

OBJECTIVES:

1. Compare the structures of **proinsulin** and **insulin**.
2. Describe the mechanism for stimulation of insulin secretion and synthesis by **glucose** by increasing intracellular calcium.
3. Delineate the steps leading to activation of the tyrosine kinase activity of the insulin receptor including the role of **interchain autophosphorylation of tyrosine residues**
4. Describe the mechanism by which insulin elicits mobilization of GLUT-4 glucose transporter protein to the plasma membrane in muscle and adipose tissue via **IRS**, p85 and PI-3K.
5. Compare the causes of **type 1 diabetes** with **type 2 diabetes** and explain what is meant by **insulin resistance** in the type 2 form.

PHYSIOLOGICAL PREMISE

Insulin receptor defects are rare autosomal recessive disorders characterized by intrauterine and postnatal growth restriction, hyperinsulinemia (elevated blood insulin), abnormal glucose homeostasis and short life expectancy. Most cases have shown affected patients to be either homozygotes (genes on both chromosomes with the same defect), or compound heterozygotes (defects in two or more locations of the same gene on a single chromosome) on mutations in the insulin receptor (IR) α -subunit. Such mutations impair insulin signaling by decreasing IR expression on the cell surface and/or IR affinity for insulin (strength of insulin attachment). One patient was identified with two mutant alleles of the IR gene (allele = any of the alternative forms of a gene that may occur at a given locus). The maternal and paternal alleles each contained a point mutation (results from a change in a single base pair in the DNA molecule, caused by the substitution of one nucleotide for another) causing structural alterations in the tyrosine kinase domain of the IR β -subunit resulting in severe impairment of insulin action.

INSULIN OVERVIEW

General Information

The physiology and biochemistry of insulin are quite significant because insulin plays an important role in fuel metabolism by promoting anabolic processes and inhibiting catabolic ones predominantly in muscle, fat and liver tissue. Insulin promotes *storage of fuels* as glycogen (from glucose), triglycerides (from fatty acids) and proteins (from amino acids). An acute action of insulin is the stimulation of *glucose uptake* into muscle and fat cells; hence, *blood glucose is lowered by insulin*.

Insulin Processing

Insulin is synthesized and released by the β -cells of the pancreas. Glucose is the primary stimulant to secretion. The insulin molecule includes two separate chains held together by disulfide bonds. These chains initially are contained within a single polypeptide chain; **preproinsulin** that is processed to **proinsulin** (Fig. 1) and eventually to **insulin**. The two chains are connected by a portion known as the C-peptide that is removed before insulin is secreted but is released together with the insulin (Fig. 1). After secretion, the C-peptide is slowly cleared by the

kidney. In type I diabetics whose β -cells have been destroyed by an autoimmune response, there is no C-peptide in the urine. The lack of insulin to store glucose causes the blood levels to become elevated. This excess glucose reacts with hemoglobin to form glycosylated hemoglobin and reflect the long-term elevated blood glucose. Eventually these can crosslink seriously impairing the oxygen carrying ability of the red blood cells.

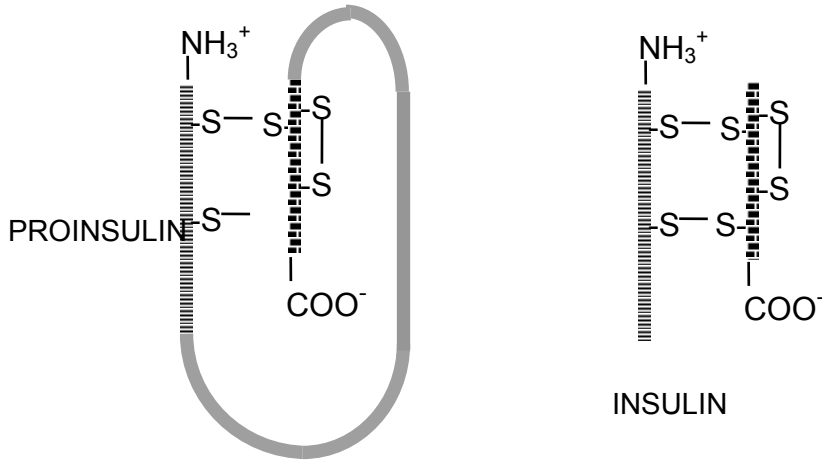


Figure 1. The structural features of proinsulin and insulin.

