

Insulin's Mechanism of Action



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A basic requirement for all vertebrates is stability of the level of blood glucose. This is essential for brain function. Regardless of large fluctuations in physical activity and food intake, blood sugar levels are held within very narrow limits. The key to this is insulin, the secretion of which is closely regulated by circulating substrates of energy metabolism. Insulin signals food abundance and initiates uptake and storage of carbohydrates, fats and amino acids. Energy supply and stability of blood sugar levels postprandial is usually accorded to glucagon and the catecholamines, but the reduction in insulin signalling postprandial is almost certainly just as important. How does insulin influence our metabolism? What are the key events in its action?

1. A brief summary of effects on key metabolic pathways and enzymes.

Control of the key enzymes of metabolism can be divided into two classes:

1. Covalent modification of enzymes, usually by phosphorylation or dephosphorylation.
2. Allosteric feedback and feed-forward regulation by metabolic intermediates.

Enzymes involved in metabolism can be either activated or inactivated by phosphorylation. Examples of this are glycogen phosphorylase and hormone-sensitive lipase which are activated when phosphorylated and glycogen synthetase and pyruvate dehydrogenase are inactivated through phosphorylation. The protein kinases that catalyze phosphorylation of these enzymes are subject to control through cyclic nucleotides (PKA and cyclic AMP), Ca^{++} and diacylglycerol (PKC) and PI(3,4,5P)P3 (PKB).

The extent of enzyme phosphorylation is controlled by the balance between protein kinases and protein phosphatases. The picture becomes extremely complex when one knows that protein kinases can activate protein phosphatases. This is clearly the case for the insulin-activation of pyruvate dehydrogenase and, therefore, crucial in insulin's stimulation of hepatic lipid synthesis.

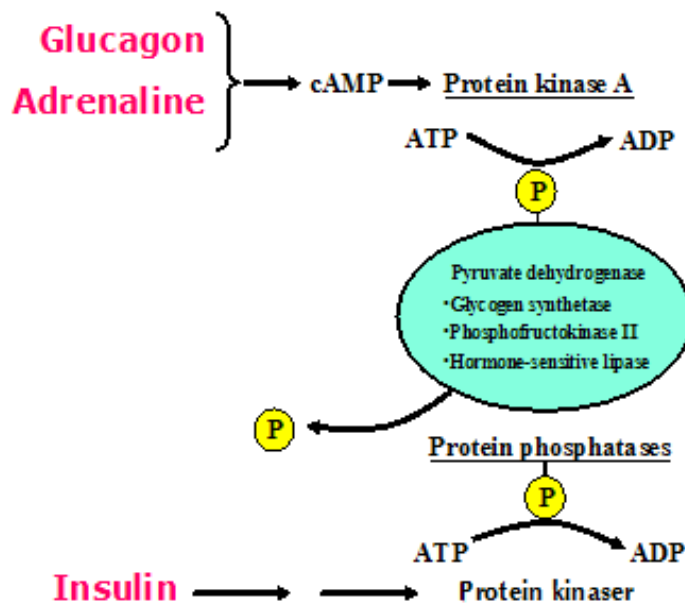
Hormone-sensitive lipase activity in fat cells is regulated largely through cAMP activation of protein kinase A (PKA). The cyclic nucleotide levels is controlled through the balance between hormone-regulated G-protein control of adenylate cyclase and breakdown of cAMP catalyzed by phosphodiesterase. Insulin regulates cAMP levels through its stimulatory effect on the esterase and reduction of cAMP levels.

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. 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

Insulin and “stress hormones” control key metabolic enzymes



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.

