}I insulin binding similarly in the 3 tissues. In contrast, as can be seen in the left lower panel, monoclonal antibody $10D_9$ inhibits insulin binding in muscle and adipose tissue but not in liver ($p < 0.01$-0.001). Furthermore, this antibody does not immunoprecipitate the liver receptors. The right lower panel demonstrates that monoclonal antibody $20H_3$ did not inhibit insulin binding in any tissue. However, it immunoprecipitated equally the insulin receptors from the 3 tissues. Interestingly, we have previously shown that monoclonal antibody $20H_3$ inhibits insulin binding in intact human adipocytes (4). The property of this antibody is seen with our polyclonal insulin receptor antibody, which also inhibits insulin binding in intact cells but not in solubilized receptors, but it immunoprecipitates solubilized insulin receptors.

Finally, figure 3 demonstrates that in the 3 tissues, insulin increased the incorporation of ^{32}P from [γ^{32}P] ATP into a protein which is specifically immunoprecipitated by polyclonal insulin receptor antibodies. This finding identifies this protein as the β-subunit of the insulin receptor. This figure and Table I also demonstrate that there are no statistically significant changes in the

electrophoretic mobility of these proteins among the 3 tissues which have an apparent molecular mass of approximately 102.5 kDa in the absence of insulin and 104.0 kDa in the presence of 1 x 10^{7}M insulin. However, a consistent difference among the tissues is that using equal numbers of receptors, the insulin-stimulated β-subunit autophosphorylation is significantly greater ($p < 0.05$) in muscle (334 ± 104 cpm ^{32}P, $n = 6$) than in liver (183 ± 68) or in adipose tissue (114 ± 10).

DISCUSSION

In our previous studies, the functional heterogeneity of insulin receptors from liver (1), muscle (2), and adipose tissue (3) from the same patients with Type II diabetes mellitus lead us to hypothesize that in man, the insulin receptors from these tissues may be different in normal individuals. In the present study, we have demonstrated that the apparent molecular mass of the insulin receptor α-subunit from liver is greater than that from either muscle or adipose tissue, and that these changes appears to be due to different sialic acid content. Furthermore, we have probed these receptors with 3 monoclonal antibodies ($5A_1$, $10D_9$, $20H_3$) directed toward different epitopes of the α-subunits (4) and have clearly demonstrated that monoclonal antibody $10D_9$ failed to recognize the insulin receptor from liver, whereas it does it in muscle and adipose tissue from the same patients. Thus, in normal man, differences in molecular weight, carbohydrate composition and antigenicity exist between insulin receptor α-subunit in liver and the two major peripheral target tissues of insulin. The molecular weight of the insulin receptor β-subunit is similar in the three tissues. However, the insulin-stimulated tyrosyl kinase activity is greater in muscle than in liver or adipose tissue. If this observation is due to different kinetics of the enzyme or different concentration of the type I insulin-like growth factor receptor among the tissues is currently under investigation. Interestingly, in the three tissues phosphorylation of the β-subunit of the receptors induced a small but consistent decrease in mobility in SDS-PAGE, being greater in β-subunit from skeletal muscle which is also the most phosphorylated. The altered mobility of the β-subunit upon phosphorylation in SDS gels could be secondary to a decrease in its ability to bind SDS or to a direct effect of phosphorylation on its tertiary structure (6). Without complete understanding of its mechanism, similar observations have been reported with other proteins. The regulatory subunit of Type II cAMP-dependent protein kinase (7), glycogen synthase kinase (8), the 21 kDa oncogene produce (9), the ovian β-adrenergic receptor (10) and phospholamban (6) have all been reported recently to have a phosphorylation-induced decrease mobility in SDS gels.

