

# How Insulin Works

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## Insulin's metabolic actions.

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 of serine, threonine or tyrosine residues.
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).

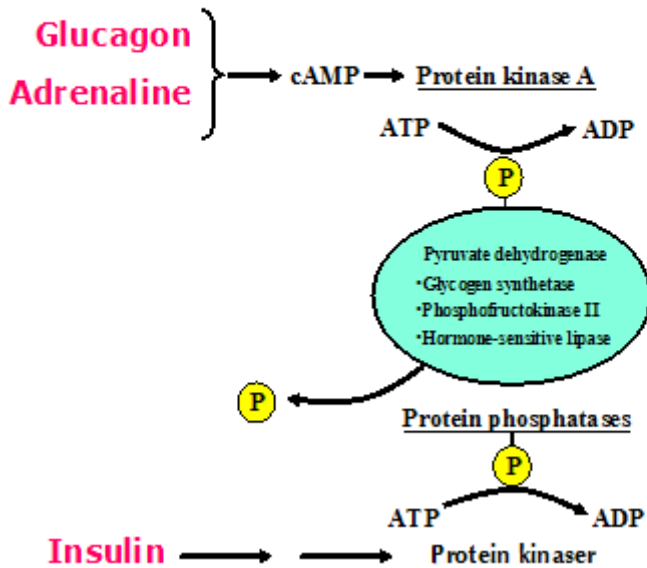
The extent of enzyme phosphorylation is controlled by the balance between protein kinases and protein phosphatases. The picture becomes extremely complex when one knows that protein kinases can activate protein phosphatases. This is clearly the case for the insulin-activation of pyruvate dehydrogenase 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.

## Insulin and "stress hormones" control key metabolic enzymes



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 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 our widely varying 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 or when the body's responsiveness is no longer coupled to blood glucose levels. The liver's gluconeogenesis progresses without alteration following reduction or loss of the insulin signal, releasing sugar in spite of high blood glucose levels. 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

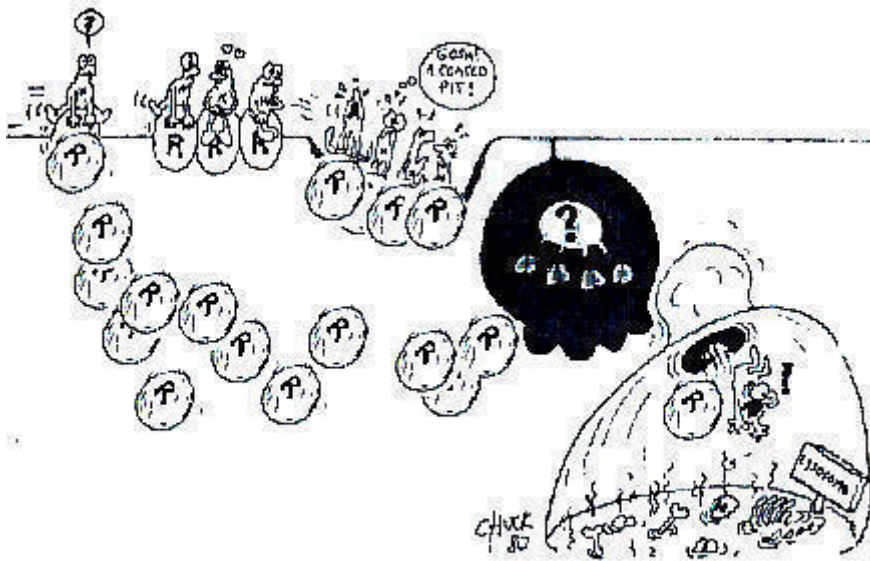
circulation and the liver converts these to ketone bodies. The high blood glucose levels lead to diuresis with loss of water,  $\text{Na}^+$ ,  $\text{K}^+$  and glucose, while the “ketones” (which are actually carboxy acids) lead to a pronounced fall in blood pH. Diabetic coma and death follow if effective treatment is not initiated.