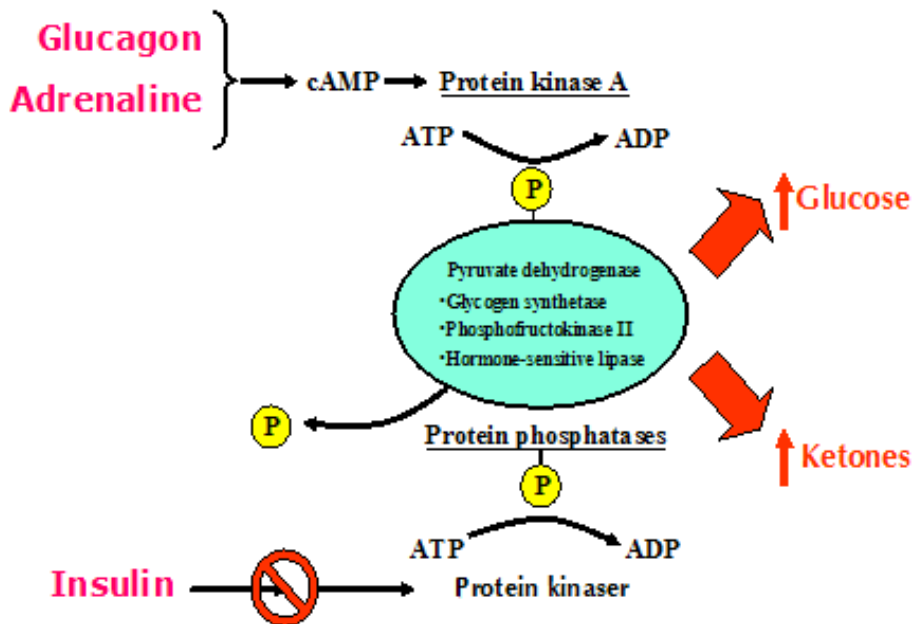
Diabetes

What happens when insulin production and secretion 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.

Glucose uptake to muscle and fat cells is dependent upon activation of GLUT4 by insulin. This system fails when insulin secretion is no longer coupled to blood glucose levels. The liver's uptake of glucose also drops

Insulin and "stress hormones" in diabetes



off because neither glycogen synthetase nor pyruvate dehydrogenase are activated by insulin. The body reacts as though glucose was not present.

Lipolysis and hepatic gluconeogenesis are 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.

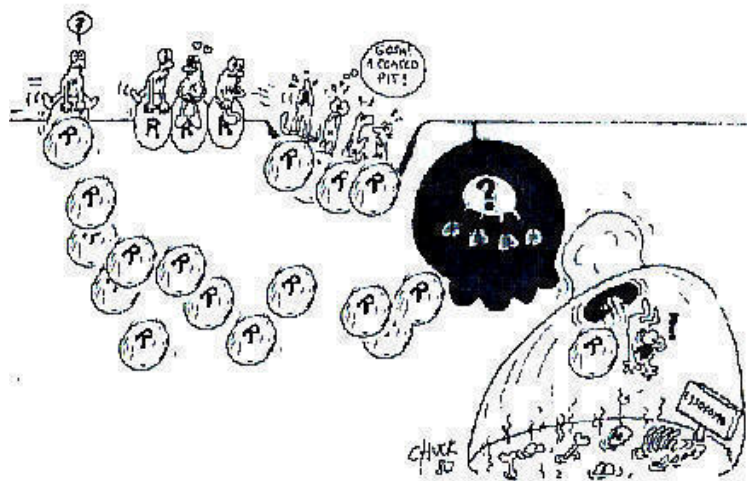
Since insulin does modify enzymatic activity and metabolic flux, allosteric effects clearly follow insulin's regulatory effects on metabolic hormones. I will not go into this large and complex field here. Please go to the literature for more information.

The metabolic effects of insulin and other hormones are discussed in detail elsewhere at Medbio.info. [Click here for more information.](#)

How does the insulin receptor work?

Insulin was the first of our hormones to be isolated and identified. Banting and Best purified insulin in 1922 and treated a diabetic patient with insulin that year. One might have expected that an understanding of the mechanism of action of insulin would quickly follow. Surprisingly, unraveling of insulin's mechanism of action as gone slowly and is still not clear.

