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RECENT DATA ABOUT INSULIN SIGNALING SYSTEM AND INSULIN RESISTANCE STATES

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SUMMARY

• *Insulin is the key hormone regulating glucose homeostasis and has many cellular effects on metabolism, growth, and differentiation. Its action is mediated through a specific cell surface receptor. The first step following insulin binding consists of receptor autophosphorylation and stimulation of its tyrosine kinase activity. Among the multiple substrates the insulin receptor substrate-1 is the major cytoplasmic substrate. It binds several Src homology 2 proteins through its multiple tyrosine phosphorylation sites: phosphatidylinositol 3-kinase, the ras guanine nucle-otide-releasing complex growth factor receptor-boundprotein-2/son of sevenlessprotein, the tyrosine phosphatase Syp, and the adapter protein Nek. Insulin receptor substrate-1 is essential for many, but not all of the insulin biological responses. Recently, a primary alternative substrate, insulin receptor substrate-2, was purified and cloned. Numerous biochemical abnormalities of the insulin signaling system lead to insulin resistance. The recent data about the molecular mechanisms of insulin action may provide new insights into the pathophysiology and therapy of diabetes mellitus and other insulin resistance states.*

INTRODUCTION

• Insulin has been the model polypeptide hormone in many ways, being the first purified, crystallized, and synthesized by chemical and molecular biology techniques. Insulin is the key hormone regulating glucose homeostasis. Its major target tissues are liver, skeletal muscle and adipose tissue. At cellular level, insulin activates glucose and amino acids transport, lipid and glycogen metabolism, protein synthesis, and transcription of specific genes. Its action is mediated by specific plasma membrane receptor. Insulin receptor binding initiates a multitude of effects on metabolism, growth, and differentiation. The insulin receptor is a transmembrane glycoprotein with intrinsic protein tyrosine kinase activity, like the receptors for epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I) etc. It is present in virtually all vertebrate tissues, although the concentration varies from 40 receptors in erythrocytes to more than 200 000 receptors in adipocytes and hepatocytes. The insulin receptor is tetrameric, consisting of two 120 kD oc- and two 90 kD p-subunits. The two a-subunits are disulphide-linked, entirely extracellular, and contain insulin-binding sites. The unoccupied a-subunit inhibits the tyrosine kinase activity of p-subunit. Each p-subunit is disulphide-linked to an ce-subunit, spans the plasma membrane, and contains a tyrosine kinase domain in its intracellular portion. The first step following insulin binding consists of receptor autophosphorylation on multiple specific sites and phosphorylation of cellular substrates. The p-subunit contains several functional regions, including ATP-binding and autophosphorylation sites in the intracellular domain and a regulatory region at the COOH (C-) terminus. The association of insulin with the receptor stimulates its tyrosine kinase activity. The C-terminus may play a regulatory role but is not essential for signaling (1-4). Recent data suggest that the acidic region including residues 1270-1280 of the insulin receptor C-terminus is involved in (/') receptor binding with histone, an exogenous substrate of the receptor kinase, and (/'/) regulation of receptor autophosphorylation and kinase activity. Thus this insulin receptor

domain could interact with cellular proteins modulating the receptor kinase (5). The intracellular domain of the (3-subumt is essential for signal transduction (4).

For a long time it was believed that the effects of insulin are mediated by a unique insulin receptor. However, there is considerable evidence suggesting that insulin receptors in brain, liver, adipocytes, and lymphocytes are heterogeneous in structure and function. This evidence is based on comparisons of concentration-response curves in cells and tissues, and on comparisons of binding and effects of insulin-derivatives and receptor antibodies. Two insulin receptor isoforms (IR-A and IR-B) generated by alternative mRNA splicing were identified but could not fully account for the observed differences in ligand binding and receptor function. It was suggested that the differences in ligand binding reflected posttranslational modifications, and that postreceptor events were responsible for the heterogeneity of insulin action (6).

The insulin receptor also mediates internalization of insulin. At least two pathways for endocytosis exist at varying degrees in all cells. The coated pit/vesicle-mediated pathway requires a functional insulin receptor, triphosphorylation in the regulatory region and intact intracellular domain. Some cells contain a constitutive pathway which does not require receptor autophosphorylation or an intact intracellular region. Insulin action may be mediated by receptor internalization and interaction with its substrate(s) associated with endomembranes. A small fraction of phosphorylated insulin receptors is sufficient for signal transduction (7). pp!20/HA4 is a hepatocyte membrane glycoprotein phosphorylated by the insulin receptor tyrosine kinase, which is part of a protein complex required for receptor-mediated internalization of insulin. It is possible that this function is regulated by insulin-induced phosphorylation of the intracellular domain of pp!20/HA4 (8).

