Insulin's Mechanism of Action

A basic requirement for all vertebrates is an adequate and stable level of blood glucose. This is essential for brain function; hypoglycemia (blood sugar levels less than around 3.0 mmol/l) or rapidly declining blood glucose levels quickly lead to dizziness, loss of consciousness and, eventually, death. In spite of large fluctuations in physical activity and food intake, blood sugar levels are held within very narrow limits. The key to this is the "hormone pair" insulin and glucagon. The secretion of these pancreatic peptides is closely regulated by circulating substrates of energy metabolism. Insulin signals food abundance and initiates uptake and storage of carbohydrates, fats and amino acids. Glucagon signals the fasting state and activates lipolysis in adipose tissue and hepatic gluconeogenesis.

Let us consider insulin and its mechanism of action first. What are the key events in its action?

1. A brief summary of insulin's 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, usually following covalent modification of enzymes.

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 by cyclic nucleotides (c-AMP and c-GMP), Ca^{++} and phosphoinositides (see Di Paolo and De Camilli, Nature 443 (2006)651-657 for a review article over the effects of phosphoinositides in cell regulation: click here if you have library conections)

The extent of phosphorylation of an enzyme is controlled by the balance between protein kinases and protein phosphatases. The picture becomes extremely complex since protein phosphatases subject to control through phosphorylation. This balance is clearly the case for the insulin-activation of pyruvate dehydrogenase and is, therefore, crucial in control of hepatic lipid synthesis by insulin.

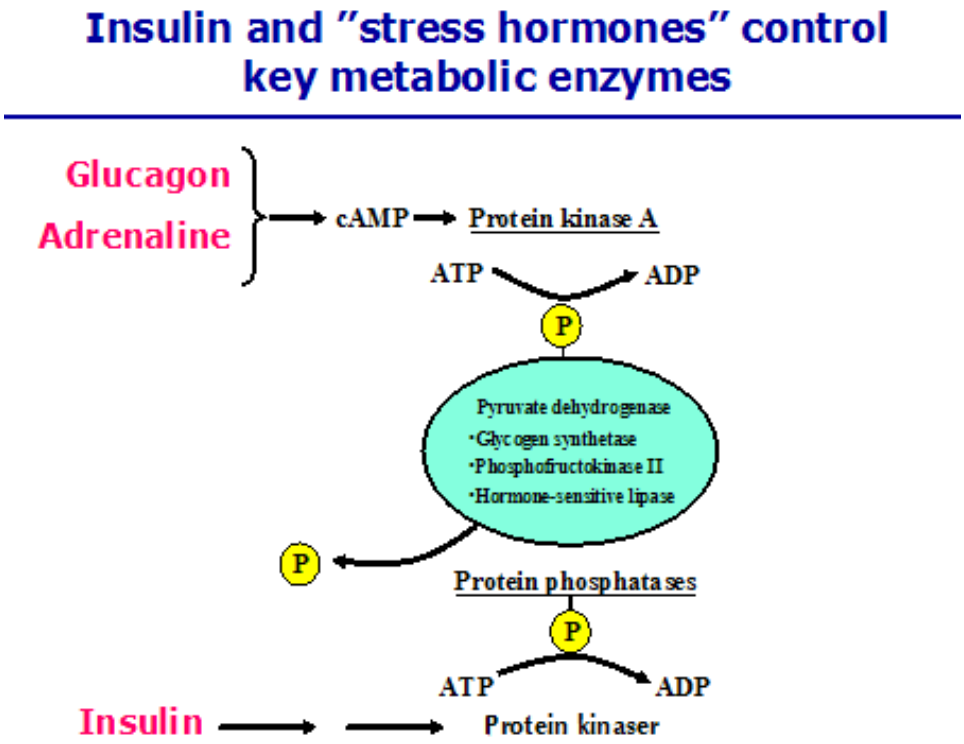
Another example of the interplay between phosphorylation and dephosphorylation is found in hormone-sensitive lipase activity in fat cells. This enzyme is regulated largely through cAMP activation of protein kinase A (PKA). The cyclic nucleotide level is controlled through the balance between hormone-regulated G-protein stimulation

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. In this manner insulin is a major actor in regulation of lipolysis in adipocytes.

Insulin and "Opposing" Hormones control Metabolism.

