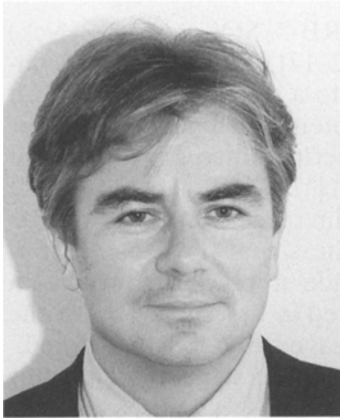
Diabetologia (1994) 37 [Suppl 2]: S 149–S 154

Diabetologia

© Springer-Verlag 1994

Hans-Ulrich Häring
Minkowski Award, 1989, Lisbon



Dr. Häring was born in 1951 in Stuttgart, Germany. He obtained an M.D. in 1976 from the Ludwig-Maximilians Universität, Munich. From 1977 to 1981 Dr. Häring was at the Institute of Diabetes Research in Munich. Between 1982 and 1983 he was a Postdoctoral Fellow at the Joslin Diabetes Research Laboratories, Harvard Medical School, Boston, Mass., USA. From 1983 Dr. Häring has been at the Third Medical Department of the Schwabing City Hospital and Institute for Diabetes Research where he is now Head of Clinical Research. Since 1992 Dr. Häring has been Professor of Internal Medicine, Ludwig-Maximilians Universität. Dr Häring is married and has two children.

Modulation of insulin receptor signalling: significance of altered receptor isoform patterns and mechanism of hyperglycaemia-induced receptor modulation

H.U. Häring, M. Kellerer, L. Mosthaf

Institut für Diabetesforschung, München, Germany

Summary Insulin resistance of the skeletal muscle plays a key role in the development of the metabolic endocrine syndrome and its further progression to non-insulin dependent diabetes (NIDDM). Available data suggest that insulin resistance is caused by an impaired signal from the insulin receptor to the glucose transport system and to glycogen synthase. The impaired response of the insulin receptor tyrosine kinase which is found in NIDDM appears to contribute to the pathogenesis of the signalling defect. The reduced kinase activation is not caused by mutations within the insulin receptor gene. We investigated two potential mechanisms that might be relevant for the abnormal function of the insulin receptor in NIDDM, i.e. changes in the expression of the receptor isoforms and the effect of hyperglycaemia on insulin receptor tyrosine kinase activity. The insulin receptor is expressed in two different isoforms (HIR-A and HIR-B). We found that HIR-B expression in the skeletal muscle is increased in NIDDM. However, the characterisation of the functional properties of HIR-A and HIR-B revealed no difference in their tyrosine kinase activity in vivo. The increased expression of HIR-B might represent a compensatory event. In contrast, hyperglycaemia might directly inhibit insulin-receptor function. We have found that in rat-1 fibroblasts which overexpressing human insulin receptor an inhibition of the tyrosine kinase activity of the receptor may be induced by high glucose levels. This appears to be mediated through acti-

Corresponding author: Dr. H. U. Häring, Institut für Diabetesforschung, Kölner Platz 1, D-80804 München, Germany

Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; NMR, nuclear magnetic resonance; IRS-1, insulin receptor substrate-1; PCR, polymerase chain reaction; PKC, protein kinase C; IRK, insulin-receptor tyrosine kinase.

vation of certain protein kinase C isoforms which form stable complexes with the insulin receptor and modulate the tyrosine kinase activity of the insulin receptor through serine phosphorylation of the receptor beta subunit. This mechanism might also be relevant in human skeletal muscle and contribute to the pathogenesis of insulin resistance. [Diabetologia (1994) 37 [Suppl 2]: S149–S154]

Key words Insulin receptor, skeletal muscle, protein-kinase C, non-insulin-dependent diabetes mellitus.