INSULIN RECEPTOR SUBSTRATE-1

• Insulin receptor substrate-1 (IRS-1) is the major cytoplasmic substrate of insulin and IGF-I receptors. This was initially detected in insulin-stimulated Fao hepatoma cells by immunoprecipitation with high affinity antiphosphotyrosine antibodies and was originally called pp!85 based on migration during SDS-PAGE. IRS-1 was purified and cloned from several sources. The amino acid sequences of rat, mouse and human IRS-1 defined from the cDNA derived from rat liver, mouse adipocytes, human muscle, and human hepatocellular carcinoma are now known. IRS-1 contains 21 potential tyrosine phosphorylation sites and about 40 potential serine/threonine phosphorylation sites in motifs recognized by various kinases, including 12 ones for casein kinase II, 5 for protein kinase A (PKA), and 16 for PKC. IRS-1 binds several Src homology 2 (SH2) proteins through its multiple tyrosine phosphorylation sites. Each SH2 domain, which consists of approximately 100 amino acids, constitutes a binding site for Tyr(P) within a specific peptide context. Tyr(P)-IRS-1 was found to associate with four SH2 domain proteins through their SH2 domains: phosphatidylinositol 3-kinase (PI,-kinase), the *ras* guanine-nucleotide-releasing complex GRB-2-SOS, the tyrosine phosphatase Syp, and the adapter protein Nek (4,9).

Insulin receptors phosphorylate IRS-1 at the plasma membrane, probably in coated pits. A desensitization mechanism is possible in which the tyrosine-phosphorylated membrane-bound IRS-1 associated with signaling molecules such as PI_3-kinase, is released into the cytoplasm in concert with its serine/threonine phosphorylation (10).

Recently, Sun *et al* (11) purified and cloned 4PS, the major substrate for the interleukin-4 (IL-4) receptor-associated tyrosine kinase in myeloid cells, which had significant structural similarity to IRS-1.

Transgenic mice lacking IRS-1 are resistant to insulin and IGF-1, but exhibit significant residual insulin action which corresponds to the presence of an alternative high molecular weight substrate in liver and muscle. 4PS is the primary alternative substrate, i.e. IRS-2, which plays a major role in physiologic insulin signal transduction *via* both Pl.-kinase activation and GRB-2/SOS association. In IRS- 1-deficient mice, 4PS/IRS-2 provides signal transduction to these two major pathways of insulin signaling (12).

In IRS-1 knockout mice, insulin signals are thought to be mediated by IRS-2 (pp!90), which is an alternative signaling molecule. pp!90 is a 190-kD IRS-1-like protein in simian COS cells, which is slightly larger than IRS-1 (pp!80) of human, rat, and mouse cells. The deduced amino acid sequence of COS pp!90 consists of 1251 amino acids and is 96.4%, 87.9% and 88.7% identical to human, mouse and rat IRS-1, respectively. The COS pp!90 binds to SH2 domains of p85, GRB-2/Ash, and Syp, as do IRS-1. The COS pp!90 may be a simian homologue of IRS-1, but not of IRS-2. Chinese hamster ovary (CHO) cells have not only the IRS-1 gene but also a gene related to the COS pp!90 (13).

IRS-1 pathway may be involved in omithine decarboxylase induction. The absence of a difference in ornithine decarboxylase and PI_3-kinase activity in the regenerating liver between IRS-1-deficient mice and wild-type ones may be related to the compensatory effects of IRS-2/ppl90 (14). IRS-1 is essential for some, if not all, of insulin biological responses. Major insulin signals such as stimulation of glucose uptake and DNA synthesis and modification of hexose metabolism are mediated by the tyrosine-phosphorylated IRS-1 in many cell species (4,10). However, several studies suggest that some insu-

lin effects, including those in the nucleus, may not be regulated by this pathway. Different mechanisms are involved in induction of *c-fos* and early-growth-regulatory-1 *(egr-l)* mRNA expression by insulin, the former by the more classic insulin receptor tyrosine kinase pathway and the latter by a not well-defined alternative signal transduction pathway (15). IRS-1 tyrosine phosphorylation is sufficient to increase the mitogenic response to insulin, whereas insulin stimulation of glycogen synthesis appears to involve other factors (16).

IRS-1 discriminates the signals generated by the insulin receptor and other receptor tyrosine kinases by binding and regulating a specific subset of SH2 domain-containing signaling molecules. For example, the receptors for insulin and PDGF are tyrosine kinases that mediate distinct effects in identical cellular backgrounds. Each receptor engages an unique subset of the signaling elements available, at least partly through the selection of proteins with SH2 proteins. Autophosphorylation sites in the PDGF receptors (PDGFR) directly bind SH2 proteins, whereas activation of the insulin receptor leads to phosphorylation of IRS-1, which in turn binds SH2 proteins. In HIR 3.5 cells, which contain similar numbers of PDGF and insulin receptors, insulin, but not PDGF, stimulates tyrosyl phosphorylation of IRS-1. Similarly, insulin, but not PDGF, treatment of HTR 3.5 stimulates the association of IRS-1 with PI,-kinase, although PDGF stimulates the association of PI_3-kinase with the tyrosine-phosphorylated PDGFR. Association with IRS-1 activates PI„-kinase more effectively than that with the PDGFR. Whereas the PDGFR associate with PI_3-kinase, *ras-GAP,* GRB-2, and phospholipase Cy, only GRB-2 andPI_3-kinase associates with IRS-1. Moreover, PDGF, but not insulin, caused tyrosine phosphorylation of phospholipase Cy in HIR 3.5 ceUs. Thus the insulin signal differs from that of PDGF by the insertion of a cytosolic, nonreceptor SH2 domain docking protein (IRS-1). Furthermore, ERS-1 binds another subset of SH2 domain-containing proteins than does the PDGFR and regulates at least one common element (PI,-kinase) differently than the PDGFR (17).