Insulin secretion

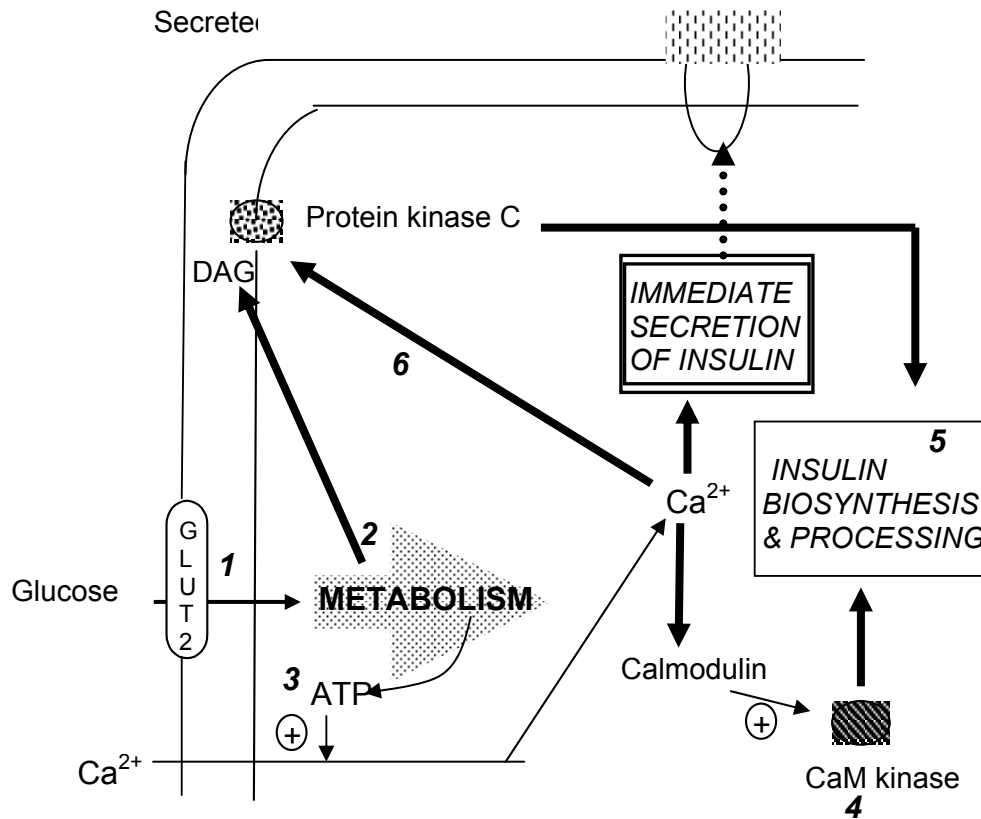


Figure 2. Control of insulin synthesis and secretion by glucose. CaM kinase is calmodulin-dependent protein kinase; DAG is diacylglycerol

Insulin release is mainly initiated by glucose, as a signal of high carbohydrate intake. An increase in the plasma concentration of glucose is the most important physiologic regulator of insulin secretion. Insulin secretion in response to glucose is biphasic. This response is characterized by an immediate, or first-phase, that begins within 1 minute and lasts for 5-10 minutes followed by a more gradual, prolonged second phase that terminates soon after the glucose stimulus is removed. These two phases likely reflect the different intracellular pools of insulin one which is at the plasma membrane surface and the other that is distant from the membrane.

The model in Figure 2 shows the most common biochemical signal transduction pathway in the control of insulin secretion. **Glucose** acts by entering the β -cell via the **GLUT-2** (Fig. 2, *I*) transporter protein. As a consequence of glucose metabolism (2), two signals are generated; ATP and diacylglycerol (DAG). The increase in the concentration of ATP in the cell causes the membrane to close potassium channels that normally facilitate the exit of K^+ . This event activates special voltage-sensitive channels that allow Ca^{2+} to enter the β -cell (3). Calcium triggers the exocytosis of secretory granules that contain insulin and C-peptide. Calcium also binds to calmodulin that activates calmodulin-dependent (CaM) protein kinase (4) to promote insulin biosynthesis (5). As discussed in an earlier lecture, **DAG** together with calcium activates **protein kinase C** (6), which plays a role in promoting insulin synthesis and processing as well. In this way, the signal from high glucose not only causes the secretion of insulin, but also initiates the events needed to replace insulin that has been secreted from the cell.