Non-insulin-dependent diabetes is characterised both by abnormal insulin secretion and by insulin resistance of the major target tissues, i. e. skeletal muscle, liver and fat [1]. There is increasing evidence that the insulin resistance of the skeletal muscle plays a key role in the development of NIDDM [1]. This evidence has been provided by several studies which have shown that the clinical onset of NIDDM is probably preceded by a period in which insulin resistance of the skeletal muscle is detectable [2–4]. Both glucose clamp studies and NMR-studies have shown that the non-oxidative glucose disposal i. e. glycogen synthesis is impaired in pre-diabetes and NIDDM while in NIDDM the oxidative glucose disposal is also reduced [1]. NMR studies suggest that stimulation of glucose transport might also be reduced [4, 5].

The molecular basis of the impaired insulin signal to glycogen synthase and to the glucose transport system is still unclear. As reviewed recently [6] several elements of the insulin signal transducing chain have been intensively investigated as possible candidates for the location of signalling defects. The results can be briefly summarized in the following way: the first element of the signal-transducing chain, the insulin receptor, appears to exhibit normal binding characteristics. However, an impaired activation of the intrinsic tyrosine kinase activity has been found in the skeletal muscle of NIDDM patients. Data concerning the function of the insulin receptor in the pre-diabetic phase are still controversial. While in a Danish pre-diabetic group an impaired tyrosine kinase activity was found [7] another study described normal tyrosine kinase activity in pre-diabetic Pima Indians [8]. Screening studies suggest that mutations of the insulin receptor are extremely rare in NIDDM patients [9–11]. This observation, together with the normal tyrosine kinase activity found in some pre-diabetic populations, suggests that the impaired tyrosine kinase activity found in NIDDM is a secondary event which develops during the transition from the pre-diabetic to the diabetic status. Possible mechanisms for the impaired receptor signal will be discussed later. Knowledge about the molecular events which transmit an insulin signal further downstream from the receptor tyrosine kinase is rapidly increasing. There is convincing evidence that a coupling protein called IRS-1 plays a central role in signal transduction [12]. IRS-1 appears to couple further signalling elements (PI-3 kinase, GRB-2, SOS, ras, SYP, Nck) to the insulin receptor [12–17]. This appears to connect the insulin receptor to a signalling chain which involves GTP-binding proteins such as ras which interact with a cascade of serine kinases including raf and ERKs (MAP-kinases) [12–17]. It is believed that this cascade links the insulin receptor to the glycogen synthase system. While the signal flow to glycogen synthase is now better understood, the downstream signalling elements involved in the signal flow to the glucose transport system have still not been defined.

As regards early downstream signalling elements (IRS-1, GRB-2, SOS, ras, raf, ERK, SYP, Nck) nothing is known about their functional properties in NIDDM. Increased expression [18] and amino acid polymorphisms [19] of IRS-1 have been found in NIDDM patients. The functional significance of this observation is, however, unclear [19]. More data are available on elements which lie further downstream in the signalling cascade to glycogen synthesis, such as phosphatases and glycogen synthase [reviewed in 6, 20]. In summary, these data suggest that glycogen synthase itself is functionally normal. The primary defect in the signal flow which leads to an impaired signal to glycogen synthase is probably located upstream of glycogen synthase but has not yet been identified. However, as outlined above and discussed earlier [6], it appears that the postulated primary signalling defect is further augmented by the development of secondary signalling defects at the receptor level.

In our own studies we have concentrated on the characterisation of possible mechanisms leading to impaired tyrosine kinase activity of the insulin receptor in NIDDM. Two aspects will be discussed here: the importance of altered isoform expression patterns of the insulin receptor in NIDDM and the mechanism of how glucose might modulate the signalling function of the insulin receptor.