In contrast, IRS-1 or DR.S-2 phosphorylation can converge signal pathways from the insulin tyrosine kinase receptor and others that do not contain an intrinsic tyrosine kinase domain (IL-4 and growth hormone [GH] receptors). IL-4 receptor activates PI,-kinase *via* IRS-1 or the IRS-1-related molecule 4PS. In primary adipocytes, tyrosine phosphorylation of Janus tyrosine kinase-2 (JAK-2) and GH receptor seems to be involved in the signal transduction leading to insulin-like effects of GH. Importantly, the signal pathways for GH and insulin clearly differ at the receptor level, but seem to converge at or before the level of IRS-1 or IRS-2 phosphorylation that occurs in response to both hormones (18).

Other signaling pathways may also be involved in the mediation of the insulin effects. PKC-oc may be involved in the insulin induced *egr-l* expression and cell proliferation, but not in the insulin suppressed hepatitis B surface antigen (HBsAg) gene expression in human hepatoma cells (19).

- **Phosphatidylinositol 3-kinase**

Many growth factors, including PDGF, insulin and IL-4, stimulate the phosphorylation of phosphoinositides on 3-position of D-myo-inosotiol ring by activating PI_3-kinase, the product of the VPS34 gene. PI,-kinase is the first SH2 protein associated with IRS-1. PI,-kinase is composed of two subunits, a 110 kD catalytic (pi 10) subunit and an 85kD regulatory (p85ot) one, which possesses two SH2 domains. At least two isoforms of p85 are known (p85a and p85[3). p85a is higher expressed and functionally better characterized. Insulin stimulates PI_3-kinase *in vivo* through interaction between the SH2 domains of p85 and IRS-1, whereas a region between both SH2 domains associates with pi 10. Insulin causes the activation of PI,-kinase through complexation of tyrosine-phosphorylated YMXM motifs on IRS-1 with the SH2 domains of PI,-kinase. Binding of PI,-kinase to phosphorylated IRS-1 leads to tenfold stimulation of its activity, accounting for the rapid rise in phosphorylated phosphatidylinositols observed in insulin-stimulated cells. For examination which tyrosine residue of IRS-1 is required for the interactions of IRS-1 with PI,-kinase, different rat IRS-1 mutants containing mutations in the tyrosine residues that interact with the SH2 domains of PI.-kinase *in vitro* were constructed. Mutation of tyrosine 608 affects the PI,-kinase activity associated with IRS-1, suggesting that this tyrosine is likely to be a principal site of interaction with the SH2 domains of p85 in response to insulin (20). PI.-kinase can be stimulated *via* IRS-1 pathway and by other ligands. GH-induced tyrosine phosphorylation of IRS-1 and the subsequent docking of PL-kinase are importantpostreceptor events in GH action (21).

PI_3-kinase regulates the trafficking of lysosomal enzymes by interfering with a mannose 6-phosphate receptor-dependent sorting event in the *trans-Golgi* network (22). PI,-kinase activity as well as an intact actin cytoskeletal network are required for insulin-stimulated glucose transport in L6 myotubes. This process occurs through the translocation of glucose transporters (GLUT1, GLUT3 and GLUT4) from the *trans-Golgi* network store to the plasma membrane. Glucose transport is also stimulated by the mitochondrial ATP production uncoupler dinitrophenol, wich induces translocation of GLUT1 and GLUT4, but not GLUT3. This response is not affected by inhibiting PI_3-kinase or disassembling the actin cytoskeletal network. Insulin, but not dinitrophenol, causes tyrosine phosphorylation of several proteins, including IRS-1 and mitogen-activated protein (MAP) kinase. Similarly, insulin, but not dinitrophenol, induces actin reorganization which is inhibited by

wortmannin. Thus insulin and dinitrophenol stimulate glucose transport by different mechanisms (23).

The activated PDGFR directly binds and activates PL-kinase, whereas the IL-4 receptor activates PI_3-kinase *via* IRS-1 or the IRS-1-related molecule 4PS. Although PDGF and IL-4 can activate PI_3-kinase in the respective cell lines, they do not possess insulin ability to stimulate glucose uptake and GLUT4 translocation to the plasma membrane. These findings indicate that activation of PI,-kinase is not sufficient to stimulate GLUT4 translocation to the plsasma membrane. Activation of a second signaling pathway by insulin, distinct from PI_3-kinase, is necessary for the stimulation of glucose uptake in insulin-sensitive cells (24).