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

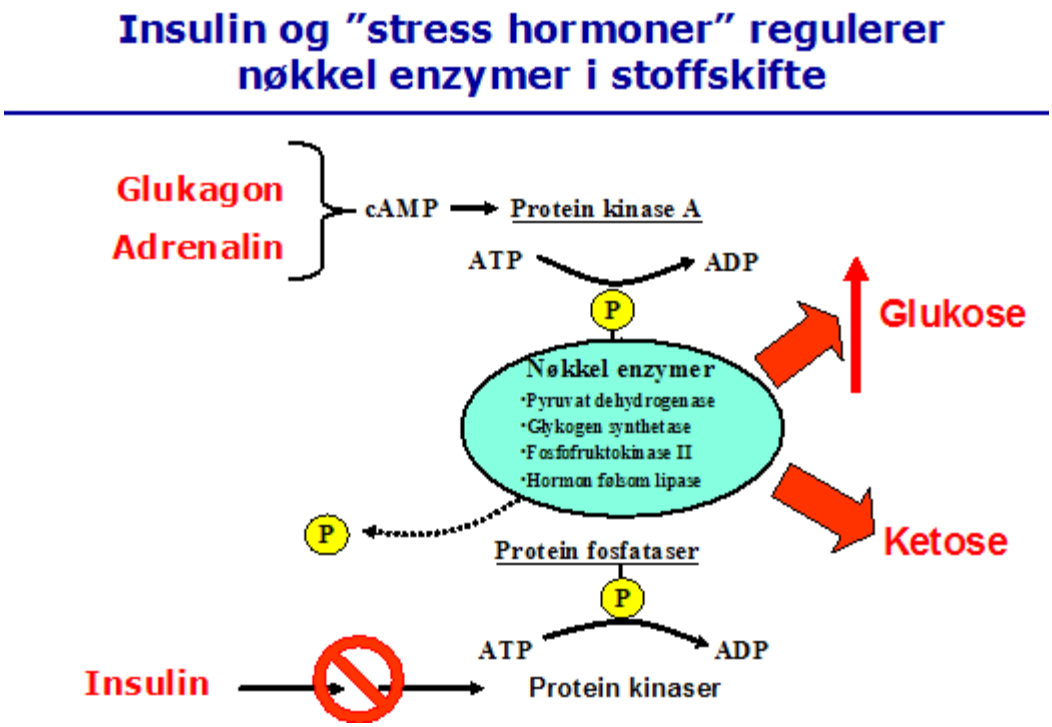
The figure below is a very rough sketch over the mechanisms involved in control of metabolism by hormones. While insulin's actions are quite complex, one can say that the catabolic hormones often work through activation of protein kinase A with



ensuing phosphorylation of key enzymes. Furthermore, insulin often activates protein phosphatases and initiates dephosphorylation of enzymes involved in energy metabolism. 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 phosphorylation of enzymes involved in metabolism is constantly adjusted 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). The next diagram depicts the metabolic result of loss of the insulin system. Please note that these diagrams give a very simplified view of hormone action.

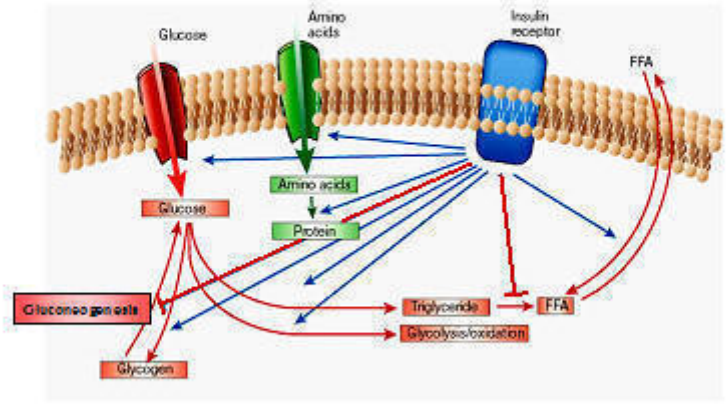


Glucose uptake to muscle and fat cells is dependent upon activation of GLUT4 by insulin. This system fails when pancreatic islet cells no longer produce insulin (diabetes type 1) or when the cellular response to insulin is diminished (insulin resistance). In spite of the increasing level of glucose in the blood, the body reacts as fasting state was entered, in spite of the high level of blood sugar. That is, insulin-sensing cells no longer register the sugar's presence. This leads to an increased release of fatty acids from adipose tissue where lipolysis is dominated by signals from catabolic hormones. The increase in serum fatty acids increases uptake in the liver with ketogenesis and export of ketone bodies. Hepatic gluconeogenesis is stimulated by catecholamines and glucagon in the absence of insulin's "countersignal". Release of glucose from the liver is therefore increased in diabetes in spite of the existing high glucose level. The body reacts as though it had entered a "starved state".

In summary, the diabetic state is marked by increased lipolysis and hepatic gluconeogenesis induced 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) can lead to a pronounced fall in blood pH. Diabetic coma and death can follow if effective treatment is not initiated.