INSULIN RECEPTOR

Structural features

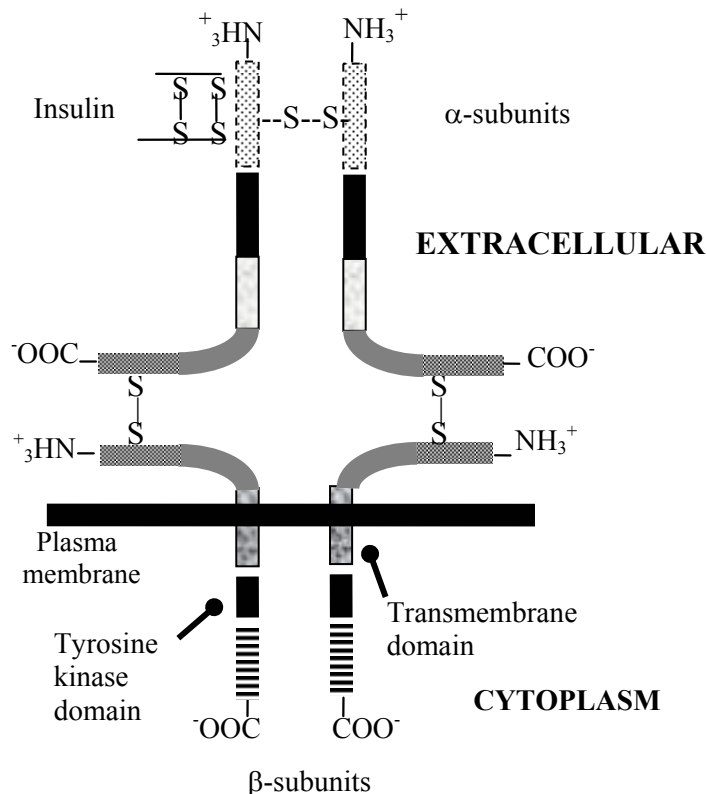


Figure 3. The insulin receptor. Insulin binding to the α -chains transmits a signal through the transmembrane domain of the β -chains to activate the tyrosine kinase activity

The insulin receptor (IR) is an integral membrane protein that exists as a dimer. Each monomer contains an α - and a β -chain (subunit) (Fig. 3). The α -subunits link to each other and to the β -subunits by disulfide bonds and lie entirely on the extracellular side of the plasma membrane. Although each α -subunit contains a binding site for insulin, binding of one insulin molecule decreases affinity for binding of a second molecule. This reduced binding for the second insulin molecule is termed negative cooperativity and is opposite to what is seen for oxygen binding to hemoglobin (see lectures for chapter 10). A cysteine-rich domain likely is involved in the binding event. The β -subunits traverse the membrane, having tyrosine kinase enzyme domains on the cytoplasmic side.

Interchain Autophosphorylation

Insulin binding ultimately *switches on the catalytic activity* of the **tyrosine kinase domain** on each β -subunit of the receptor (Fig. 4). The activated insulin receptor tyrosine kinase (IRTK) then propagates the insulin signal by catalyzing the phosphorylation of proteins in the cytoplasm. After binding insulin, the receptor undergoes a conformational change that causes the IRTK, on the same half of the receptor to which insulin binds (Fig. 4, β -subunit L), to become active (Fig. 4, steps 1 \rightarrow 2). Once this IRTK is activated, it phosphorylates, via **interchain autophosphorylation**, the tyrosine kinase domain on the opposing β -subunit (R) (steps 2 \rightarrow 3). Phosphorylation of tyrosine residues on the latter β -subunit (R) activates that tyrosine kinase domain which in turn phosphorylates the initially activated β -subunit (L) (steps 3 \rightarrow 4). Thus phosphorylation of the tyrosine kinase domains leads to *enhanced catalytic activity of the tyrosine kinase, independent of insulin binding*.