Studies with inhibitors have indicated that activation of PI_3-kinase is required for the stimulation of glucose transport in adipocytes. Short peptides containing two tyrosine-phosphorylated or thiophosphorylated YMXM motifs potently activate PI_3-kinase in the cytosol from 3T3-L1 adipocytes. Introduction of the phosphatase-resistant thiophosphorylated peptide into 3T3-L1 adipocytes through permeabilization with *Staphylococcus aureus* a-toxin stimulates PL-kinase as strongly as insulin. However, under the same conditions, this peptide increases glucose transport into the permeabilized cells only 20% as well as insulin. Determination of the distribution of GLUT4 by confocal immunofluorescence shows that its translocation to plasma membrane can account for the effect of the peptide. Thus, one or more insulin-triggered signaling pathways, besides the PL-kinase one, participate in the stimulation of glucose transport (25). It has been shown that pervanadate (H,O_2 and vanadate), but not H,O_2 or vanadate alone, mimics most insulin actions in CHO cells or CHO cells that overexpress wild-type or mutant insulin receptor and/or IRS-1. Pervanadate-induced tyrosine phosphorylation of the insulin receptor enhances insulin receptor tyrosine kinase activity toward IRS-1 *in vivo* and fifteenfold IRS-1-associated PI,-kinase activity. However, pervanadate treatment does not induce insulin receptor/IRS-1 association (26).

- **Growth factor receptor-bound protein-2**

Growth factor receptor-bound protein-2 (GRB-2) is a 25 kD cytosolic protein that contains two SH3 domains and one SH2 domain that binds to Tyr^{895} in IRS-1. GRB-2 is an "adapter molecule" that links the 150 kD guanine nucleotide exchange factor for p21ras termed mammalian son-of-sevenless (mSOS) to tyrosyl phosphoprotein, such as the EOF receptor, IRS-1, and BCR-abl. mSOS catalyses the release of GTP from *ras.* The GRB-2-mSOS unit is considered a complex of a regulatory and a catalytic subunit similar to the complex of PI $_3$-kinase. The GRB-2 -mSOS complex thus may activate p21 *ras* by stimulating GTP-binding. The dissociation of GRB-2 from SOS proteins caused by insulin in 3T3-L1 cells mediates p21ras deactivation and desensitization (27). Thus the binding of GRB-2-mSOS to IRS-1 mediates the insulin stimulation of p2 *Iras* which in turn binds directly Raf-1 serine/threonine kinase, the first element of the MAP kinase cascade. Raf-1 activates MAP kinase kinase which in turn activates MAP kinase. Insulin activates NF-kappa B in mammalian cells through a posttranslational mechanism requiring both insulin receptor tyrosine kinase and Raf-1 kinase activities (28). However, alternative pathways for insulin regulation of ras may exist, including insulin-stimulated tyrosine phosphorylation of She or the direct binding of p21 *ras* to the insulin receptor (4).

Insulin or EGF stimulation induces a rapid increase in p2 Iras levels but after several minutes they decline toward basal despite ongoing hormone stimulation. The deactivation of p2*Iras* correlates closely with phosphorylation of SOS and dissociation of SOS from GRB-2. The inhibition of MAP kinase kinase blocks both events, resulting in prolonged p21 ras activation. Thus, a negative feedback loop exists whereby activation of the RafTMAP-ERK-kinase (MEK) cascade by p21 ras causes SOS phosphorylation and, therefore, SOS/GRB-2 dissociation, limiting the duration of p2Iras activation by growth factors. A serine/threonine kinase downstream of MAP kinase kinase mediates this desensitization feedback pathway (29).

The stimulation of the MAP kinase pathway by insulin is required for stimulation of pp90 and *c-fos,* but is not required for many of the metabolic activities of the hormone in cultured fat and muscle cells, basal and insulin-stimulated glucose uptake, lipogenesis, glycogen synthesis, insulin receptor autophosphorylation, activation of protein phosphatase-1 and Pl.-kinase (30).

Other data suggest that signaling molecules other than IRS-1, including She, are critical for insulin stimulation of p2Iras. IRS-1 phosphorylation is either not sufficient or not involved in insulin stimulation (16).

A SH2 domain-containing protein was identified by yeast two-hybrid screen, performing with the cytoplasnuc domain of the human insulin receptor as bait to trap high-affinity interacting proteins encoded by human liver or HeLa cDNA libraries. It binds with high affinity to the autophosphorylated insulin receptor *in vitro.* The mRNA for this protein was found by Northern blot analysis to be highest in skeletal muscle and was also detected in adipocytes by polymerase chain reaction. To study the role of this protein in insulin signaling, a full-length cDNA encoding this protein (called GRB-IR) was isolated and stably expressed in CHO cells overexpressing the human insulin receptor. Insulin treatment of these cells leads to *in situ* formation of a complex of the insulin receptor and the 60 kD GRB-IR. Although almost 75% of the GRB-IR pro-

tein is bound to the insulin receptor, it is only weakly tyrosine-phosphorylated. The formation of this complex appears to inhibit the insulin-induced increase in tyrosine phosphorylation of two endogenous substrates, a 60 kD GTPase-activating protein and, to a lesser extent, IRS-1. The subsequent association of this latter protein with PI_3-kinase is also inhibited. GRB-IR is probably a SH2-domain-containing protein that directly complexes with the IR and serves to inhibit signaling or redirect the insulin receptor signaling pathway (31).