Actions of Insulin



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

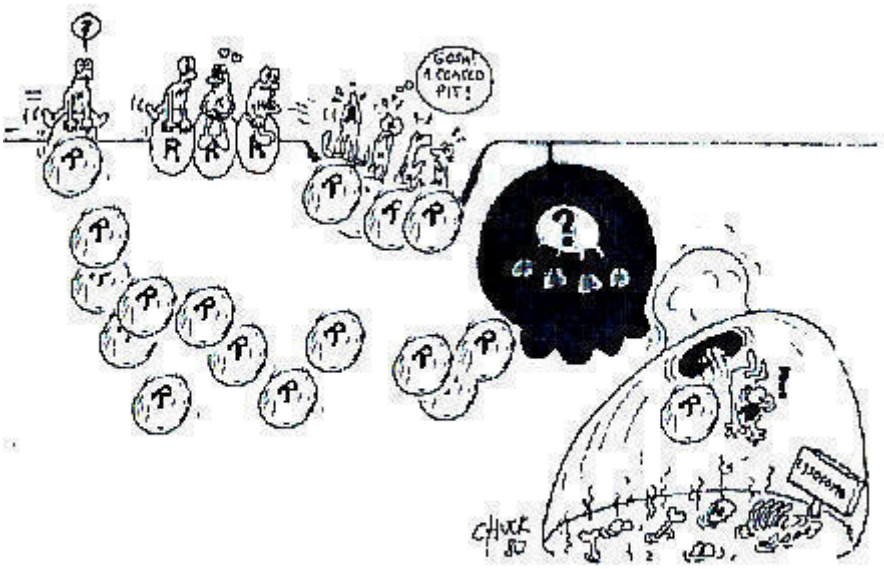
Insulin's actions are summarized in the next figure. The active receptor speeds uptake of amino acids and glucose, activates protein synthesis from amino acids and glycogen and triglyceride synthesis from glucose. Insulin inhibits breakdown of triglycerides in adipose tissue and gluconeogenesis in the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

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.

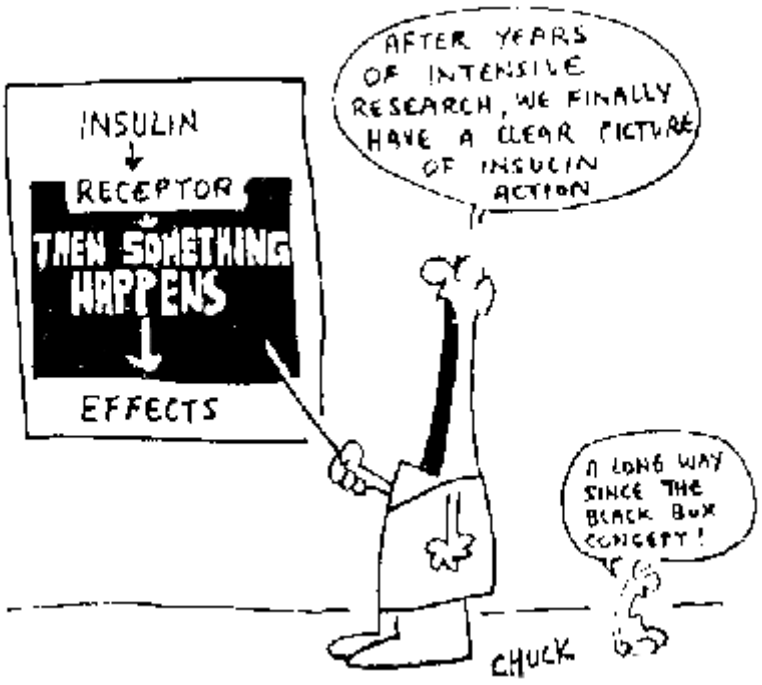
We can our discussion begin by looking at a few cartoons from the 1970-80s. By that time the insulin receptor had been identified and its movements in the target cell's plasma membrane had been noted.