Heterogeneity in the molecular weight of the insulin receptor has been previously reported. In rat brain, both the α- (11,12) and the β- (13) subunits have smaller Mrs than in peripheral tissues. The decreased Mr in the α-subunit of brain receptor is due to changes in sialic acid content (12). The β-subunit of endothelial cells is larger than that in a hepatocyte cell line (14). The α- and β-subunit in a monocyte cell line are larger than in IM-9 lymphocytes (15). The α-subunit and β-subunit of rat liver-derived receptor is larger than that from rat muscle (16).

The high affinity of the insulin receptor for its ligand provides the mechanism for target recognition. We have demonstrated that this property is similar in the three major target organs of insulin in man. Following this initial interaction, a cascade of incompletely understood events (17,18) results in a great array of biological functions, some of which are tissue-specific. The tissue-specific differences of insulin receptors in liver versus muscle and adipose tissue (α-subunit molecular weight) and in muscle versus liver and adipose tissue (β-subunit tyrosyl kinase activity) might be involved in the tissue-specific biological functions of insulin under physiological and pathological conditions in man.

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REFERENCES

1. Caro, J.F., O. Ittoop, W.J. Pories, D. Meelheim, E.G. Flickinger, F. Thomas, M. Jenquin, J.F. Silverman, P.G. Khazanie, and M.K. Sinha. (1986) *J. Clin. Invest.* 78:249-258.
2. Caro, J.F., W.J. Pories, E.G. Flickinger, D. Meelheim, O. Ittoop, M. Jenquin, M.K. Sinha, and G.L. Dohm. (1987) *J. Clin. Invest.* 79:1330-1337.
3. Sinha, M.K., W.J. Pories, E.G. Flickinger, D. Meelheim, and J.F. Caro. (1987) *Diabetes* 36:620-625.
4. Forsayeth, J.R., J.F. Caro, M.K. Sinha, B.A. Maddux, and I.D. Goldfine. (1987) *Proc. Nat. Acad. Sci. (USA)* 84:3448-3451.
5. Burant, C.F., M.K. Treutelaar, and M.G. Buse. (1986) *J. Clin. Invest.* 77:260-270.
6. Wegener, and L.R. Jones. (1984) *J. Biol. Chem.* 259:1834-1341.
7. Hofmann, F., J.A. Beavo, P.J. Bechtel, and E.G. Krebs. (1975) *J. Biol. Chem.* 250:7795-7801.
8. Ahmad, Z., A.A. Depaoli-Roach, and P.J. Roach. (1982) *J. Biol. Chem.* 257, 8348-8355.
9. Stadel, J.M., P. Nambi, R.G.L. Sharr, D.F. Sawyer, M.G. Caron, and R.J. Lefkowitz. (1983) *Proc. Natl. Acad. Sci., U.S.A.* 80:3173-3177.
10. Shih, T.Y., M.O. Weeks, H.A. Young, and E.M. Scolnick. (1979) *Virology* 96:64-79.
11. Heidenreich, K.A., N.R. Zahniser, P. Berhanu, D. Brandenburg, and J.M. Olefsky. (1983) *J. Biol. Chem.* 258:8527-8530.
12. Yip, C.C., M.L. Moule, and C.W.T. Yeung. (1980) *Biochem. and Biosphys. Res. Commun.* 96:1671-1678.
13. Rees-Jones, R.W., S.A. Hendricks, M. Quarum, and J. Roth. (1984) *J. Biol. Chem.* 259(6): 3470-3474.
14. Jialal, I., M. Crettaz, H.L. Hachiya, C.R. Kahn, A.C. Moses, S.M. Buzney, and G.L. King. (1986) *Endocrinology* 117:1222-1229.
15. Elduff, A., G. Grunberger, and P. Gorden. (1985) *Diabetes* 34:686-690.
16. Burant, C.F., M.K. Treutelaar, N.E. Blocks, and M.G. Buse. (1986) *J. Biol. Chem.* 261:14361-14364.
17. Espinal, J. (1987) *Nature (Lond)* 328:574-575.
18. Rosen, O.M. (1987) *Science* 237:1452-1458.