- **Protein tyrosine phosphatase Syp**

Syp is an ubiquitously expressed protein tyrosine phosphatase (FTP) that contains two amino-terminal SH2 domains responsible for its association with tyrosine-phosphorylated proteins. This 65 kD protein is also known as PTP2C and SH-PTP2. Mutation of tyrosine^{1172} and tyrosine^{1222} totally prevents the insulin-induced association of IRS-1 with the SH2 domains of PTP2C, demonstrating that both tyrosines are key elements in the binding sites for the SH2 domains of PTP2C. The ability of purified PTP2C to dephosphorylate IRS-1 depends on the association of PTP2C with phosphorylated IRS-1 (20). The association of Syp with phosphopeptides containing a consensus binding motif, YVNI, activates the phosphatase, suggesting a role for IRS-1 in the activation of this signal pathway. One role of this assotiation is likely to be the termination of signaling from Tyr(P)IRS-l, since Syp rapidly dephosphorylates purified recombinant Tyr(P)-IRS-l. It is also possible that Syp has a positive role in signaling, since there is genetic evidence that the homologous phosphatase in *Drosophila,* the corkscrew gene product, augments signal transduction in a pathway involving a receptor tyrosine kinase, *ras* and Raf (9). Expression of dominant interfering mutants of Syp inhibits insulin stimulation of *c-fos* reporter gene expression and activation of the 42 kD (ERK2) and 44 kD (ERK1) MAP kinases. Cotransfection of dominant interfering SHPTP2 mutants with *v-ras* or GRB-2 indicates that SHPTP2 regulates insulin signaling either upstream of or in parallel to *ras* function. Furthermore, phosphotyrosine blotting and immunoprecipitation identifies the 125 kD focal adhesion kinase (pp!25^{FAK}) as a substrate for insulin-dependent tyrosine dephosphorylation. Thus SHPTP2 functions as a positive regulator of insulin action and that insulin signaling results in the dephosphorylation of tyrosine-phosphorylated pp!25^{FAK} (32).

FTP play a key role in the regulation of insulin action. Insulin mediates inhibition of nuclear protein phosphatase-2A (PP-2A) which is associated with an increase in phosphorylation of the transcription factor cAMP response element-binding protein. Insulin inhibits nuclear PP-2A activity, and C-terminal domain of insulin receptor is important for this effect (33). There is relative abundance and subcellular distribution of specific PTP in muscle tissue, the major site of insulin-mediated glucose disposal *in vivo* involved in the regulation of reversible tyrosine phosphorylation in this tissue (34).

- **Protein Nek**

Nek is a 47 kD protein containing three SH3 domains in addition to the SH2 domain. It associates through its SH2 domain with Tyr(P)-IRS 1 in CHO cells overexpressing the insulin receptor. Since the 85 kD subunit of PI_3-kinase contains two proline-rich motifs that can associate with the v-Src SH3 domain, consideration should also be given to the possibility that these proline-rich segments interact with the Nek SH3 domains within a tertiary complex of IRS-1, PI_3-kinase and Nek (9).

INSULIN RESISTANCE

• All forms of diabetes mellitus in man are associated with some degree of peripheral insulin resistance. In type I diabetes, it is unclear whether the insensitivity to the actions of insulin on glucose transport is a direct result of low circulating insulin or a response to hyperglycemia. Non-insulin-dependent diabetes mellitus (NTDDM) is a complex metabolic disorder that involves numerous biochemical abnormalities, a heterogenous clinical picture, and a polygenic hereditary component. The primary causal event in NIDDM is a decrease in the maximal stimulation of *in vivo* glucose disposal. The pathophysiologic state involves increased basal hepatic glucose production, decreased insulin-mediated glucose utilization in target tissues, and altered pancreatic function with decreased p-cell function and enhanced glucagon secretion. Prospective studies indicate that insulin resistance and hyperinsulinemia exist in the prediabetic state at time when glucose tolerance is normal. When hyperglycemia supervenes both insulin secretion and insulin-mediated glucose utilization are further compromised, and mediated in part by sustained hyperglycemia itself. Insulin resistance may occur at any level in the biological action of insulin, from initial binding to cell surface receptors to the phosphorylation cascade initiated by autophosphorylation of the insulin receptor. Receptors isolated from patients with NIDDM have defective autophosphorylation kinase activity when isolated from adipocytes, liver, erythrocytes, and skeletal muscle. The magnitude of the decrease in insulin receptor kinase activity correlates with the degree of fasting hyperglycemia. However, the defect in insulin receptor kinase activity normalizes after weight reduction or other measures that reduce hyperglycemia indicating the secondary nature of the defect. Clarification of the mechanisms underlying insulin resistance in NIDDM may lead to new treatment modalities for this disease (35). Insulin resistance is a common clinical feature of obesity and NIDDM, and is characterized by elevated serum levels of glucose, insulin, and lipids. The mechanism of acquisition of insulin resistance is unknown (2).

Insulin resistance of skeletal muscle, liver and fat combined with an abnormality of insulin secretion characterizes NIDDM. There is increasing evidence that the insulin resistance of the skeletal muscle plays a key role early in the development of NIDDM. Recent research efforts focuss on the characterization of insulin signal transduction elements in the muscle which are candidates for a localization of a defect causing insulin resistance, i.e. the insulin receptor, phosphatases related to insulin action, glycogen synthase and the glucose transporters (36).

Glucagon, probably acting through cAMP, may impair insulin signaling in the three early steps in insulin action after binding. There is a decrease in the insulin-stimulated receptor and ER.S-1 phosphorylation levels which is paralleled by a reduced association between IRS-1 and PI_3-kinase *in vivo* in liver and muscle of glucagon-treated rats (37).

