# Insulin and Glucagon
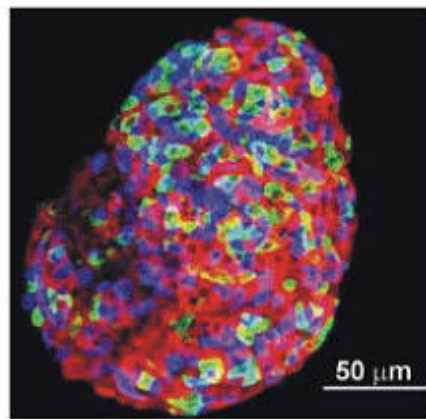
## The islets of Langerhans

The pancreatic Islets of Langerhans are the sites of production of insulin, glucagon and somatostatin.  The figure below shows an immunofluorescence image in which antibodies

### The Pancreatic Islet

Red = ß-cells: Insulin
Green = α-cells: Glucagon
Blue = γ-cells: Somatostatin

50 μm

Rorsman, P., Br J Diabetes Vasc Dis. 2005;5(4): 187-191

specific for these hormones have been coupled to differing fluorescence markers.  We can therefore identify those cells that produce each of these three peptide hormones.  You can see that most of the tissue, around 80 %, is comprised of the insulin-secreting red-colored beta cells (ß-cells).  The green cells are the α-cells (alpha cells) which produce glucagon.  We see also some blue cells; these are the somatostatin secreting γ-cells (gamma cells).  Note that all of these differing cells are in close proximity with one another.  While they primarily produce hormones to be circulated in blood (endocrine effects), they also have marked paracrine effects.  That is, the secretion products of each cell type exert actions on adjacent cells within the Islet.

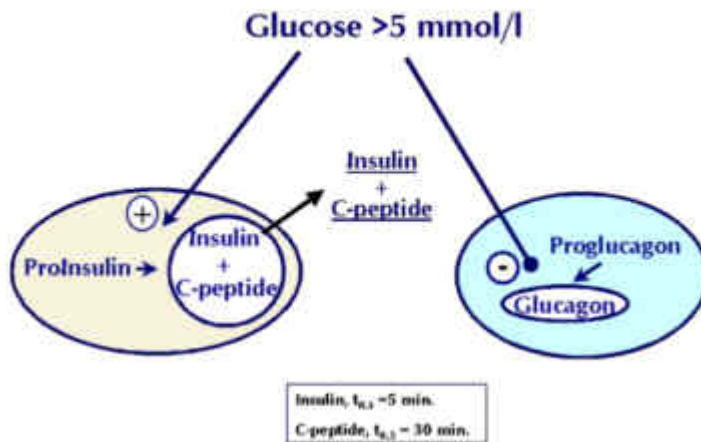## An Introduction to secretion of insulin and glucagon

The nutrient-regulated control of the release of these hormones manages tissue metabolism and the blood levels of glucose, fatty acids, triglycerides and amino acids.  They are responsible for

homeostasis; the minute-to-minute regulation of the body's integrated metabolism and, thereby, stabilize our inner milieu.   The mechanisms involved are extremely complex.  Modern medical treatment of diabetes (rapidly becoming "public enemy number one") is based on insight into these mechanisms, some of which are not completely understood.  I will attempt to give an introduction to this complicated biological picture in the following section.  Somewhat deeper insight will come later.

# The Basics: secretion

Let us begin with two <u>extremely</u> simplified figures (that perhaps belong in a newspaper and not here).  The beta-cell lies to the left and the alpha-cell to the right in these figures.  There are several things to note here.  Both insulin and glucagon are initially synthesized as larger "prepro-hormones" and, after cleavage, "pro-hormones".  These are then once again split to yield the give active peptide hormones and the remains of the longer peptide chains.  In the case of insulin, the excess peptide is known as C-peptide or "connecting peptide".  C-peptide appears to be inactive but is of interest because it has a much longer half-life than insulin and is released simultaneously with the hormone.  Insulin is destroyed in the liver, the half-life being approximately 5 minutes.  The rate of secretion of insulin is, therefore, difficult to measure.  One can, however, estimate the rate of insulin secretion by measuring the level of C-peptide as this has a half-life of about 30 minutes.



So to the main point.  When blood glucose levels increase over about 5 mmol/l the beta-cells increase their output of insulin and C-peptide.  The glucagon-producing alpha-cells remain quiet, and hold on to their hormone.
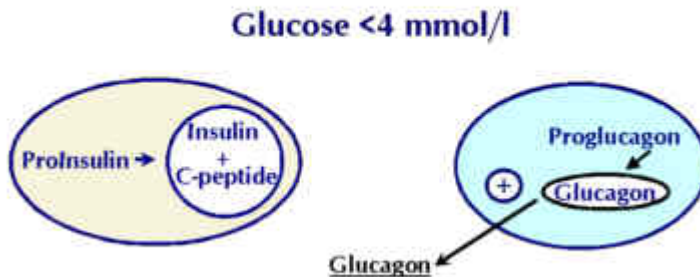