We can our discussion begin by looking at a few cartoons from the



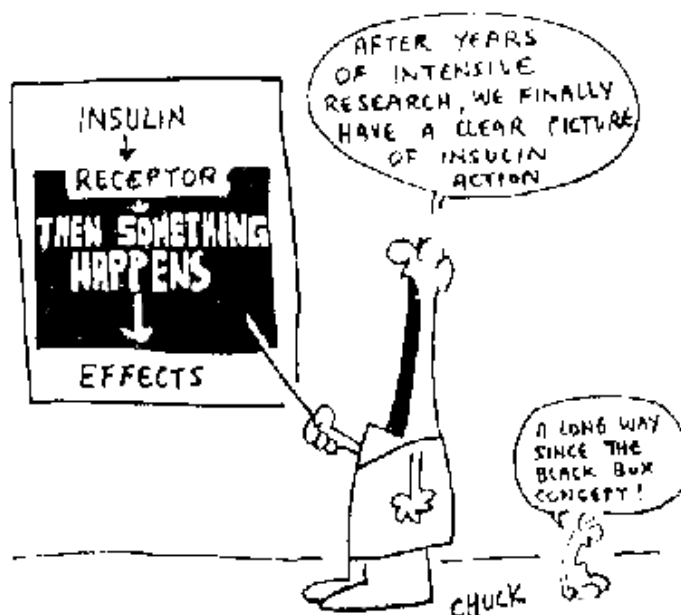
Modified from "Chuck", TIBS 1980

1980's. By that time the insulin receptor had been identified and its movements in the target cell's plasma membrane had been noted.

Insulin combined with a protein-receptor molecule, moved into clathrin-coated pits and, after a while disappeared into the cell's interior. These were thought to wind up in lysosomes and be destroyed there.

Later it was established that some of the "ingested" receptor were actually recovered, repaired in the Golgi apparatus and reappeared in the cell's plasma membrane.

This was really exciting at that time, and many of us went to work to find out what happened when the hormone bound to its receptor. We were looking for a "simple" signal substance that went from the activated hormone-receptor complex into the cell and which started up all of those mystical actions of insulin.

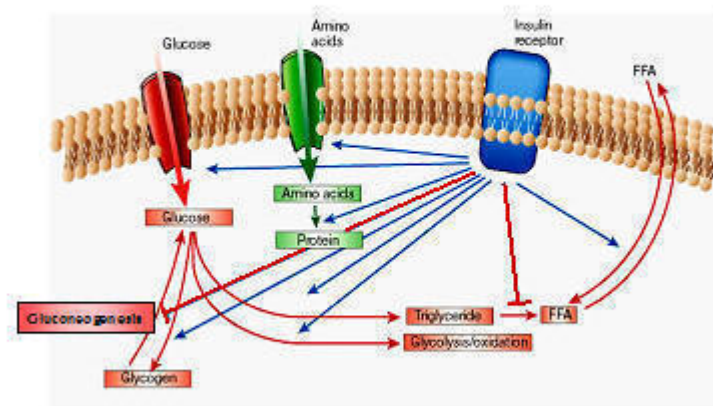


The situation was summarized in another beautiful cartoon by "Chuck", published in TIBS. We were just lacking that single second messenger for insulin that could carry out all of the complex

actions the hormone had.

Well, soon afterward it became clear that one of the very first things that happened after hormone binding was initiation of autophosphorylation reactions, whereby the intracellular parts of the receptor became tyrosine-phosphorylated by the protein kinase activity of these same receptors. A phosphorylation cascade followed and started up a whole

Actions of Insulin



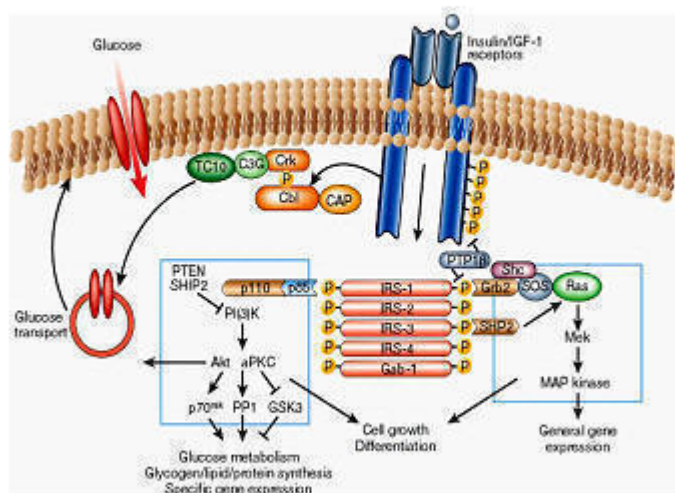
Modified from Sattler and Kahn, Nature 414, 799-806, 2001

series of enzyme phosphorylation/dephosphorylation reactions which are now thought to account for the effects of insulin. Insulin's action are summarized in the next figure. The active receptor speeds uptake of amino acids and glucose, activates protein synthesis from amino acids and glycogen and triglyceride synthesis from glucose. Insulin inhibits breakdown of triglycerides in adipose tissue and gluconeogenesis in the liver. A whole series of intracellular signal substances seemed to be responsible for the many actions of insulin.