Impaired insulin-stimulated glucose uptake in skeletal muscle from severely obese subjects is accompanied by a deficiency in insulin receptor signaling, which may contribute to decreased insulin action. Insulin-stimulated 2-deoxyglucose uptake is lower in muscle strips from obese subjects. Insulin stimulation of IRS-1 immunoprecipitable PI_3-kinase activity also is markedly lower in obese subjects compared with controls. The obese individuals have a lower abundance of insulin receptors, IRS-1, and p85 subunit of PI_3-kinase. In their skeletal muscle, insulin is less effective in stimulating tyrosine phosphorylation (38). Hyperglycemia stimulates glucose transport by Ca^{2+}-dependent activation that does not involve PI_3-kinase activity and is separated from the mass-action effect of glucose on glucose transport (39).

Incubation of Rat 1 fibroblasts expressing human insulin receptors for 4 days in 27 mM D-glucose leads to impaired insulin stimulation of both oc-aminoisobutyric acid uptake and phosphorylation of pp!85 and receptor p-subunits *in vivo. In vitro* autophosphorylation and tyrosine kinase activities toward poly Glu^{80} Tyr^{20} of insulin receptors from cells exposed to high glucose media are also impaired, although the binding of insulin to high glucose media cells is unchanged (40).

In hyperthyroidism, increased insulin-stimulated glucose transport is associated with an increase of GLUT4 which may be responsible for the increment of peripheral glucose utilization in hyperthyroidism. The effect of hypothyroidism on insulin action in adipocytes is characterized by a state of increased insulin sensitivity as indicated by the increase in insulin receptor affinity and tyrosine kinase activity. Despite the marked reduction of GLUT4, insulin-stimulated glucose transport is not diminished, suggesting that functional activity of plasma membrane glucose transporters is enhanced in hypothyroidism (41). The regulation of the insulin receptor, IRS-1, and PI_3-kinase contributes significantly to the insulin resistance induced by chronic hyperinsulinemia, but the glucocorticoid-induced insulin resistance is located beyond these early steps in insulin action (42).

Upon chronic treatment of fibroblasts with insulin, conditions that mimic the hyperinsulinemia associated with insulin resistance, the membrane-associated insulin receptor P-subunit is proteolytically cleaved, resulting in the generation of a cytosolic fragment of the P-subunit (beta'), and that is inhibited by the thiol protease inhibitor E64. In 3T3-L1 adipocytes, (/) cytosolic beta' is generated by chronic insulin administration to the cells, and E64 inhibits the production of beta', *(ii)* chronic administration of insulin to the adipocytes leads to an insulin-resistant state, as measured by lipogenesis and glycogen synthesis, and E64 totally prevents the generation of this insulin-induced insulin resistance, *(Hi)* E64 has no effect on the insulin-induced downregulation of IRS-1, and therefore insulin resistance is not mediated by the down-regulation of insulin receptor substrate-1, (iv) under *in vitro* conditions, partially purified beta' stoichiometrically inhibits the insulin-induced autophosphorylation of the insulin receptor P-subunit, and (v) administration of E64 to obese Zucker fatty rats improves the insulin resistance of the rats compared with saline-treated animals. These data indicate that beta' is a mediator of insulin resistance, and the mechanism of its action is inhibition of insulin-induced autophosphorylation of p-subunit of the insulin receptor (43).

The insulin resistance in muscle maybe due to triacylglycerol accretion in the tissue itself. Diacylglycerol mimicks insulin action by increasing 2-deoxyglucose uptake and this is also reduced by triacylglycerol preloading, suggesting that the effect is not mediated at the insulin receptor. Thus triacylglycerol may exert a direct effect on muscle cell insulin sensitivity possibly at the level of diacylglycerol second messenger pathway (44).

CONCLUSIONS

Insulin binding activates the receptor tyrosine kinase toward the ER.S-1. Phosphorylated ER.S-1 then interacts with p85 a-subunit of PI,-kinase, Nek, GRB-2, and Syp, thus branching insulin signals both for mitogenic and metabolic responses. The levels of these proteins are compared in adipose and liver tissues of nondiabetic mice and obese insulin-resistant diabetic KKAy mice. IR and PI_3-kinase p85 a protein levels are significantly lower in KKAy mice than in control nondiabetic mice, whereas IRS-1 protein levels are not altered. In contrast, the protein levels of GRB-2, Nek, Syp, and GLUT1 are dramatically elevated in KKAy fat, with less striking changes in liver. Treatment of diabetic animals with pioglitazone, an insulin-sensitizing antihyperglycemic agent, partially corrects the expression of some of these proteins. Taken together these

findings suggest that the insulin-resistant diabetic condition is characterized by changes in expression of insulin signal transduction components that may be associated with altered glucose metabolism (45). :</'V.GO

In effect, the recent data about the molecular mechanisms of insulin action may provide new insights into the pathophysiology and therapy of diabetes mellitus and other insulin resistant states.