The isoforms of the insulin receptor

The human insulin receptor exists in two isoforms which are generated by alternative splicing of a single gene transcript [21–24]. The two receptor isoforms differ by a 12 amino acid peptide at the C-terminal end of the receptor alpha-subunit which is en-

coded by exon 11 of the insulin receptor gene [23]. Expression of the two receptor isoforms seems to occur in a tissue-specific manner [21]. We have characterised the expression pattern of the two receptor isoforms in the skeletal muscle of NIDDM patients and non-diabetic control subjects. Based on RT-PCR-studies we found an altered pattern of receptor isoform expression at the RNA-level in the skeletal muscle [24]. These studies suggested that in non-diabetic skeletal muscle the isoform HIR-A is almost exclusively expressed while in NIDDM skeletal muscle both HIR-A and HIR-B isoforms are expressed [24]. Using PCR-technology, this finding has been reproduced by several investigators, while other groups could not confirm differences between the two groups [25–29]. The controversy is restricted to the results in non-diabetic subjects where some investigators have found both isoforms. As the controversy might be caused by methodological problems related to the PCR technique, we determined the insulin receptor isoform pattern of the skeletal muscle of NIDDM patients at the protein level using antibodies which discriminate between the two insulin receptor isoforms [30]. These data suggested that normal subjects have variable amounts of the isoform B, however, there was a clear shift toward higher levels of HIR-B in the skeletal muscle of NIDDM patients [31]. To elucidate whether this phenomenon, i.e. a tendency toward increased expression of the isoform HIR-B occurs with the onset of NIDDM or whether this phenomenon precedes the onset of the disease we performed PCR-studies in muscle biopsies of insulin-resistant subjects who exhibited the characteristics described for the potentially pre-diabetic situation of the metabolic endocrine syndrome. In these subjects PCR also suggested an increased expression of HIR-B mRNA in the insulin resistant skeletal muscle while predominantly HIR-A mRNA was found in the skeletal muscle of insulin-sensitive individuals [32]. This study demonstrated that the tendency to increased expression of HIR-B is a phenomenon which is linked to insulin resistance of the skeletal muscle. To understand the meaning and the functional consequences of an increased expression of HIR-B, in particular to understand whether this might be a potential cause for the impaired signalling of the receptor in this tissue, we characterised functional properties of the two receptor isoforms in cell culture systems.

Functional characteristics of HIR-A and HIR-B

The functional properties of the two receptor isoforms were characterised in different cell systems. We used rat-1 fibroblasts stably overexpressing HIR-A or HIR-B and 293 cells transiently overexpressing HIR-A or HIR-B. Earlier studies with these cell lines had suggested that the receptor isoforms differ with respect to their binding affinities towards insulin [21]. We studied the activation and autophosphorylation of the tyrosine kinase activity of the receptor [33]. In the in vitro system using isolated insulin receptors HIR-B exhibited an increased tyrosine kinase activity [33]. In contrast, no difference in receptor autophosphorylation was detectable when both receptor isoforms were studied in the intact cell situation [33]. These data suggested that in the solubilized condition the receptor isoform B undergoes a conformational change which favours activation of the tyrosine kinase, however, obviously this modulation of the receptor does not occur when the receptors are embedded in their natural environment in the plasma membrane. Therefore, it is likely that in the intact cell both receptors behave identically with respect to their tyrosine kinase activity. In agreement with this no differences were detectable concerning the downstream signalling steps of the receptors. Both isoforms phosphorylate IRS-1 in a similar manner and induce a comparable translocation and activation of phosphatidylinositol-3-kinase [34, 35]. Furthermore, no differences were detectable with respect to activation of phospholipase C [36], release of phosphatidyl inositol glycanes [37] and further downstream signalling such as activation of 3-O-methyl-glucose uptake [38]. In summary, striking differences in the signalling functions of the two receptor isoforms were not detectable. It is, however, interesting to note that HIR-A and HIR-B behave differently with respect to receptor recycling in rat-1 fibroblasts. While both receptor isoforms are internalized with roughly similar kinetics, a rapid recycling was only observed for isoform A, not for isoform B [39].