These are shown in the next figure from the work of Saltiel and Kahn. Here we can see that the phosphorylation of the insulin receptor starts up serine-threonine phosphorylation of a series of proteins, the so-called insulin-receptor substrates (IRS1-4). These are coupled to several additional protein kinase signal systems:

1.

Mechanisms of Insulin Action



Saltiel and Kahn, *Nature* 414, 799-806, 2001

Pathways signaling through PI 3-kinase and phosphatidylinositol (3,4,5)P3 (PI-3 kinase and protein kinase B/Akt).

2. Mitogen-activated protein kinases (MAPKineses).

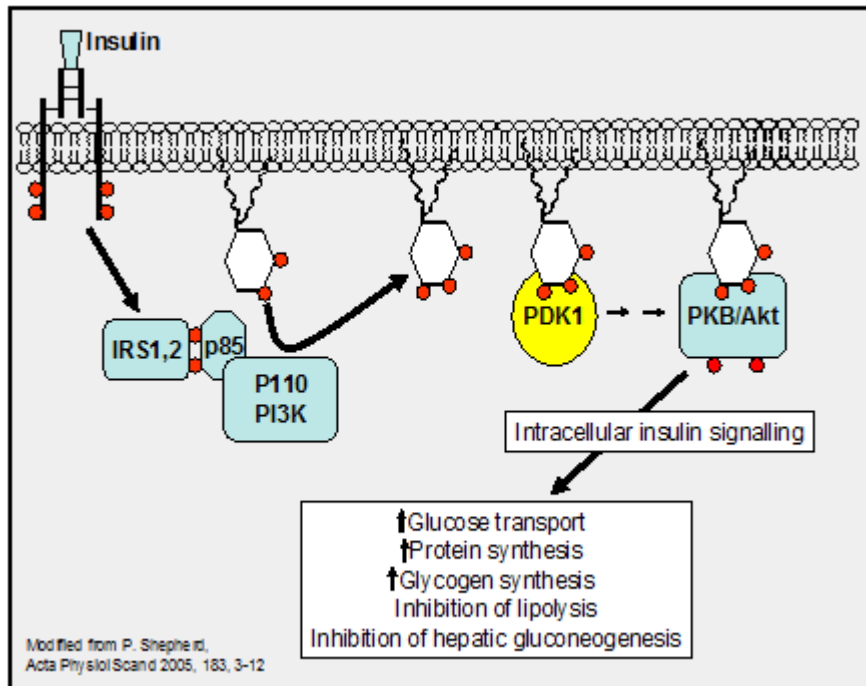
NB: Both group 1 and 2 signals also activate protein kinase C γ and Protein kinase C ζ .

3. Possible interaction via kinases not coupled to IRS proteins.

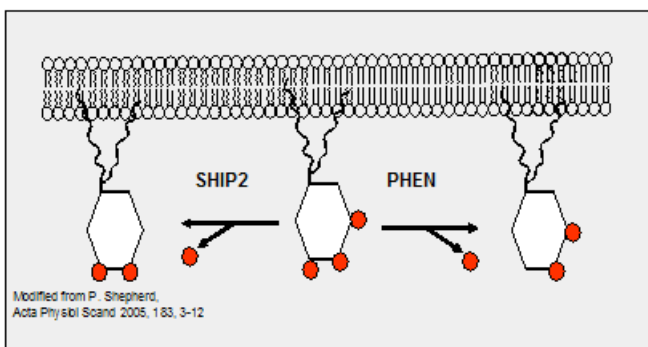
It has been suggested that the most dominant is the first group (PI 3-kinase) which converts phosphatidylinositol 3,4 bisphosphate [PI (3,4)P₂] to phosphatidylinositol 3,4,5 triphosphate (PI 3,4,5)P₃. These nucleotides act as anchors, binding down-line protein kinases to the plasma membrane and activating them. These nucleotides seem to be responsible for the alterations in carbohydrate, protein and lipid metabolism that are initiated by insulin.