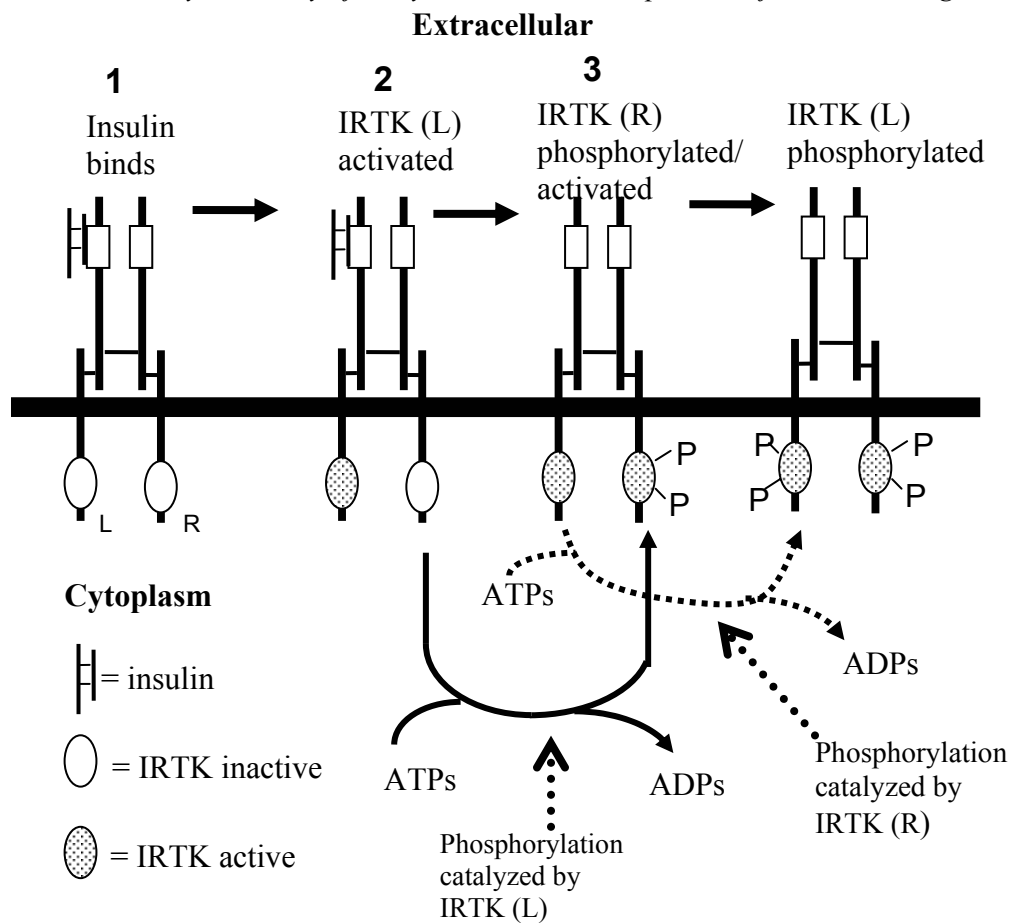


Figure 4. Activation of the tyrosine kinase domains of the insulin receptor by insulin binding, followed by interchain autophosphorylation

Inactivation of the insulin receptor may occur by phosphorylation of the receptor on serine residues by an as yet unidentified serine kinase. Therefore control of the insulin receptor comes about by: 1) insulin binding and subsequent dissociation, 2) autophosphorylation of tyrosine residues (activation), and 3) phosphorylation of serine residues (inactivation). Cessation of these events also occurs with destruction of the insulin. Insulin has a plasma half-life of 3 to 5 minutes, and the liver and kidneys primarily degrade it.

INSULIN SIGNAL TRANSDUCTION:

Actions of insulin

Understanding how the insulin signal is transduced is a fundamental problem in medicine. It is crucial to understand this cascade in order to diagnose and treat patients with noninsulin-dependent (type 2) diabetes mellitus (NIDDM).

In the absence of insulin, tyrosine residues adjacent to the tyrosine kinase domains remain dephosphorylated. The insulin-receptor complex undergoes an activation sequence involving conformational changes and autophosphorylations on the β -subunits of the receptor, as described above. This triggering of tyrosine kinase activity leads to phosphorylation of several cytoplasmic proteins that can carry the insulin signal into the interior of the cell. Besides phosphorylating the β -subunits of the receptor, activated insulin receptor tyrosine kinase phosphorylates a variety of proteins including **insulin receptor substrate (IRS)**, **phospholipase C**, and **SHC protein**. The responses can be divided into metabolic and growth (mitogenic). The principal metabolic responses include activation of the insulin-sensitive glucose transport system in adipose and muscle cells, and of protein phosphatase in most tissues. Protein phosphatase is responsible for dephosphorylating a variety of regulated enzymes that have been phosphorylated in response to hormone signals from glucagon or epinephrine, as will be discussed in later lectures. Recall that glucagon or epinephrine activates adenylyl cyclase to produce cyclic AMP that activates protein kinase A. Protein kinase A, in turn, phosphorylates various enzymes associated with pathways of carbohydrate or fat metabolism. Growth responses are mediated following phosphorylation of SHC through nuclear processes that include increases in synthesis of DNA and mRNA, culminating in augmentation of cell growth and replication, and protein synthesis, respectively.