## The Insulin Signaling System

### 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, insulin's mechanism of action is exceedingly complex and is still not completely known.

We can our discussion begin by looking at a few cartoons from the 1980's. By that time



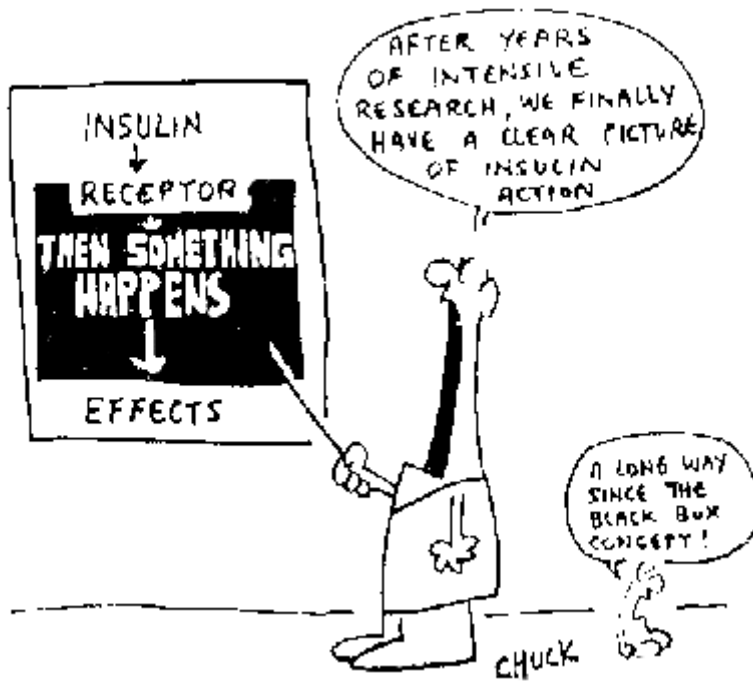
Modified from "Chuck", TIBS 1980

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 which then disappeared into the cell's interior. These insulin-carriers were thought to wind up in lysosomes and to be destroyed there. Later it

was established that some of the "ingested" receptors were actually recovered, repaired in the Golgi apparatus and reappeared as active receptors 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. This was the pattern we were accustomed to see with several other hormones.

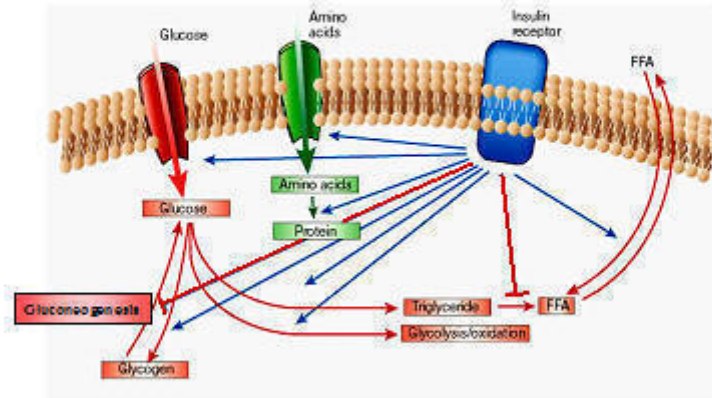


The situation was summarized in another beautiful cartoon by "Chuck", published in TIBS. We were just lacking that single second messenger for insulin that could carry out all of the complex actions the hormone had.