It has recently been suggested that PKB or Akt is THE central element in the actions of insulin. Look at the next figure from the work of Professor Peter Shepherd (*Acta Physiol Scand* 183, 3-12, 2005). Phosphorylation of the insulin receptor and IRS1 and 2 lead to binding and activation of phosphatidylinositol 3 kinase (PI3K) and formation of PI (3,4,5)P₃. This

then binds to the plasma membrane and associates with phosphoinositol- dependent kinase-1 (PDK-1) and this leads to



phosphorylation and activation of protein kinase B, otherwise known as Akt. And, finally, the activated Akt is thought to initiate many of the metabolic actions of insulin.

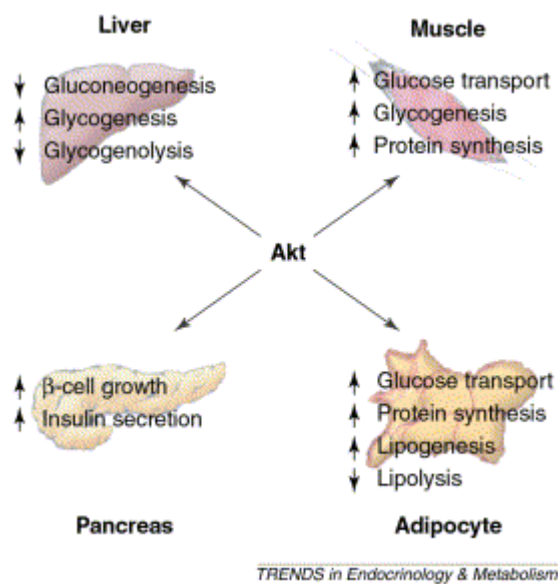


Just to complicate things a little more, the levels of PI(3,4,5)P3 are under control of two additional enzymes, also closely tied to metabolism. Both SHIP2 and PHEN inactivate PI(3,4,5)P3 by converting it back to a diphosphate. SHIP2 removes the 5' phosphate while PHEN takes the 3' phosphate. SHIP2 is regulated by a tyrosine kinase and PHEN activity by the cell's oxidative state. The point is that Akt or PKB activity is closely controlled and a major contributor to the overall stability of our inner milieu. SHIP2 and PHEN have been

implicated as controllers of insulin signaling in a recent publication by Decker and Saltiel ([Nature Medicine 11, 123-124 \(2005\)](#)).

Theory is one thing, clinical observations another. In a recent publication, Barbra Kahn and coworkers at Harvard University have shown that protein kinase C isoforms gamma and zeta are involved in development of insulin resistance in humans. They observed that the levels of these isoforms in skeletal muscle were reduced in obese diabetic patients and that weight reduction reversed insulin resistance and enhanced PKC/PI-3K activity. [You can pluck up that article by clicking here.](#)

The very central role of Akt was emphasized in a recent review article article in TIBS (TRENDS in Endocrinology and Metabolism 13, 444-451 (2002). See the next figure.



Here we can see that Akt is suggested to be involved in almost all of the actions of insulin. This means that receptor phosphorylation and ensuing protein phosphorylation cascades and phosphatidylinositol phosphorylation are all closely involved in the day-to-day control of metabolism. Knowledge of the details in this process may make possible new approaches to control of diabetes without use of insulin.

I include another reference for those who are interested in protein kinase signaling. This is a most complicated field but is absolutely essential for an understanding of endocrinology. Click on the following if your library has a subscription: [Protein Kinase A Signaling, "Cross-talk" with other Pathways in Endocrine Cells, A. Robinson-White and C. A. Stratakis, Ann. N. Y. Acad. Sci. 968: 256-270 \(2002\).](#)