Modified from "Chuck", TIBS 1980

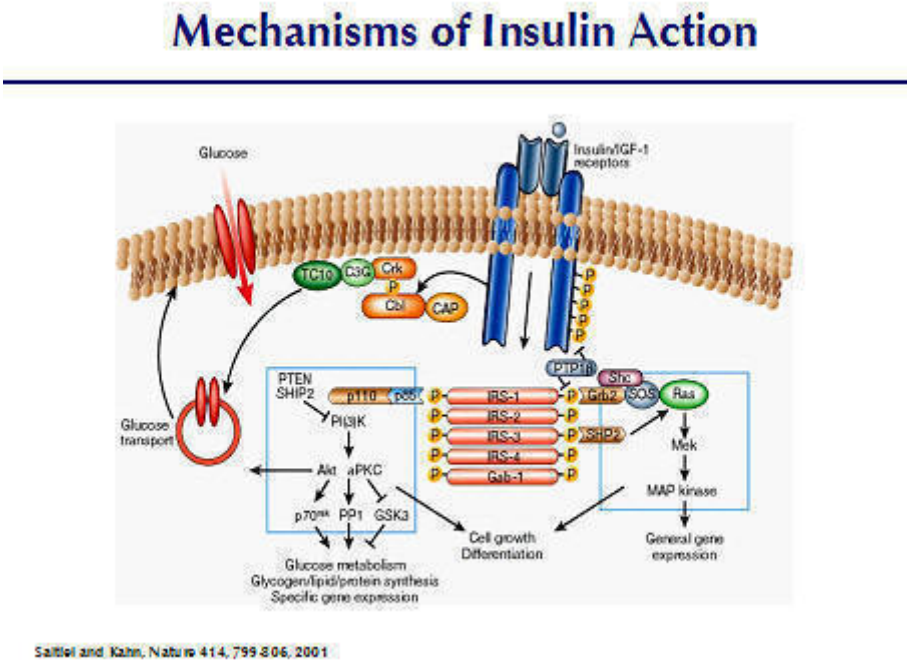
Insulin combined with its receptor moved into clathrin-coated pits and, after a while, disappeared into the cell's interior. These complexes were thought to wind up in lysosomes and be destroyed there. Later it was established that some of the "ingested" receptors were actually recovered, repaired in 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 in 1979. We were just lacking that single second messenger for insulin that could carry out all of the hormone's complex actions.

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 series of enzyme phosphorylation and dephosphorylation



reactions which are now thought to account for the effects of insulin. These are shown in the next figure from the work of Saltiel and Kahn. Here we can see that the autophosphorylation of the insulin receptor tyrosine residues starts up a protein phosphorylation cascade. First out are a set of proteins known as insulin-receptor substrates (IRS-1-4). These are coupled to several additional protein kinase signal systems:

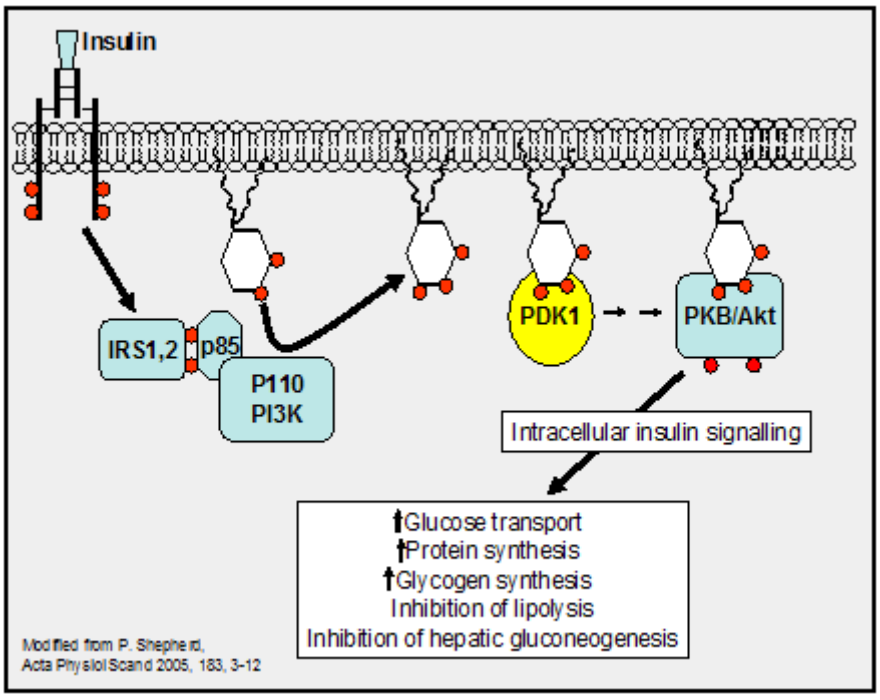
1. Pathways signaling through PI 3-kinase and phosphatidylinositol (3,4,5)P3 (PI-3 kinase and protein kinase B/Akt).

2. Mitogen-activated protein kinases (MAPKinases).

NB: Both group 1 and 2 signals also activate protein kinase Cγ and Protein kinase Cζ.