At present the physiological significance of the different isoforms is not clear. The differences of the isoform pattern observed in the skeletal muscle in NIDDM offer no explanation for the reduction of the insulin receptor kinase activity found in the skeletal muscle of these patients. It appears that a tendency to increased expression of receptor isoform B might represent a compensatory event for an impaired signal caused by a defect at another level of the insulin signalling chain. It is interesting to note that such putative compensatory up-regulation has also been observed in some NIDDM patients for the glucose transporter GLUT-1 [40] or for signalling elements such as IRS-1 [18].

Glucose-induced inhibition of the insulin receptor

As outlined above partial inactivity of the insulin receptor kinase is found in NIDDM while in the prediabetic situation at least in Pima Indians a normal insulin receptor kinase activity has been reported [8]. Furthermore, there is no evidence for frequent muta-

tions of the receptor in NIDDM patients [9–11]. This suggests that the impaired response of the insulin receptor kinase in NIDDM is a secondary event which might be a consequence of the metabolic derangements which occur after the transition from the prediabetic situation to clinically overt NIDDM. Among the metabolic derangements hyperglycaemia is the most prominent feature. We have investigated whether hyperglycaemia modulates the insulin receptor function. In earlier studies we had shown that insulin receptors isolated from phorbolester or catecholamine-treated rat fat cells or human fat cells displayed a reduced tyrosine kinase activity in vitro [41–43]. However, the reduced tyrosine kinase activity was only observed when the receptors were isolated in the presence of serine phosphatase inhibitors, suggesting that serine phosphorylation of the insulin receptors in the intact cell is proably involved in the reduced tyrosine kinase activity. This concept has recently been applied to study the effect of high glucose on the insulin receptor in rat adipocytes [44]. Insulin receptors isolated from glucose-treated fat cells in the presence of serine phosphatase inhibitors displayed a similar reduction of tyrosine kinase activity in the solubilized condition as has been observed earlier with receptors from phorbol- or catecholamine-treated fat cells. The inhibitory effect of high glucose was not detectable after the addition of PKC inhibitors, suggesting that a serine phosphorylation of the insulin receptor by glucose-activated PKC was responsible for the inhibition [44]. As this reduced tyrosine kinase activity had only been determined in the solubilized condition it was important to analyse whether a relevant glucose inhibition also occurred in the intact cell. Therefore, we studied the effect of hyperglycaemia on the tyrosine kinase activity of rat-1 fibroblasts stably over-expressing the insulin receptor.

Rat-1 fibroblasts stably over-expressing high levels of human insulin receptor were used as a model system to study the effects of hyperglycaemia on IRK activity and PKC translocation in parallel in the intact cell. Glucose (10–25 mmol/l) induced a significant reduction of IRK activity (tyrosine phosphorylation of IR-beta-subunit and IRS-1) within 10 min [45]. This effect was paralleled by a rapid translocation of several PKC isoforms (cPKC alpha, nPKC delta, nPKC epsilon, aPKC zeta) to the plasma membrane within 1 min [45]. Kinetics of IRK inhibition and PKC translocation are consistent with the idea that the glucose effect on IRK is mediated by PKC activation. This hypothesis is supported by further observations: The addition of the PKC inhibitor H-7 can prevent the effect of glucose on IRK [45]. Inhibition of IRK is also seen after stimulation of the cells with the phorbolester 12-O-tetradecanoylphorbol-13-acetate (TPA), which can substitute for a physiological activator of PKC. Glucose (25 mmol/l) increases the [^{32}P] incorporation in serine residues of the β-subunit of IRK [45]. This shows that high glucose levels induce inhibition of IRK in vivo. There is indirect evidence that this effect is mediated by a glucose-induced PKC translocation/activation and serine phosphorylation of the insulin receptor [45].

