Although insulin was the first endocrine hormone to be isolated and identified, its mechanism of action is still only partially understood. Once more, I refer the reader to one of the many excellent textbooks of medical biochemistry for a discussion of the insulin receptor and its interaction with insulin. Let me just state quickly that the hormone binds to its receptor which has both extra and intracellular domains. Binding of insulin to the tetrameric receptor initiates tyrosine protein kinase activity bringing about an autophosphorylation of the intracellular domains of the receptor. This marks the beginning of a phosphorylation cascade. When these processes were first identified in the early 1980s, the following cartoon appeared in Trends in Biochemical Sciences (TIBS). It gives a pretty good impression of the frustration that many researchers felt at that time. We knew that phosphorylation was involved in insulin's action, but the specific substrates and relationship to physiological processes remained in the dark.
Another observation that caused a lot of speculation was that the insulin-receptor complexes merged in the target cell's plasma membrane and gathered at clathrin-coated "pits". These underwent endocytosis and uptake into endosomes where the receptors and insulin parted company. The separated partners were then sent to lysosomes and destruction. Later, it was found that many of the receptors were reused, being sent back to the plasma membrane from the endosomes after a little control-and-reparation visit in the Golgi apparatus. Again, a great cartoon appeared in TIBS showing the state of knowledge at that point (I have added the "receptor return pathway").

Modified from "Chuck", TIBS 1980
Insulin and Glucagon regulate minute to minute metabolism.

Our metabolism is controlled by so-called feedback mechanisms. Control sensors measure blood levels of lipids, amino acids and sugar and send this information to hormone-secreting cells. In all animals, insulin is THE anabolic hormone, maintaining stores of energy and building these up after meals. Glucagon, growth hormone, adrenalin and noradrenalin oppose insulin’s actions, freeing energy in the form of fatty acids or glucose when these are needed. Insulin’s main effects are summarized in the following figure.

**Actions of Insulin**

Insulin stimulates glucose uptake in the liver, fat cells and skeletal muscle. Glucose is stored as either glycogen in liver and muscle, or as triglycerides in adipocytes. Important actions of insulin include inhibition of lipolysis, glycogenolysis, and gluconeogenesis. Insulin controls metabolism from minute-to-minute. About 50% of that insulin released from beta cells during the day is NOT associated with meals. Insulin and its “opposing hormones” constantly control our metabolism to provide optimal efficiency.
Mechanisms Involved in Insulin Action.

The interaction of insulin and its receptor initiate a protein phosphorylation cascade. Initially, tyrosine residues on the intercellular domains of the receptor become phosphorylated. This leads to phosphorylation of serine residues on insulin-receptor-substrate molecules (IRS-1 -IRS-4). Thus far, it would appear that phosphorylation of IRS-1 and IRS-3 are most important in intracellular insulin signalling. Further reactions are divided into at least three categories:

1. Those following phosphatidylinositol-3-kinase activity.
2. Those following Ras and MAP kinase activation.
3. Reactions occurring independently of IRS phosphorylation. The first group is mainly concerned with control of metabolism; the second with steering of nuclear reactions and the third is involved with glucose uptake and GLUT4 movement. These are shown in the next figure.

Mechanisms of Insulin Action

Taken together, these mechanisms control the body’s most important physiological functions; energy storage, energy use, cellular differentiation and cell growth.

It is important to keep in mind that all of these processes are controlled by a kind of “accelerator-and –brake at the same time ” mechanism. That is, both anabolic and catabolic reactions proceed simultaneously. It is the balance between these control mechanisms that determines metabolic activity.
Insulin and “Opposing” Hormones control Metabolism

Insulin is an anabolic hormone, causing cells to take up energy substrates at times of excess. Insulin action is countered by the catabolic hormones glucagon, adrenalin, and noradrenalin, and growth hormone. These act primarily through cyclic AMP (cAMP) and protein kinase A. Look at the figure below.