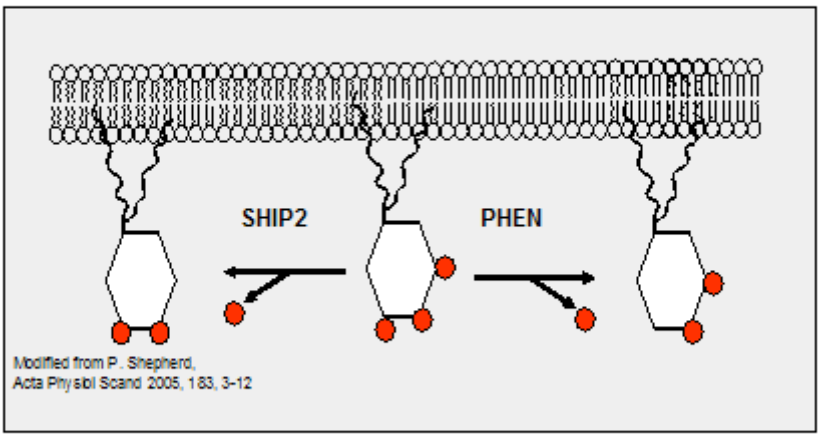
3. Possible interaction via kinases not coupled to IRS proteins.

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 (PI3,4,5)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.

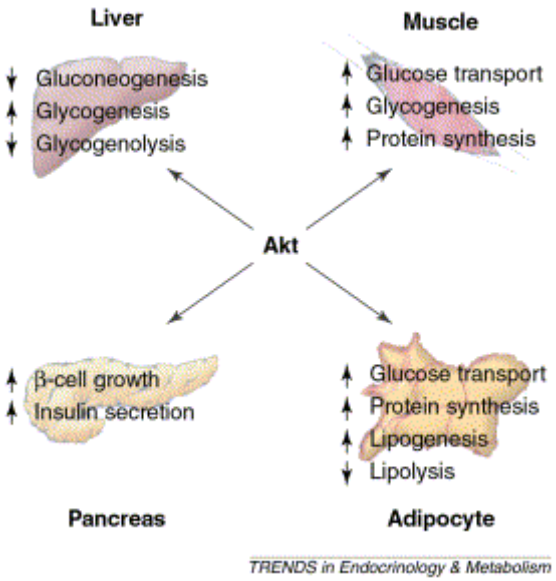


Professor Peter Shepherd has suggested that PKB or Akt is THE central element in the actions of insulin. Look at the next figure from the work of Professor Peter Shepherd (Acta Physiol Scand 183, 3-12, 2005). Phosphorylation of the insulin receptor and IRS1 and 2 lead to binding and activation of phosphatidylinositol 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 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 Endochrine CElls, A. Robinson-White and C. A. Stratakis, Ann. N. Y. Acad. Sci. 968: 256-270 (2002).

More recently, Biddinger og Kahn have published a review article in Annu. Rev. Physiol. 2006. 68:123-58 entitled "From Mice to Men: Insights into the Insulin Resistance Syndrome". Click here if you have library services. Two figures are presented with the authors permission from that article. They present our understanding of the insulin receptor's action pathways and their possible involvement in the insulin resistance syndrome. Insulin's target areas are control of