It is evident that rat-1 fibroblasts over-expressing the human insulin receptor are an artificial system and that results obtained in this system are not necessarily relevant for the situation of the insulin receptor in human skeletal muscle. Data on the effects of acute or chronic hyperglycaemia on human skeletal muscle, the consequences for PKC activation and downregulation and further consequences for the insulin receptor kinase activity are not available at present. However, we were recently able to show that acute hyperglycaemia has a similar effect on rat skeletal muscle as on the rat-1 fibroblast system [46]. We found that acute hyperglycaemia induces a translocation of the PKC isoform beta in rat skeletal muscle. This observation points at least in the direction that PKC regulation by hyperglycaemia is relevant in skeletal muscle.

More indirect evidence for a potential role for this mechanism in the pathogenesis of skeletal muscle insulin resistance in humans is provided by the following observations. An orally administered hypoglycaemic agent (CS045, Troglitazone) which improves insulin resistance in humans is able to prevent the inhibitory effects of hyperglycaemia on insulin receptor function in the rat-1 fibroblast system [47]. Again, this is only indirect evidence and it is clear that further studies in human target tissues are needed to extrapolate the in vitro observations to the situation in patients with hyperglycaemia. To perform these studies in human skeletal muscle, identification of the relevant isoform of PKC and of the regulatory domain of the receptor beta-subunit involved in the receptor modulation through hyperglycaemia is important.

Acknowledgements. We thank L. Berti, E. Capp, P. Galante, Dr. G. Kroder, J. Mushack, E. Seffer and S. Tippmer for their valuable contributions. Furthermore, we wish to thank Drs. A. Ullrich and M. F. White for their constant support and collaboration.

References

1. DeFronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM: a balanced overview. Diabetes Care 15: 318–368
2. Eriksson J, Franssila-Kallunki A, Ekstrand A et al. (1989) Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med 321: 337–343
3. Warram HJ, Martin BH, Krolewski AS, Soeldner JS, Kahn CR (1990) Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic patients. Ann Intern Med 113: 909–915