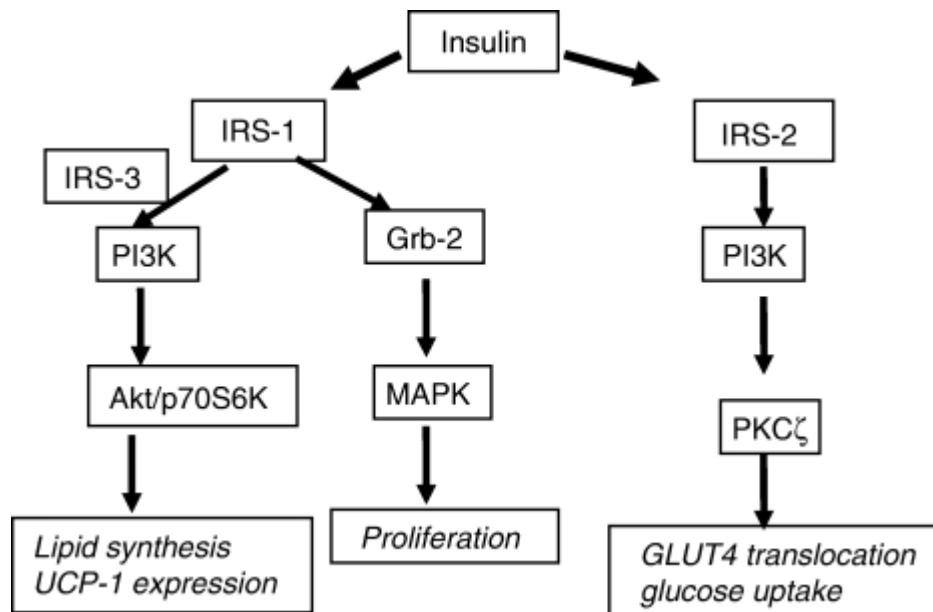
A Model System for Insulin Action

Another article in Acta Physiologica Scandinavia volume 183 deals with brown fat tissue (BAT) as a model for insulin action in human tissues ([A. M Valverde et al, Acta Physiol Scand 2005, 183, 59-73](#)). The human brown fat cell is insulin-sensitive and insulin stimulates glucose uptake into the cells and triglyceride synthesis. Glucose transport is mainly

carried out by GLUT4, as is the case in skeletal muscle, the body's major insulin target organ. Valverde and coworkers have studied the effects of various enzymatic inhibitors on BAT's responses to insulin. Their data show that several protein kinase systems are involved here.

Activation of IRS-2/PI3K/Akt and the protein kinase zeta (PKC ζ) were shown to be required for translocation of the glucose transport protein from storage areas to the cell surface. Phospholipase C γ activation with release of IP $_3$ and Ca $^{++}$ are also involved.

The roles of the IRS proteins in transmitting the insulin signal in BAT are summarized in the next figure, taken from the same article.



This "simple" model system involves a series of enzymes coupled to the phosphatidylinositol and protein kinase systems. Most members of these groups can interact. Insulin resistance and type 2 diabetes could follow malfunction at any of these steps.

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 very 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 movement of the insulin-sensitive glucose transporter and new findings 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.

The Molecular Pathway of Insulin Resistance

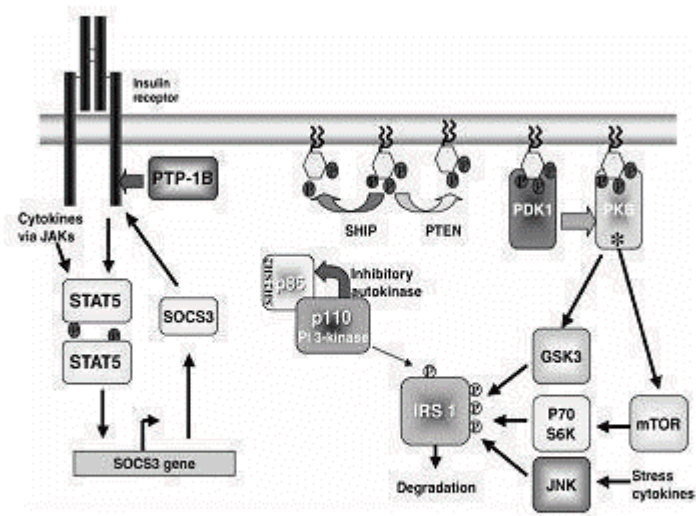
Resistance to insulin and the following increased secretion of the hormone from β -cells is characteristic for people with glucose intolerance. They are on the way toward developing type two diabetes. That is, while their insulin receptors usually are activated by the hormone and these do phosphorylate IRS1 and 2, the insulin signal is dampened further down the line. Which of the protein kinase cascades leading from IRS1/2 is effected in the resistant cells?