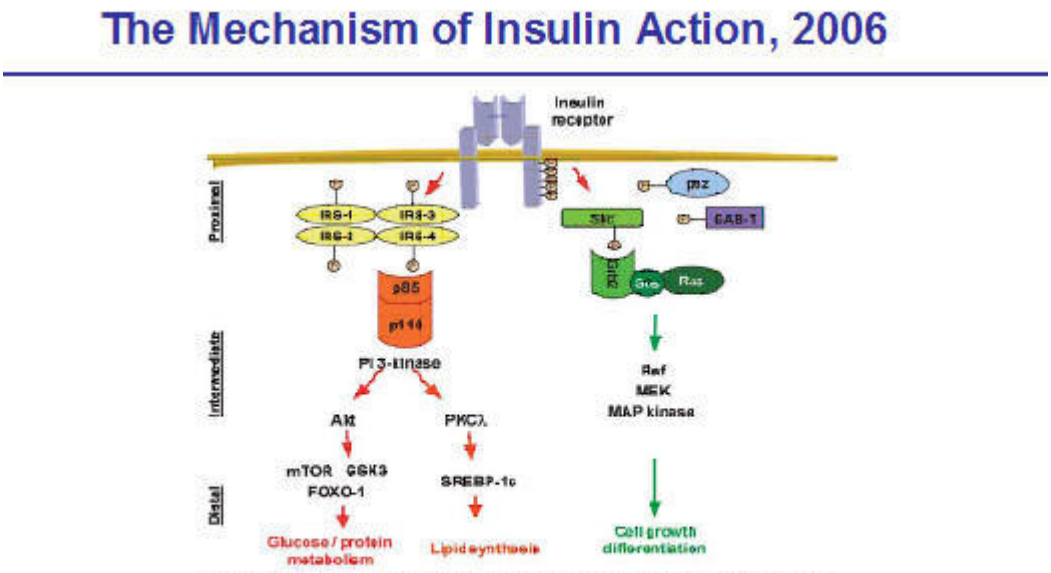
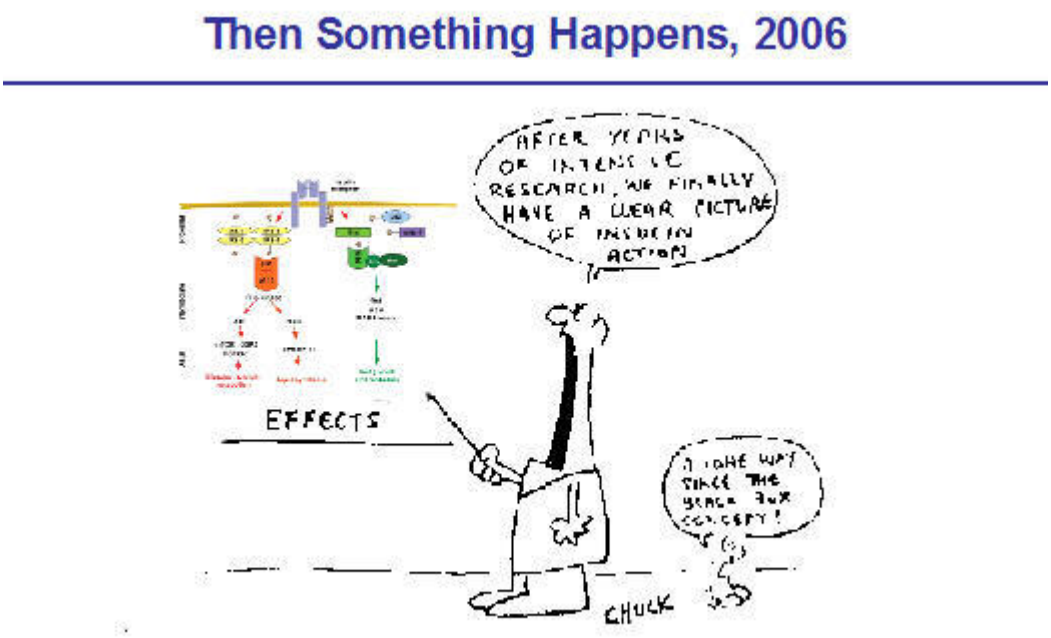


Figure 6 The insulin signaling network. Insulin signaling impacts many cellular processes, including the metabolism of glucose, protein, and lipids, as well as cell growth and differentiation. Thus, the insulin signaling network is broad and complex.

S. B. Biddinger and C. R. Kahn, Annu. Rev. Physiol. 2006. 68:123-158

lipid metabolism, glucose and protein metabolism and cell growth and differentiation. Each of these is coupled to the insulin receptor by defined separate pathways and signal substances. Both glucose and protein metabolism and control of lipid metabolism follow activation of the IRS-system and conversion of phosphatidylinositol (4,5)P_2 [PtdIns(4,5)$P_{2]}$ to the triphosphate PtdIns(3,4,5)P_3 by PI 3-kinase. The pathways then split with several new factors for protein and glucose metabolism one side, and others for lipid metabolism. Insulin effects on the nucleus involve still other factors. The bottom line is that we still do not know all of the players and steps in insulin action.

I have taken the liberty of combining a few figures to give a indication of today's knowledge. A simplification, of course! Read more below.



The links between PKB/Akt and insulin's physiological actions.

The links between Akt/PKB and the elicited effects of insulin and glucagon have been reported to consist of several response elements. These are small proteins which modify genetic activity and synthesis of several enzymes involved in carbohydrate and lipid metabolism. With time, these may become targets for therapy for type 2 diabetes. Interested readers can click on the following links:

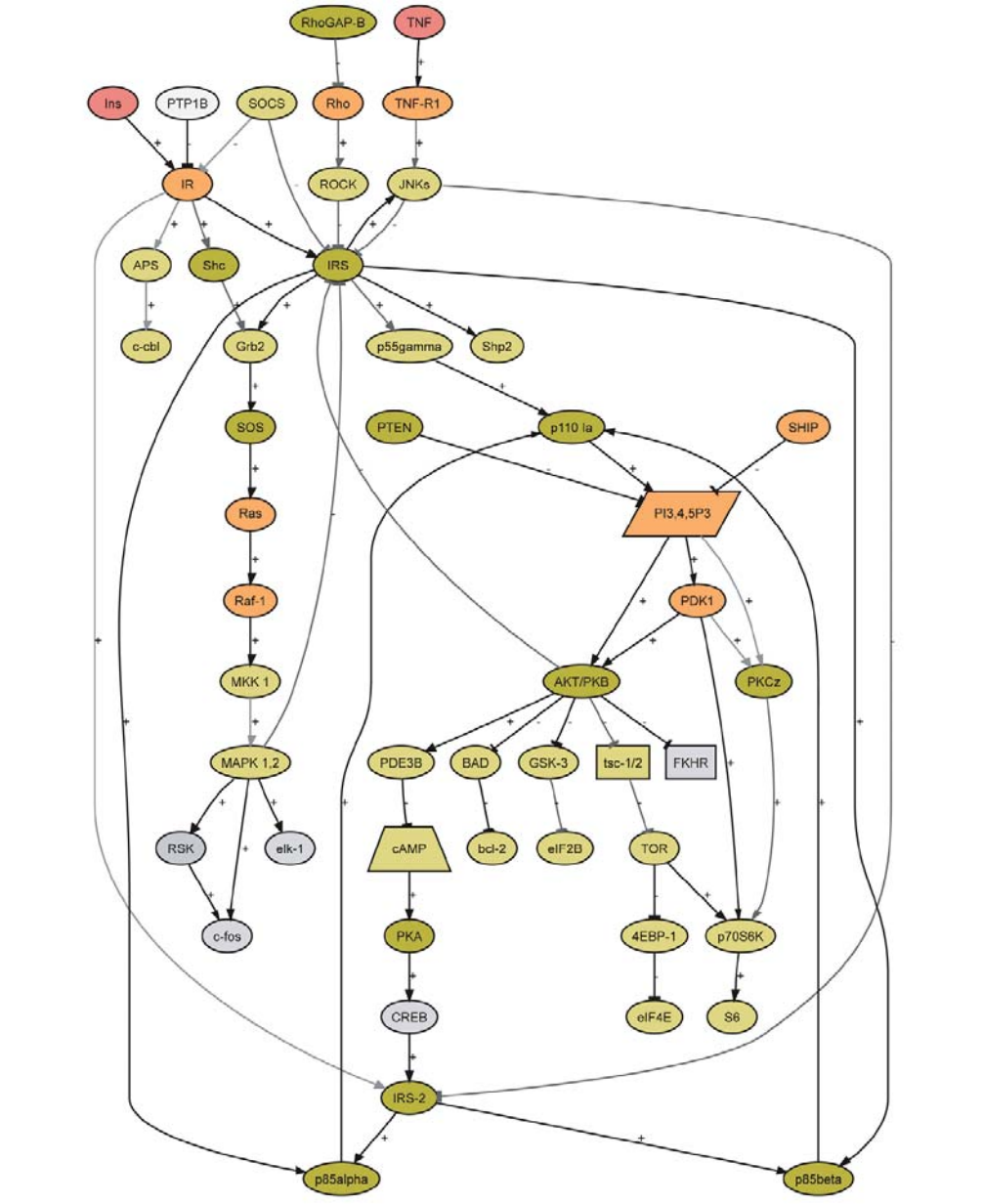
1. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1, Nature 2001, 413, 179-183.

2. Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. Nature 2004, 432, 1027-1032.

3. Nutrient control of glucose homeostasis through a complex of PCG-1 and SIRT1. Nature 2005, 434, 113-118.

4. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism, Nature 2005, 437, 1109-1114.

An Overview of the Insulin Signaling System; August 2005.



M.F. White, Insulin Signaling Pathway. *Sci. STKE* (Connections Map in the Database of Cell Signaling, as seen 23 March 2007), http://stke.sciencemag.org/cgi/cm/stkecm;CMP_12069.

We have become increasingly aware of the enormous consequences of overweight, obesity and resulting resistance to insulin over the past few years. An understanding of the cause of resistance would very greatly aid treatment of diabetes type II patients. However, that understanding remains out of reach. The reason for this is the extreme complexity of the cellular insulin-signaling system. An overview of which has recently been published in Science Magazines "Signal Transduction Knowledge Environment". The figure is from that

article. Not only are there many factors involved here, but interplay and feedback regulation occurs at many levels. Please go to the original paper to come further with this. Click here.

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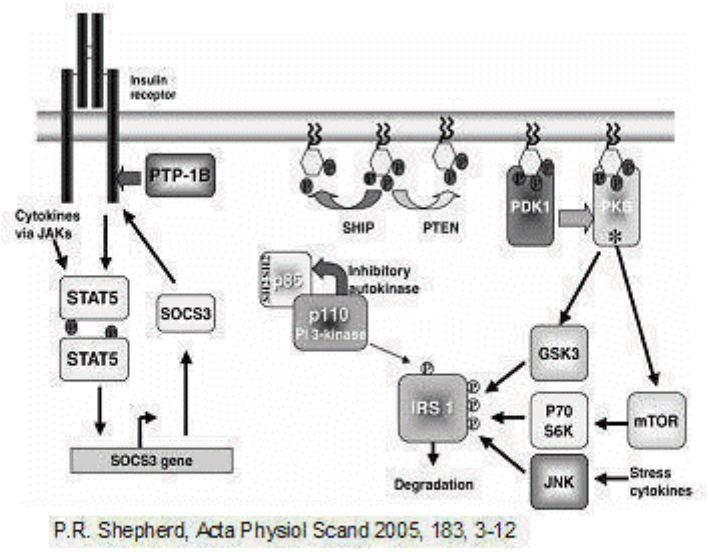
Some Approaches to an Understanding of

The Molecular Pathway of Insulin Resistance.