4. Shulman GI, Magnusson I, Cline GW et al. (1993) Muscle glucose transport/phosphorylation is reduced in subjects with a high risk of developing non insulin dependent diabetes mellitus (NIDDM). Exp Clin Endorinol 101 [Suppl 2]: 306
5. Rothman DL, Shulman RG, Shulman GI (1992) ^{31}P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Invest 89: 1069–1075
6. Häring HU, Mehnert H (1993) Pathogenesis of type II (non-insulin-dependent) diabetes mellitus: candidates for a signal transmitter defect causing insulin resistance of the skeletal muscle. Diabetologia 36: 176–182
7. Handberg A, Vaag A, Vinten J, Beck-Nielsen H (1993) Decreased tyrosine kinase activity in partially purified insulin receptors from muscle of young, non-obese first degree relatives of patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 36: 668–674
8. Bulangu LN, Ossowski VM, Bogardus C, Mott D (1990) Insulin sensitive tyrosine kinase: relationship with in vivo insulin action in humans. Am J Physiol 258: E964-E974
9. O'Rahilly S, Choi WH, Patel P, Turner RC, Flier JS, Moller DE (1991) Detection of mutations in insulin-receptor gene in NIDDM patients by analysis of single-stranded conformation polymorphisms. Diabetes 40: 777–782
10. Kusari J, Verma US, Buse JB, Hanry RR, Olefsky JM (1991) Analysis of the gene sequences of the insulin receptor and the insulin-sensitive glucose transporter (GLUT4) in patients with common-type non-insulin-dependent diabetes mellitus. J Clin Invest 88: 1323–1330
11. Cocozza S, Porcellini A, Riccardi G et al. (1992) NIDDM associated with mutation in tyrosine kinase domain of insulin receptor gene. Diabetes 41: 521–526
12. White MF, Asano T, Backer JM et al. (1993) The new elements of insulin signalling: IRS-1 and proteins with SH2 domains. Exp Clin Endocrinol 101 [Suppl 2]: 98
13. Skolnik EY, Lee C-H, Batzer A, Vicentini LM et al. (1993) The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. EMBO J 12/5: 1929–1936
14. Skolnik EY, Batzer A, Lee C-H et al. (1993) The function of GRB2 in linking the insulin receptor to Ras signaling pathways. Science 260: 1953–1955
15. Baltensperger K, Kozma LM, Chernick AD et al. (1993) Binding of the ras activator son of sevenless to insulin receptor substrate-1 signaling complexes. Science 260: 1950–1952
16. Vojtek AB, Hollenberg SM, Cooper JA (1993) Mammalian ras interacts directly with the serine/threonine kinase raf. Cell 74: 205–214
17. Kuhné MR, Pawson T, Lienhard GE, Feng G-S (1993) The insulin receptor substrate 1 associates with the SH2-containing phosphotyrosine phosphatase Syp. J Biol Chem 268/16: 11479–11481
18. Kellerer M, Mühlhöfer A, Zierath J, Wallberg-Henriksson H, White MF, Häring HU (1993) Expression of IRS-1 and the 85 kDa subunit of PI-3 kinase in skeletal muscle of patients with non-insulin-dependent diabetes mellitus (NIDDM). Exp Clin Endocrinol 101 [Suppl 2]: 300
19. Almind K, Bjorbaek Ch, Vestergaard H, Hansen T, Echwald S, Pedersen O (1993) Aminoacid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. Lancet 342: 828–832
20. Vestergaard H, Lund S, Larsen FS, Bjerrum OJ, Pedersen O (1993) Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin-resistant patients with non-insulin-dependent diabetes mellitus. J Clin Invest 91/6: 2342–2350
21. Mosthaf L, Grako K, Dull TJ, Coussens L, Ullrich A, McClain DA (1990) Functionally distinct insulin receptors generated by tissue-specific alternative splicing. EMBO J 9: 2409–2414
22. Moller DE, Yokota A, Caro JF, Flier JS (1989) Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol 3: 1263–1269
23. Seino S, Bell GI (1989) Alternative splicing of human insulin receptor messenger RNA. Biochem Biophys Res Comm 159: 312–316
24. Mosthaf L, Vogt B, Häring HU, Ullrich A (1991) Altered expression of insulin receptor types A and B in the skeletal muscle of non-insulin-dependent diabetes mellitus patients. Proc Natl Acad Sci USA 88: 4728–4730
25. Sesti G, Marini MA, Tullio AN et al. (1991) Altered expression of the two naturally occurring human insulin receptor variants in isolated adipocytes of non-insulin-dependent diabetes mellitus patients. Biochem Biophys Res Commun 181: 1419–1424
26. Van der Vorm ER, Kuipers A, Krans HMJ, Möller W, Maassen JA (1992) A change in alternative splicing of the insulin receptor gene may contribute to severe insulin resistance in leprechaunism. Diabetes 41 [Suppl]: 54 A (Abstract)
27. Benecke H, Flier JS, Moller DE (1992) Alternatively spliced variants of the insulin receptor protein. Expression in normal and diabetic human tissues. J Clin Invest 89: 2066–2070
28. Hansen T, Bjoerbeak C, Vestergaard H, Bak JF, Pedersen O (1992) Alternatively spliced variants of the insulin receptor and its functional correlates in muscle from patients with type 2 diabetes and normal subjects. Diabetologia 35 [Suppl 1]: A76 (Abstract)
29. Norgren S, Zierath J, Galuska D, Wallberg-Henriksson H, Luthman H (1993) Differences in the ratio of RNA encoding two isoforms of the insulin receptor between control and NIDDM patients. Diabetes 42: 675–681
30. Sesti G, Marini MA, Montemurro A et al. (1992) Two naturally occurring immunologically distinct human insulin receptor α-subunit variants. Diabetes 41: 6–11
31. Kellerer M, Sesti G, Seffer E et al. (1993) Altered patter of insulin receptor isotypes in skeletal muscle membranes of type-2 (non-insulin-dependent) diabetic patients. Diabetologia 36: 628–632
32. Mosthaf L, Eriksson J, Häring HU, Groop L, Widen E, Ullrich A (1993) Insulin receptor isotype expression correlates with risk of non-insulin-dependent diabetes. Proc Natl Acad Sci 90: 2633–2635
33. Kellerer M, Lammers R, Ermel B, Tippmer S, Vogt B, Ullrich A, Häring HU (1992) Distinct α-subunit structures of human insulin receptor A and B-variants determine differences in tyrosine kinase activities. Biochemistry 31: 4588–4596
34. Carrascosa JM, Vogt B, Ullrich A, Häring HU (1991) Activation of phosphatidylinositol-3-kinase by insulin is mediated by both A and B human insulin receptor types. Biochem Biophys Res Commun 174: 123–127
35. Mühlhöfer A, Kellerer M, Berti L et al. (1993) Characterization of IRS-1 interaction with both insulin receptor isoforms. Exp Clin Endocrinol 101 [Suppl 2]: 125
36. Kellerer M, Machicao F, Seffer E, Mushack J, Ullrich A, Häring HU (1991) Stimulation of phospholipase C activity by insulin is mediated by both isotypes of the human insulin receptor. Biochem Biophys Res Commun 181: 566