Mechanisms of insulin action via IRS

The several mechanisms by which insulin signals are transduced remain incomplete. The student should note that many details remain hypothetical so that the events may differ when reviewing different texts. Conflicting ideas on these mechanisms may reflect the fact that studies have been conducted in different systems, so that the final mechanisms of insulin action will depend on the tissue of interest. Until such time that the picture is complete, it is best to refer to all of these as hypotheses. The sheer complexity of insulin signaling is the main reason why it remains so difficult for researchers and clinicians to identify appropriate treatments of NIDDM.

IRS (insulin receptor substrate) is a principal target of the phosphorylated (active) IRTK (insulin receptor tyrosine kinase). Several IRS proteins have been identified that have different functions. Phosphorylation of tyrosine residues on IRS, by IRTK, activates it. Activated IRS functions in this pathway as a docking protein. The active IRS can dock with a variety of other proteins to trigger several responses. For instance IRS-2 (*probably*) has been linked to regulation of the insulin-sensitive glucose transport system in adipose and muscle cells (Fig. 5). In this mechanism, IRS, after phosphorylation by IRTK (Fig. 5, [1]), docks with p85 to facilitate the interaction of p85 with **phosphatidylinositol kinase (PI-3K)** (Fig. 5, [2]). PI-3K then phosphorylates phosphatidylinositol to phosphatidyl 4-phosphate that in turn activates protein kinase B. Protein kinase B, through an unknown mechanism, signals the Golgi to mobilize GLUT-4 protein that is stored in this organelle, for trafficking to the plasma membrane (Fig. 5, [3]). Thus in the fed state, via this mechanism insulin increases the capacity of muscle and adipose tissue to use blood glucose.