REFERENCES

1. Mitev V. Molecular mechanisms of insulin action. *Adv Mol* 5/0/1992; 12:49-81

2. SirakovL, Mitev V. Insulin. Sofia, 1991, p.221

3. Baron V, van Obberghen E. Mechanism of insulin action. *CR Seanc Soc Biol Fil* 1995; 189: 25-41

4. White M, C.R. Kahn. The insulin signaling system. *Biol Chem* 1994; 269: 1-4

5. Baron V, Kaliman P, Alengrin F, Van Obberghen E. Interaction of the C-terminal acidic domain of the insulin receptor with histone modulates the receptor kinase activity. *EurJBiochem* 1995; 229: 27-34

6. Joost HG. Structural and functional heterogeneity of insulin receptors. *Cell Signal* 1995; 7: 85-91

7. Kublaoui B, Lee J, Pilch PF. Dynamics of signaling during insulin-stimulated endocytosis of its receptor in adipocytes. *J Biol Chem* 1995; 270: 59-65

8. Formisano P, Najjar SM, Gross CN, Philippe N, Oriente F, Kern Buell CL *et al.* Receptor-mediated internalization of insulin. Potential role of pp!20/HA4, a substrate of the insulin receptor kinase. *J Biol Chem* 1995; 270: 24073-24077

9. Keller S, Lienhard G. Insulin signalling: the role of insulin receptor substrate-1. *Trends Cell Biol* 1994; 4:115-119

10. Heller-Harrison RA, Morin M, Czech MP. Insulin regulation of membrane-associated insulin receptor substrate-1. *JBiol Chem* 1995; 270: 24442-24450

11. Sun XJ, Wang LM, Zhang Y, Yenush LP, Myers MG, Glasheen Jr E *et al.* Role of IRS-2 in insulin and cytokine signaling. *Nature* 1995; 377: 173-177

12. Patti ME, Sun XJ, Bruening JC, Araki E, Lipes MA, White MF *et al.* 4PS/insulin receptor substrate (IRS)-2 is the alternative substrate of the insulin receptor in IRS-1-deficient mice. *JBiol Chem* 1995; 270: 24670-24673

13. Wang L, Hayashi H, Mitani Y, Ishii K, Ohnishi T, Niwa Y *et al.* Cloning of a cDNA encoding a 190 kD insulin receptor substrate-1-like protein of simian COS cells. *Biochem BiophysRes Commun* 1995; 216: 321-328

14. Furusaka A, Nishiyama M, Nishimaki H, Ogasawara Y, Tamemoto H, Yamauchi T *et al.* Ornithine decarboxylase induction during liver regeneration in IRS-1-deficient mice. *Biochem Biophys Res Commun* 1995; 216: 284-290

15. Harada S, Smith RM, Smith JA, Shah N, Hu DQ, Jarett L. Insulin-induced egr-1 expression in Chinese hamster ovary cells is insulin receptor and insulin receptor substrate-1 phosphorylation-independent. Evidence of an alternative signal transduction pathway. *J Biol Chem* 1995; 270: 26632-26638

16. Chen D, Van Horn DJ, White MF, Backer JM. Insulin receptor substrate 1 rescues insulin action in CHO cells expressing mutant insulin receptors that lack a juxta-membrane NPXY motif. *Mol Cell Biol* 1995; 15: 4711-4771

17. Myers Jr MG, Cheatham B, Fisher TL, Jachna BR, Kahn CR, Backer JM *et al.* Common and distinct elements in insulin and PDGF signaling. *Ann N Y Acad Sci* 1995; 766:369-387

18. Eriksson H, Ridderstrale M, Tornqvist H. Tyrosine phosphorylation of the GH receptor and Janus tyrosine kinase-2 is involved in the insulin-like actions of GH in primary rat adipocytes. *Endocrinology* 1995; 136: 5093-5101

19. Lin YL, Chen HC, Yeh SF, Chou CK. Differential pathways of insulin action upon the hepatitis B surface antigen gene expression and cell proliferation in human hepatoma cells. *Endocrinology* 1995; 136: 2922-2927

20. Rocchi S, Tartare-Deckert S, Mothe I, van Obberghen E. Identification by mutation of the tyrosine residues in the insulin receptor substrate-1 affecting association with the tyrosine phosphatase 2C and phosphatidylinositol 3-kinase. *Endocrinology* 1995; 136: 5291-5297

21. Ridderstrale M, Degerman E, Tornqvist H. Growth hormone stimulates the tyrosine phosphorylation of the insulin receptor substrate-1 and its association with phosphatidylinositol 3-kinase in primary adipocytes. *Biol Chem* 1995; 270: 3471-3474

22. Brown WJ, DeWald DB, Emr SD, Plutner H, Balch WE. Role for phosphatidylinositol 3-kinase in the sorting and transport of newly synthesized lysosomal enzymes in mammalian cells. / *Cell Biol* 1995; 130: 781-796

23. Tsakiridis T, Vranic M, Klip A. Phosphatidylinositol 3-kinase and the actin network are not required for the stimulation of glucose transport caused by mitochondrial uncoupling: comparison with insulin action. *Biochem J* 1995; 309: 1-5

24. Isakoff SJ, Taha C, Rose E, Marcusohn J, Klip A, Skolnik EY. The inability of phosphatidylinositol 3-kinase activation to stimulate GLUT4 translocation indicates additional signaling pathways are required for insulin-stimulated glucose uptake. *Proc NatlAcadSci USA* 1995; 92: 10247-10251