Insulin and “Stress Hormones” Regulate Key Metabolic Enzymes

This is merely a rough sketch over the mechanisms involved in control of metabolic hormones. Insulin’s actions are far more complex than control of enzyme phosphorylation. However, as a generalization, one can say that the catabolic hormones work through activation of protein kinase A with ensuing phosphorylation of key enzymes. Insulin activates protein phosphatases and dephosphorylates these enzymes. Some of these are activated by phosphorylation, other are inactivated through the same mechanism. Insulin activates glycogen synthetase and pyruvate dehydrogenase, and inactivates phosphofructokinase II and hormone-sensitive lipase. Complicated control mechanism steer hormone secretion such that metabolism is constantly adjusted by hormones to meet energy intake and expenditure, assuring a constant internal milieu.
Diabetes

What happens when insulin production and secretions fails? How does the body react to a collapse of the insulin signalling system? This can follow either destruction of Islet beta cells (diabetes type 1) or loss of response to insulin (diabetes type 2/insulin resistance). Several other forms of diabetes are known. The next diagram depicts the metabolic result of loss of the insulin system.

Insulin and "Stress Hormones" Regulate Key Metabolic Enzymes

Glucose uptake to muscle and fat cells is dependent upon activation of GLUT4. This system fails when insulin secretion is no longer coupled to blood glucose levels. The liver's uptake of glucose also drops off because neither glycogen synthetase nor pyruvate dehydrogenase are activated by insulin. The body reacts as though glucose was not present.

Lipolysis is activated by glucagon, growth hormone and catecholamines to meet this "low energy crisis". Massive amounts of fatty acids are released to the circulation and the liver converts these to ketone bodies. The high blood glucose levels lead to diuresis with loss of water, Na⁺, K⁺ and glucose, while the "ketones" (which are actually carboxy acids) lead to a pronounced fall in blood pH. Diabetic coma and death follow if effective treatment is not initiated.
Insulin Activation of Glucose Transport over the Plasma Membrane

The best known of the many actions of insulin is control of glucose transport over the plasma membrane of skeletal muscle and fat cells. Remember, molecules as large as glucose (MW 180) cannot simply diffuse through cell membranes (discussed here). Glucose transport protein 4 (GLUT4) carries out insulin-stimulated glucose transport. It moves from inactive stores in the Golgi apparatus to the plasma membrane following insulin-receptor interaction. GLUT4 is active as long as it is localized in the plasma membrane.

It is important to realize that muscular work (utilization of glucose and glycogen as energy substrates in muscle) can also activate movement of GLUT4 to the plasma membrane. Plasma insulin levels fall under conditions of hard work. Never the less, glucose uptake into muscle cells increases under exercise due to the response of the muscle cell to substrate use (Refer to the earlier discussion of the effects of work on glucose transport). Movement of GLUT4 to and from the plasma membrane requires the use of ATP. Furthermore, movement of GLUT4 is dependent upon an intact microtubular system: Cholchicine, a drug used in the treatment of gout, binds to microtubular proteins and causes depolymerization of these. Cholchicine blocks insulin activation of glucose transport in muscle and fat.

A recent publication by Saltiel and Kahn demonstrated insulin’s effect on GLUT4 in isolated fat cells. They used a fluorescent antibody to mark
GLUT4 and exposed cells to insulin. You can clearly see that GLUT4 was restricted to an internal region in the basal state that the GLUT4-antibody complex moved to the fat cell's outer membrane in the presence of insulin.

**Insulin Activates Glucose Uptake Through Translocation of Glucose Transport Protein 4 (GLUT4)**

Insulin has many actions in addition to regulation of glucose uptake by muscle and fat. Insulin is strongly involved in regulation of cyclic AMP levels through its effects on phosphodiesterase. Thus, insulin counters actions of the many hormones that modify metabolism through activation of adenyl cyclase. Perhaps the most important of these in regulation of homeostasis is glucagon. Insulin reduces the rate of lipolysis and is a major element in regulation of hepatic gluconeogenesis. Insulin activates amino acid uptake in most cells and is necessary for activation of protein synthesis at the nuclear level.