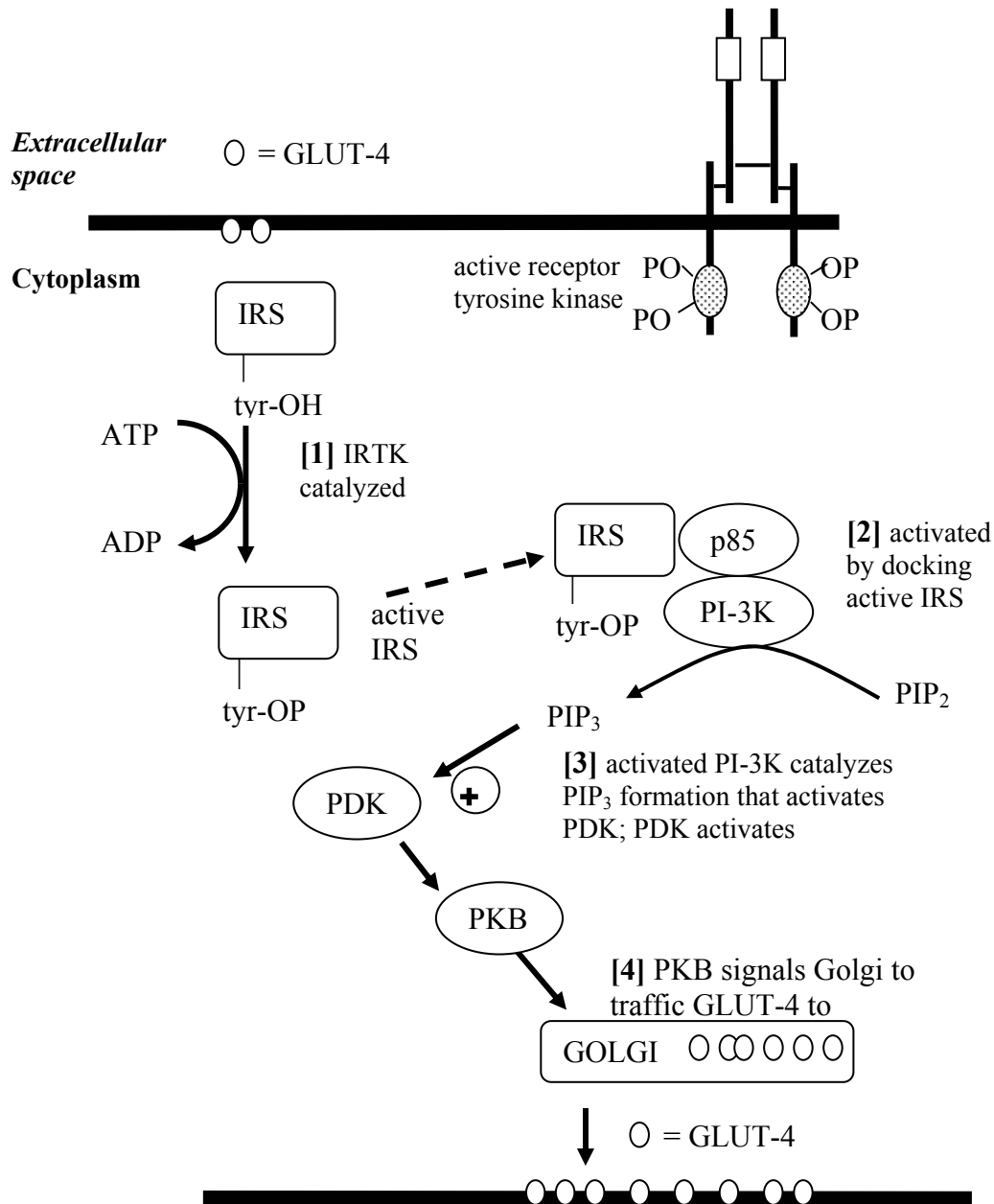


Figure 5. Hypothetical mechanism for insulin to mobilize GLUT-4 glucose transporter protein to the plasma membrane in muscle and adipose tissue. Abbreviations used: IRS, insulin-receptor substrate; IRTK, insulin receptor tyrosine kinase; PI-3K, phosphatidylinositol kinase; PKB, protein kinase B.

Further detail of the recruitment of GLUT-4 transporters in muscle and adipose tissue cells is depicted in Figure 6. D-Glucose enters these cells by carrier-mediated-facilitated diffusion, a process enhanced in adipose and muscle cells by insulin. This involves a V_{max} effect (increased number of transporters) rather than a K_m effect (increased affinity of binding). This is accomplished, after insulin binding (Fig. 6, step 1), by recruiting **glucose transporters** (*GLUT-4 protein*) from an inactive pool in the Golgi body. These transport proteins are then moved (step 2) to the plasma membrane. They then bind and fuse with the membrane (step 3) to become available for glucose transport (step 4). Once the insulin receptor is inactivated (step 5), the excess GLUT-4 returns to the Golgi (step 6).