In a recent article in Medscape by Wang, Goldstone and Draznin, it is argued that there is good evidence that only the PI 3K pathway loses its response to the insulin receptor's interaction with IRS1 and 2. The Grb2/MAPK pathway appears to be normal or, in fact, stimulated in people with type 2 diabetes. The body seems to adjust insulin secretion to normalize the metabolic effects of the hormone through the PI 3K cascade. The authors put forth the argument that the compensatory hyperinsulinemia required to normalize the metabolic actions of insulin via PI 3K, PDK and Akt lead to over-stimulation of the MAPK pathway. The authors suggest that the CVD that is often seen in diabetes patients is caused both by a loss of NO production (PI 3K activated) and by increased MAPK expression. [Go to the original article for more information \(click here\).](#)

Additional control mechanisms

Activation of hormone receptors often initiates reactions that dampen the effects of that hormone. One of the first systems which was identified was the adrenergic beta receptor kinase or "Bark" reaction. Here, activation of the beta adrenergic receptor activates a kinase (Bark) that phosphorylates and inactivates the beta adrenergic receptor. A similar "feedback" effect is seen in the case of the insulin receptor. A family of proteins known as "suppressor of cytokine signaling" or SOCS bind to the insulin receptor substrate (IRS 1 and 2) and inhibit their activity. This is but one of the many feedback reactions controlling insulin signaling. Many of these are described in the article by Shepherd. A figure from that article is reproduced below. Here, we can see that activation of the insulin receptor pathway not only activates metabolic enzymes etc., but also initiates "turn-off" reactions. The activated

metabolic enzymes phosphorylate members of the signaling family and



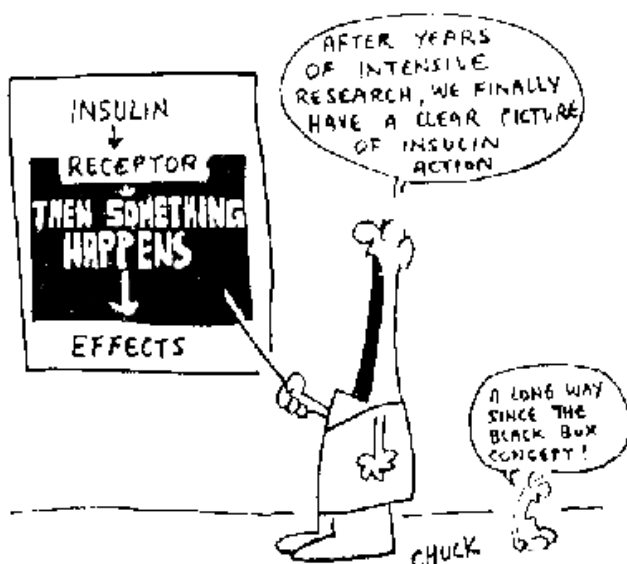
reduce their activity. Additionally, the SOCS gene is activated and SOCS (or SOCS3 here) is turned on and inhibits the insulin receptor. This diagram is quite complex but in no way underestimates the control mechanisms involved in insulin's action.

A simple conclusion (for now).

Most of the publications concerning insulin's mechanism of action conclude that, although the authors have made progress in understanding how insulin works, much remains unclear and activity must and will continue.

To put it simply, that drawing from TIBS is still actual, insulin combines with its receptor, activates a lot of enzymes and cofactors "and then something happens". Defining "something" will require perception of interplay between all of the many factors involved in the endocrinologic control of metabolism.

We are still a long way from a good understanding of the mechanisms through which insulin controls metabolism. However, this is an extremely active research area and progress is reported almost every



day. Go to [Acta Physiol Scand 183 \(2005\)](#) or Science Magazine <http://www.sciencemag.org/sciext/diabetes/> the latest views in this area. Another article giving a detailed overview of today's knowledge is [Modification of Insulin Action](#), L. Pirola, A. M. Johnston and E. Van Obberghen, Diabetologica (2004) 47, 170-184. Just click on the title to call it up if you have access to a library.