Let us go back to Saltiel and Kahn’s rather complex figure. Protein phosphorylation cascades are involved in most of the actions of insulin. Hormone-receptor interaction initiates an autophosphorylation of tyrosine residues on the intracellular domain of the receptor. This triggers phosphorylation of serine residues on a number of "insulin-receptor substrates" (IRS1-4 and Gab-1). The phosphorylated substrates activate a series of small "G-proteins" linked to gene expression and phosphoinositol 3-kinase that directs metabolism. Thus,
insulin controls gene expression, lipid, protein and carbohydrate metabolism and cell growth and differentiation.

Mechanisms of Insulin Action

The insulin receptor substrate proteins (IRS proteins) have been subject to further investigation by Kahn and his associates. There are a series of studies in mice with disruption of the IRS genes. Knockout of IRS-1 production slows growth, but such mice do not develop diabetes in spite of development of mild insulin resistance and glucose intolerance. Knockout of IRS-2 genes gives only a slight effect upon growth. However IRS-2 deficient mice develop insulin resistance and diabetes. The functions of IRS-3 and IRS-4 are less clearly defined. In a recent publication Kahn et al have studied the effects of combined IRS-1/IRS-3 and IRS-1/IRS-4 deficiency in mice. The first knockout pair IRS-1/IRS-3 resulted in severe lipoatrophy. This depression of adipose tissue activity also resulted in a major decrease in leptin production and release. Hyperglycemia accompanied by high insulin levels was found in these animals. The IRS-1/IRS-4 animals did not differ from mice lacking IRS-1. See Genes and Development 16 3213-3222, 2003 for more information.

One of the many studies aimed at finding a key common signal for insulin’s many actions was published in 1998 by Sheperd et. al. (Biochemical J., 333, 471-490). Here the authors summarize work that demonstrates that many of insulin’s actions appear follow activation of phosphoinositol 3-kinase, leading to formation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) and phosphatidylinositol 3,4 bisphosphate [PI(3,4)P₂]. It would appear that PIP₃ is the more important of these. PIP₃ is thought to act as a membrane anchor for elements in insulin’s

Saftel and Kahn, Nature 414, 709-806, 2001
mechanism of action as well as acting as an allosteric regulator in these complex processes.

Insulin's effects on phosphodiesterase seem to be independent of these mechanisms.

While this section may appear to be beyond the interests of second-year medical students, it does present data that is actively being pursued in an effort to discover new approaches to the treatment of diabetes.

Insulin-activation of GLUT4 transport is mediated by GTP-binding proteins.

The various actions of insulin have been shown to follow exceedingly complex mechanisms and the transport of GLUT4 is no exception. An informative publication has recently appeared in *Molecular and Cellular Endocrinology* 235 (2005) 1-9, entitled *Functional role of Rab11 in GLUT4 trafficking in cardiomyocytes*, M. Uhlig, W. Passlack and J. Eckel. This article presents an excellent review of current knowledge about the role of GTP-binding proteins in these processes. Their data show that one of these, RAB11, is intimately involved in movement of GLUT4 in response to insulin. Please go to the original article if you wish to examine the details.
Glucagon’s Mechanism of Action.

Glucagon is one of the many hormones that act through activation of adenyl cyclase, increasing the level of cyclic AMP in target cells. Glucagon receptors are found primarily in adipose tissue (where the hormone initiates lipolysis and release of fatty acids) and the liver (where it promotes glucose release through activation of glycogenolysis and gluconeogenesis). Skeletal muscle does not have receptors for this hormone. Cyclic AMP in general opposes insulin’s diverse actions, and insulin action goes at least partially through activation of phosphodiesterase and reduction of cAMP levels. This is the basis for insulin’s antilipolytic effect shown in the figure above.

The increase in the rate of lipolysis seen in diabetes is due to glucagon’s stimulation of hormone-sensitive lipase and the simultaneous loss of the braking effect of insulin on this enzyme. Hepatic gluconeogenesis is accelerated in diabetes in spite of the high levels of glucose in the blood. As stated above, the body interprets the inability to transport glucose over cell membranes as a lack of glucose. In other words, as an energy crisis requiring mobilization of stored fat and production of sugar in the liver. Normalization of blood glucose levels in mild type 2 diabetes often can be achieved by diet and inhibition of hepatic gluconeogenesis with metformin.