37. Kellerer M, Machicao F, Berti L et al. (1993) Inositol phospho-oligosaccharides from rat-1 fibroblasts and adipocytes stimulate 3-O-methylglucose transport. Biochem J 295: 699–704
38. Müller HK, Seidl G, Ullrich A, Häring HU (1991) Both insulin receptor isoforms (HIR-A, HIR-B) are able to induce the synthesis of glucose carrier m-RNA in rat-1 fibroblasts. Diabetologia 34 [Suppl]: A 26 (Abstract)
39. Vogt B, Carrascosa JM, Ermel B, Ullrich A, Häring HU (1991) The two isotypes of the human insulin receptors (HIR-A and HIR-B) follow different internalization kinetics. Biochem Biophys Res Commun 177: 1013–1018
40. Vogt B, Mühlbacher C, Carrascosa J et al. (1992) Subcellular distribution of GLUT4 in the skeletal muscle of lean type 2 (non-insulin-dependent) diabetic patients in the basal state. Diabetologia 35: 456–463
41. Häring HU, Kirsch D, Obermaier B, Ermel B, Machicao F (1986) Reduced tyrosine kinase activity of insulin receptor isolated from rat adipocytes rendered insulin resistant by catecholamine treatment in vitro. Biochem J 234: 59–66
42. Häring H, Kirsch D, Obermaier B, Ermel B, Machicao F (1986) Tumor promoting phorbolesters increase the K_m of the ATP-binding site of the insulin receptor kinase from rat adipocytes. J Biol Chem 261: 3869–3875
43. Obermaier B, Kirsch D, Ermel B et al. (1987) Catecholamines and tumour promoting phorbolesters induce insulin resistance in isolated human adipocytes by modulation of the insulin receptor kinase. Diabetologia 30: 93–97
44. Müller HK, Kellerer M, Ermel B et al. (1991) Prevention by protein kinase C inhibitors of glucose-induced insulin-receptor tyrosine kinase resistance in rat fat cells. Diabetes 40: 1440–1447
45. Berti L, Mosthaf L, Kroder G et al. (1994) Glucose induced translocation of protein kinase C isoforms in rat-1 fibroblasts is paralleled by inhibition of the insulin receptor tyrosine kinase. J Biol Chem 269: 3381–3386
46. Galante P, Mosthaf L, Berti L, Häring HU (1993) Translocation of protein kinase C isoforms upon in vivo insulin stimulation of rat skeletal muscle. Diabetologia 36 [Suppl 1]: 165 (Abstract)
47. Kellerer M, Kroder G, Tippmer S, Kiehn R, Mosthaf L, Häring HU (1994) Troglitazone prevents glucose-induced insulin resistance of insulin receptor in rat-1 fibroblasts. Diabetes 43: 447–453