25. Herbst JJ, Andrews GC, Contillo LG, Singleton DH, Genereux PE, Gibbs EM *et al.* Effect of the activation of phosphatidylinositol 3-kinase by a thiophosphotyrosine peptide on glucose transport in 3T3-L1 adipocytes. *JBiol Chem* 1995; 270: 26000-26005

26. Wilden PA, Broadway D. Combination of insulinomimetic agents H,O, and vanadate enhances insulin receptor mediated tyrosine phosphorylation of IRS-1 leading to IRS-1 association with the phosphatidylinositol 3-kinase. *J Cell Biochem* 1995; 58: 279-291

27. Cherniack AD, Klarlund JK, Conway BR, Czech MP. Disassembly of Son-of-sevenless proteins from Grb2 during p2*Iras* desensitizationby insulin. *JBiol Chem* 1995; 270: 1485-1488

28. Bertrand F, Philippe C, Antoine PJ, Baud L, Groyer A, Capeau J *et al.* Insulin activates nuclear factor kappa B in mammalian cells through a Raf-1-mediated pathway. *JBiol Chem* 1995; 270: 24435-24441

29. Langlois WJ, Sasaoka T, Saltiel AR, Olefsky JM. Negative feedback regulation and desensitization of insulin- and epidermal growth factor-stimulated p21ras activation. *JBiol Chem* 1995; 270: 25320-25323

30. Lazar DF, Wiese RJ, Brady MJ, Mastick CC, Waters SB, Yamauchi K *et al.* Mitogen-activated protein kinase kinase inhibition does not block the stimulation of glucose utilization by insulin. *J Biol Chem* 1995; 270: 20801-20807

31. Liu F, Roth RA. Grb IR: a 8112-domain-containing protein that binds to the insulin receptor and inhibits its function. *Proc NatlAcadSci USA* 1995; 92: 10287-10291

32. Yamauchi K, Milarski KL, Saltiel AR, Pessin JE. Protein-tyrosine-phosphatase SHPTP2 is a required positive effector for insulin downstream signaling. *Proc NatlAcad Sci USA* 1995; 92: 664-668 :

33. Reusch JE, Hsieh P, Bhuripanyo P, Carel K, Leitner JW, Olefsky JM *et al.* Insulin inMbits nuclear phosphatase activity: requirement for the C-terminal domain of the insulin receptor. *Endocrinology* 1995; 136: 2464-2469

34. Ahmad F, Goldstein BJ. Purification, identification and subcellular distribution of three predominant protein-tyrosine phosphatase enzymes in skeletal muscle tissue. *Biochim BiophysActa* 1995; 1248: 57-69

3 5. Olefsky JM, Nolan JJ. Insulin resistance and non-insulin-dependent diabetes mellitus: cellular and molecular mechanisms. *Am J Clin Nutr* 1995; 61(Suppl): 980S-986S

36. Haring H. Pathophysiology of insulin resistance. *Herz* 1995; 20: 5-15

37. Saad MJ, Hartmann LG, de Carvalho DS, Galoro CA, Brenelli SL, Carvalho CR. Effect of glucagon on insulin receptor substrate-1 (IRS-1) phosphorylation and association with phosphatidylinositol 3-kinase (PI 3-kinase). *FEBSLett* 1995; 370: 131-134

38. Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ, Dohm GL. Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J Clin Invest* 1995; 95: 2195-2204

39. Nolte LA, Rincon J, Wahlstrom EO, Craig BW, Zierath JR, Wallberg-Henriksson H. Hyperglycemia activates glucose transport in rat skeletal muscle *via* a Ca^{2+}-dependent mechanism. *Diabetes* 1995; 44: 1345-1348

40. Ide R, Maegawa H, Kashiwagi A, Kikkawa R, Shigeta Y. High glucose condition desensitizes insulin action at the levels of receptor kinase. *EndocrJ* 1995; 42: 1-8

41. Matthaei S, Trost B, Hamann A, Kausch C, Benecke H, Greten H *et al.* Effect of *in vivo* thyroid hormone status on insulin signalling and GLUT1 and GLUT4 glucose transport systems in rat adipocytes. *J Endocrinol* 1995; 144: 347-357

42. Saad MJ, Folli F, Kahn CR. Insulin and dexamethasone regulate insulin receptors, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in Fao hepatoma cells. *Endocrinology* 1995; 136: 1579-1588

43. Knutson VP, Donnelly PV, Balba Y, Lopez-Reyes M. Insulin resistance is mediated by a proteolytic fragment of the insulin receptor. *JBiolChem* 1995; 270:24972-24981

44. Shillabeer G, Chamoun C, Hatch G, Lau DC. Exogenous triacyl-glycerol inhibits insulin-stimulated glucose transport in L6 muscle cells *in vitro. Biochem Biophys Res Commun* 1995; 207: 768-774

45. Bonini JA, Colca JR, Dailey C, White M, Hofmann C. Compensatory alterations for insulin signal transduction and glucose transport in insulin-resistant diabetes. *Am J Physiol* 1995; 269: E759-E765

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