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

## Actions of Insulin

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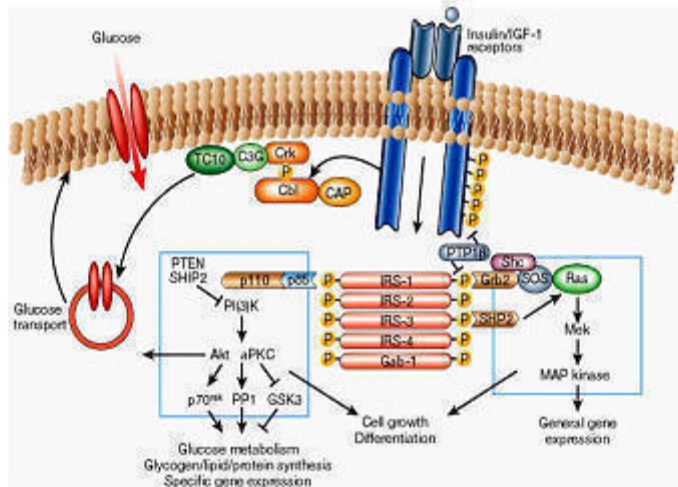
Modified from Saltiel and Kahn, *Nature* 414, 799-806, 2001

whole series of enzyme phosphorylation/ dephosphorylation reactions which are now thought to account for the effects of insulin.

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.

## Mechanisms of Insulin Action



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

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. Pathways

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

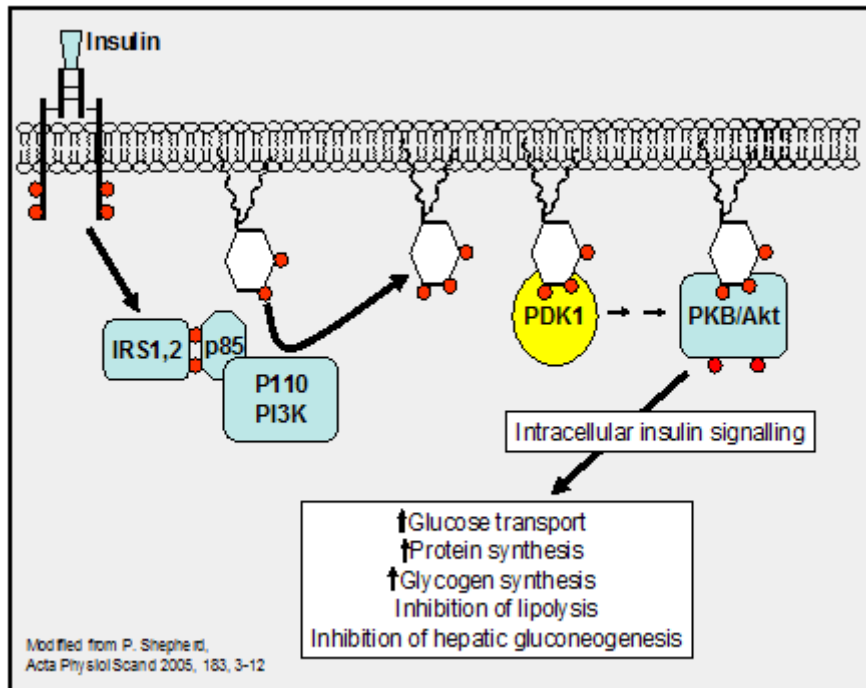
2. Mitogen-activated protein kinases (MAPKinases).

NB: Both group 1 and 2 signals also activate protein kinase C $\gamma$  and Protein kinase C $\zeta$ .

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 (PIP<sub>2</sub>) or [PI(3,4)P<sub>2</sub>] to phosphatidylinositol 3,4,5 triphosphate PIP<sub>3</sub> or (PI 3,4,5)P<sub>3</sub>. 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 is now clear that a serine-threonine kinase "Akt" (otherwise known as protein kinase B) is THE central element in the actions of insulin. Akt is activated by PIP3. Recent work has shown that PIP3 binding opens Akt to phosphorylation by phosphoinositide-