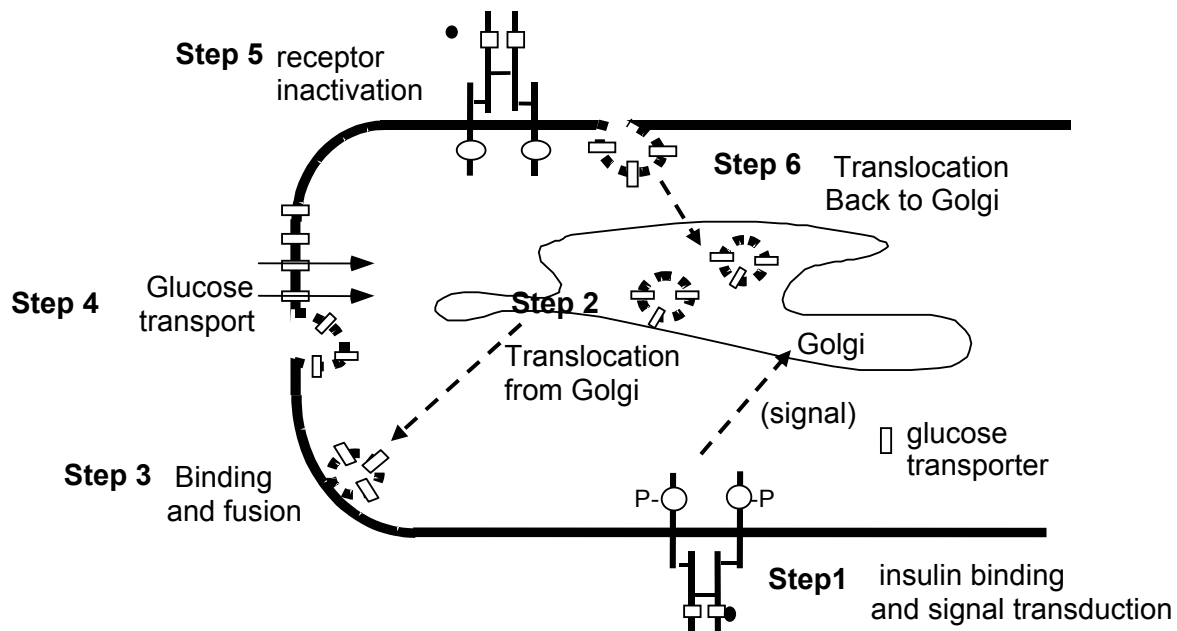


Figure 6. Insulin stimulated glucose transport (GLUT-4) in adipose or muscle cells. Glucose transporters are stored in Golgi and translocated to the plasma membrane in response to insulin binding.

INSULIN-DEPENDENT (TYPE 1) AND –INDEPENDENT (TYPE 2) DIABETES:

In diabetic patients, there is major problem processing fats and especially carbohydrates for storage. Consequently these patients present with elevated blood glucose concentrations (hyperglycemia) and this excess sugar may spill into the urine (glucosuria). The glucose takes water with it so that diabetes is associated with frequent urination and the danger of dehydration. Patients with type 1 diabetes are also susceptible to onset of ketoacidosis (overproduction of ketone bodies; to be discussed in lecture 31) if they do not take insulin injections regularly.

Despite similarities in symptoms there are important differences between type 1 diabetes and type 2 diabetes. Type 1 generally appears in childhood, though there are a significant number of cases where the disease appears between the ages of 20 and 30. In type I diabetes, the β -cells of the pancreas are destroyed by an autoimmune (self-destruction) response. Consequently the individual loses the ability to produce insulin and must be maintained with insulin injections for the rest of their life. Hence the disease is termed insulin-dependent and is also referred to as juvenile-onset because most cases appear in childhood. Patients who fail to inject their insulin as prescribed face severe long-lasting side effects due to the elevated blood glucose that can even non-enzymatically attach to hemoglobin interfering with its function.

Type 2 diabetes is often referred to as adult-onset though there has been an alarming increase in the appearance of this disease in teenagers. The initiating event in type 2 diabetes is generally the failure of tissues such as muscle and adipose tissue to decrease their ability to respond to insulin despite normal or even elevated amounts in the blood. This is termed **insulin resistance** because the cells are resisting the effects of insulin. This disease is generally insulin-independent because the pancreas continues to produce insulin. However as the disease progresses, the β -cells of the pancreas may lose some function because of their constant overstimulation by elevated blood glucose. Overstimulation generally results from the intake of dietary excesses of carbohydrates, so that obesity often precedes the onset of type 2 diabetes. The alarming increase of type 2 diabetes observed in teenagers relates to unhealthy life styles in which teenagers are overweight. This problem may be a particular issue in certain ethnic groups because of a propensity to become overweight. Because the pancreas still produces insulin, this ability generally prevents the development of ketoacidosis that may be seen in type 1 diabetics.

Table 1. Comparison of Type 1 and Type 2 Diabetes Mellitus

<i>Characteristic</i>	<i>Type 1</i>	<i>Type 2</i>
AGE AT ONSET	childhood and young adulthood but in rare instances may be as old as late 40s	middle and old age (though now starting to appear in pre-teenage patients)
RACE	predominantly Caucasians	all races; high incidence in Native-Americans; Blacks
FAMILY HISTORY OF DIABETES MELLITUS	rare	common (95%)
KETOSIS PRONE	yes	no (unless insulin-dependent)
OBESITY	rare	common (80%)
MECHANISM	autoimmune destruction	variable, often unknown
INSULIN SENSITIVITY	sensitive	resistant
REQUIRES INSULIN	yes	about half of the patients
FIRST FUNCTIONAL ABNORMALITY	decrease in insulin secretion	decrease in response to insulin
INITIAL PATHOLOGY	insulinitis; reduction in β -cell mass	little or none
LATE PATHOLOGY	absence of β -cells; increase in α and δ cells	amyloid deposition; fibrosis; β -cell mass normal or moderate reduction