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 "ßark" reaction. Here, activation of the beta adrenergic receptor activates a kinase (ßark) 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 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

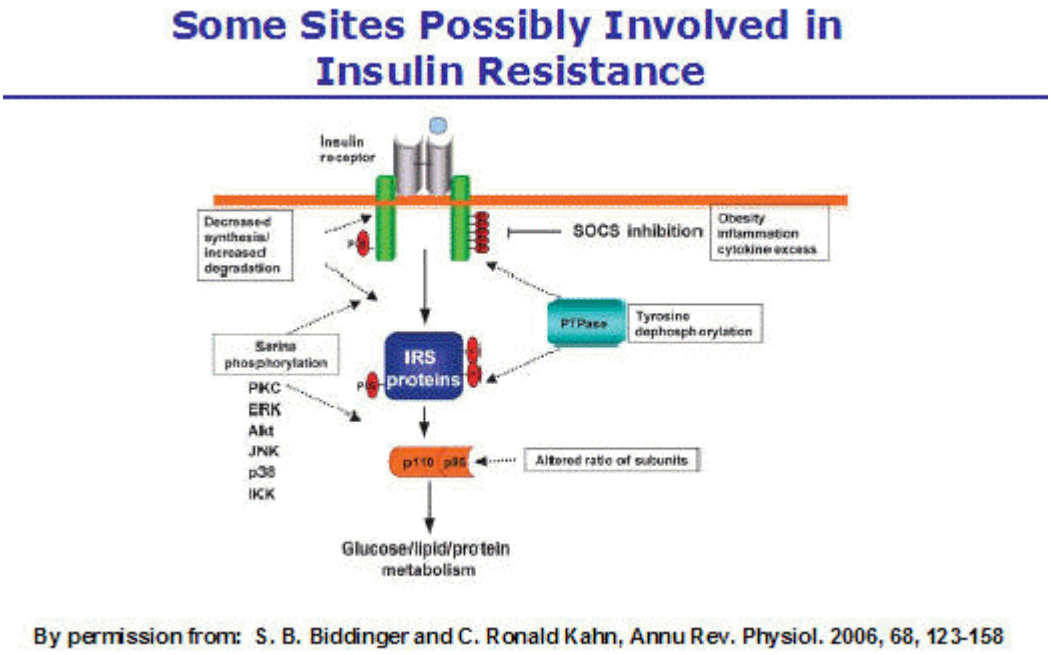


P.R. Shepherd, Acta Physiol Scand 2005, 183, 3-12

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.

Resistance to insulin and the following increased secretion of the hormone from ß-cells is characteristic for people with glucose intolerance. They are on the way toward developing type two diabetes. In most patients the insulin receptors respond normally to the hormone and phosphorylate IRS1and 2. The insulin signal is dampened further down the line. Which of the protein kinase cascades leading from IRS1/2 is affected in the resistant target cells?

Bidding and Kahn have summarized many possible sites for loss of sensitivity to insulin in the following figure. No single fail can be pointed out as the cause for insulin resistance. A reduction in the numbers of receptors is found in some few persons. A very small percentage develop antibodies to the receptor. Decreased autophosphorylation of tyrosine residues can follow inflammation of target cell



By permission from: S. B. Biddinger and C. Ronald Kahn, Annu Rev. Physiol. 2006, 68, 123-158

membranes and obesity. Phosphotyrosine and phosphoserine phosphatases can reduce the outcome of receptor activation. Phosphatidylinositol-3 kinase is subject to modification by a number of factors and these will alter IP(3,4,5)P3 levels and insulin signaling.

Additionally, alterations in the effects of various response elements can modify the effects of insulin on gene expression.

In a recent article in Medscape by Wang, Goldstone and Draznin, it is argued that there is good evidence that only the PI3K 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 PI3K cascade. The authors put forth the argument that the compensatory hyperinsulinemia required to normalize the metabolic actions of insulin via PI3K, 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 (PI3K activated) and by increased MAPK expression. Go to the original article for more information (click here).

NB: For a good and very clear discussion of the clinical consequences of diabetes type 2 and current treatment methods, go to the following CME from Medscape (Click on the title): New Mechanisms of Glucose Control: Expanding Therapeutic Options with Incretin Mimetic Therapies.

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.