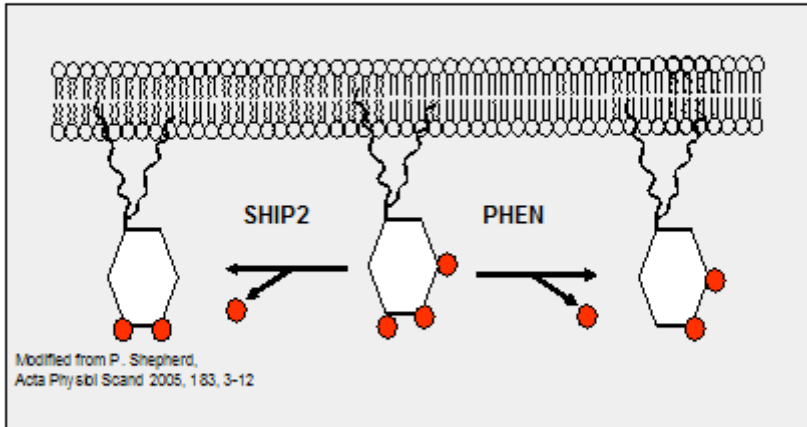


dependent protein kinase PDK-1 and the mammalian target of rapamycin (mTOR) complex 2 (mTORC2). Look at the next figure from the work of Professor Peter Shepherd (Acta Physiol Scand 183, 3-12, 2005). Phosphorylation of the insulin receptor and IRS1 and 2 lead to binding and activation of phosphatidylinositol 3 kinase (PI3K) and formation of PI(3,4,5)P3. This

then binds to the plasma membrane and associates with phosphoinositide-dependent kinase-1 (PDK-1) and leading to phosphorylation and activation of PKB/Akt. Activated Akt is initiates many of the metabolic actions of insulin.

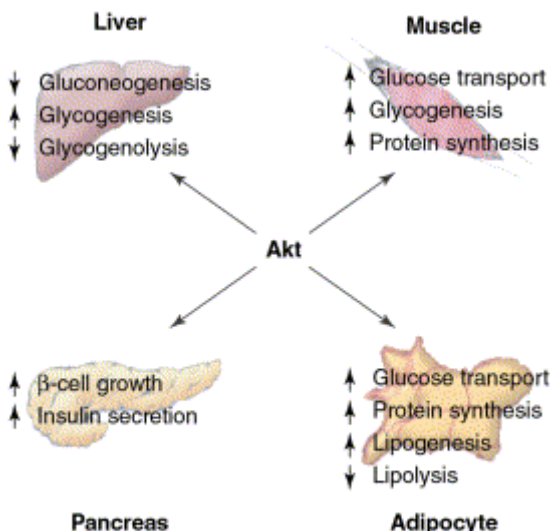
Just to complicate things a little more, the levels of PI(3,4,5)P3 are under control of two

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 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 \(2002\).](#)

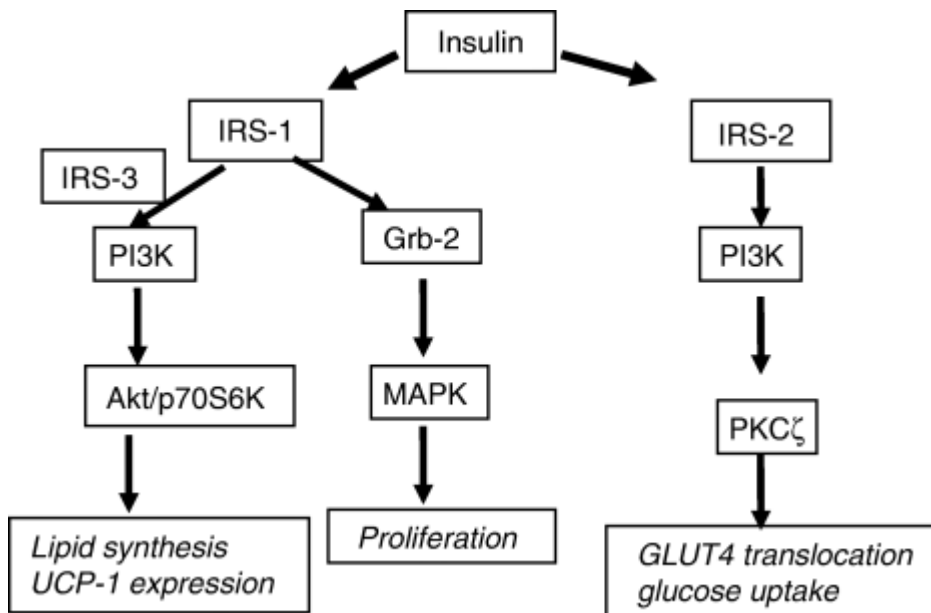
**NB: The requirement for Akt and its substrate Foxo1 for insulin action in the liver has been recently questioned. Please check the march 2012 addition (\*) for an updated reference.**

## **A Model System for Insulin Action**

Another article in Acta Physiologica Scandinavica 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 $\zeta$ ) were shown to be required for translocation of the glucose transport protein from storage areas to the cell surface. Phospholipase C $\gamma$  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.



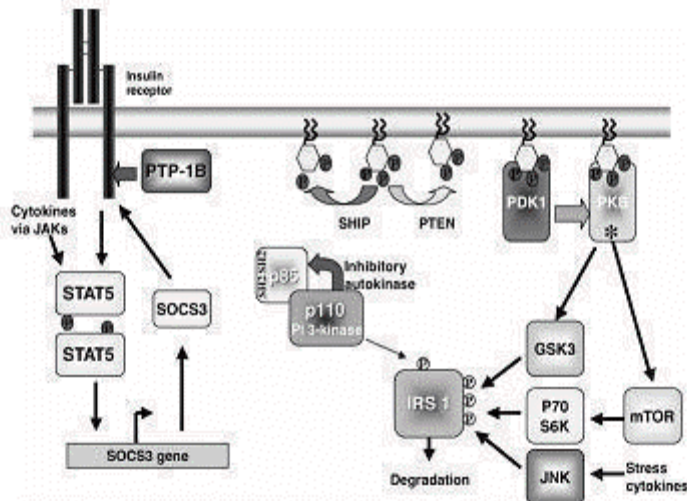
This "simple" model system involves a series of enzymes coupled to the phosphatidylinositol and protein kinase systems. Most members of these groups can interact. Insulin resistance and type 2 diabetes could follow malfunction at any of these steps.

## 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.

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



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

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

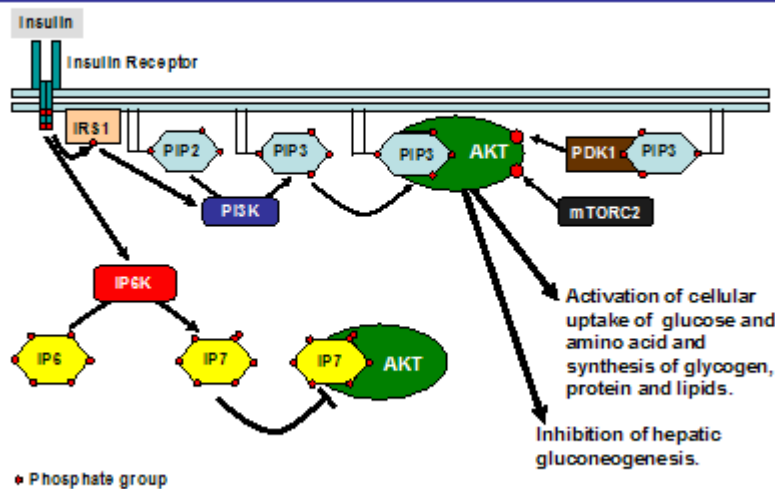
signaling family and reduce their activity. Additionally, the SOCS gene is activated and SOCS (or SOCS3 here) is turned on and inhibits the insulin receptor. This diagram is quite complex but in no way underestimates the control mechanisms involved in insulin's action.

## Inositol phosphate kinase, a newly found (2010) signal system for Insulin.

Our metabolic balance is dependent upon acute and transient responses to insulin. One common way to achieve good control of many metabolic systems is so-called feedback regulation. That is, correct metabolic activity is often the result of a balance between an

"off" and an "on" reaction or control system. Is this also found for insulin signaling?

### Insulin Signaling and Control of Metabolism



Modified from: B. D. Manning, Cell 143, DOI 10.1016/j.cell.2010.11.040

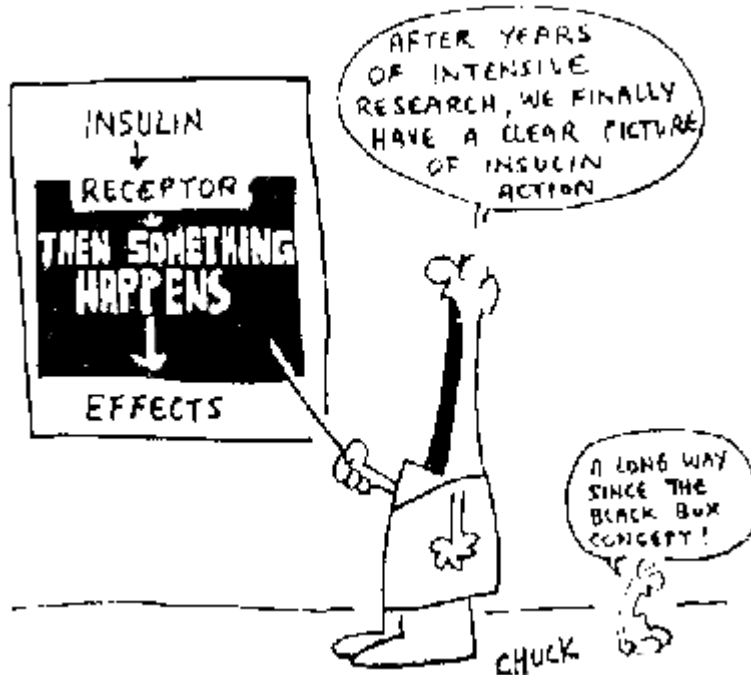
In a recent paper in Cell 143, 897-910 2010, Chakraborty et. al. report a new player in the insulin story. This is reviewed in the same issue of Cell by Brendan D. Manning Cell 143 861-862 2010 (DOI 10.1016/j.cell.2010.11.040). They have discovered an insulin receptor product which has not been reported earlier. Activation of the insulin

receptor stimulates both phosphorylation of IRS1 and activation of inositol hexakisphosphate (IP6) kinase (IP6K1). The latter yields 5-diphospho-inositolpentakisphosphate (5-PP-IP5 or IP7). IP7 binds to Akt, inactivating it and preventing its function as a substrate for mTORC2 and PDK1 phosphorylation. That is, insulin produces signal substances that both activate and deactivate Akt. The "on" signal is the PIP2-PIP3 sequence. The "off" signal is the IP6-IP7 sequence. The balance between these may dominate insulin regulation of metabolism. Perhaps even more important is the possibility that the key to understanding "insulin resistance" may lie in the balance between these.

## 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.

## \*NB: March 2012

Insulin's regulation of hepatic metabolism is a major element in control of blood glucose levels. Inappropriate gluconeogenesis in postprandial periods is a key to the hyperglycemia seen in type 2 diabetes.

The protein kinase Akt and the transcription factor Foxo1 have been classically viewed as essential in control of hepatic gluconeogenesis. This view has been seriously questioned in a recent publication by a leading research group (Lu *et al*, Nature Medicine, 18,3, 2012). It appears that a hepatic response to insulin can occur in the absence of these factors. This raises the possibility of new approaches to control of type 2 diabetes. Click [here for the Lu publication](#) and [here for a Nature Medicine News](#) presentation and discussion of this paper.

A possible control point external to the IRS-Akt\_Foxo1 system may lie in the cAMP-protein kinase A system activated by glucagon. This is not mentioned in the two publications

cited here. However, the balance between insulin and glucagon is a major factor in control of hepatic gluconeogenesis.

The results of further investigations in the control of hepatic glucose synthesis will be intriguing.