A fall in blood glucose under about 4 mmol/l leads to a pronounced decrease in insulin secretion.  The alpha-cells become active and deliver glucagon to the blood. Do not get the impression that there is a total secession of secretion of insulin or glucagon at high or low glucose levels.  Both cell types release their hormones simultaneously at a basal
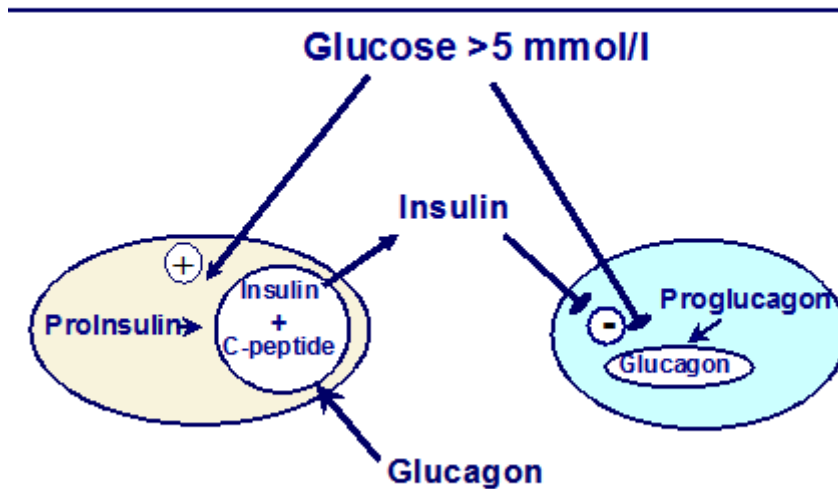
level*.  This is augmented in response to alterations in blood glucose levels or consumption of food.  It is the balance between insulin and glucagon (the resulting molar ratios of these hormones) that controls metabolism.

*  About one half of the daily output of insulin occurs between meals.

# Paracrine Actions of Insulin and Glucagon

As I mentioned above, the cells of the Langerhans Islets are tightly packed.  This results in high concentrations of each hormone within the organelle.  Increases in insulin levels inhibit glucagon release from α-cells.  This paracrine effect is a basic element in insulin's control of glucagon's activation of both hepatic gluconeogenesis and lipolysis in adipose tissue.

### Paracrine Actions of Insulin and Glucagon

Glucose >5 mmol/l

Insulin

ProInsulin➤  Insulin + C-peptide  (+)

(-)  Proglucagon   Glucagon

Glucagon

Glucagon secretion increases when blood sugar levels are diminished, countering insulin's effects upon glucose and fat metabolism.  One might expect that glucagon would have a negative paracrine action on ß-cells, but that is NOT the case.  Glucagon does affect these insulin-secreting cells, but that effect is positive!  That is, glucagon released between meals primes ß-cells so that they release INCREASED amounts of insulin WHEN GLUCOSE LEVELS RISE.  Glucagon's action is coupled to production of a "second messenger" known as cyclic AMP or cAMP.  Increased levels of cAMP are an important stimulating factor in insulin secretion.  This seems to be the basic mechanism for a new class of drugs now coming in use for regulation of insulin secretion in diabetes type 2.  Click here for more information.

# Insulin and glucagon secretion is coordinated

Insulin has many actions, the most well known is stimulation of glucose and amino acid uptake from the blood to various tissues.  This is coupled with stimulation of anabolic processes (or synthetic reactions) such as glycogen, protein and lipid synthesis.  Glucagon has opposing

## Both Carbohydrate and Protein Meals Alter Serum Insulin and Glucagon Levels
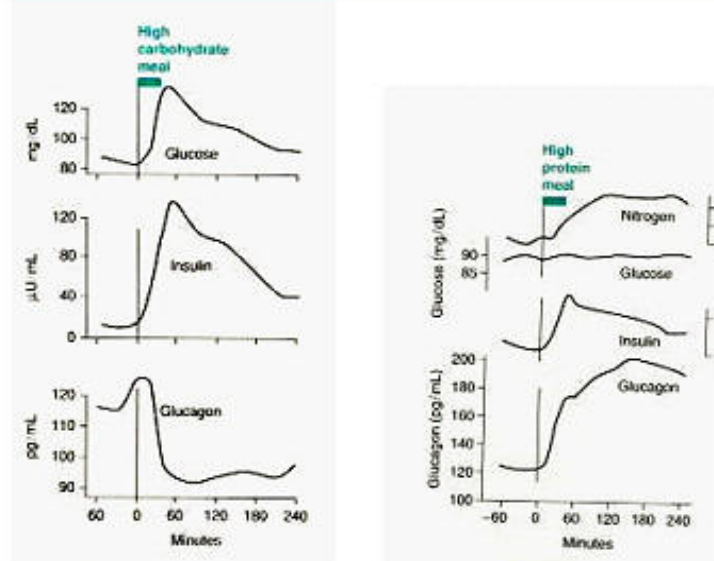


Modified from Marks, Basic Medical Biochemistry, 2. ed, 2004

effects, causing release of glucose from glycogen, release of fatty acids from stored triglycerides and stimulation of gluconeogenesis.  The balance between these two hormones holds metabolism "on the line", promoting a stable inner metabolic milieu (or homeostasis).

Consumption of carbohydrates triggers release of insulin from beta cells.  Alpha cells become inhibited and cease to secrete glucagon.  Taken together, these actions produce a rapid return to fasting blood sugar levels and storage of glucose as glycogen or lipid.

A protein-rich meal leads to release of both insulin and glucagon.  The latter stimulates gluconeogenesis and release of the newly formed glucose from the liver to the blood stream.  The very moderate rise in insulin associated with the protein meal stimulates uptake of the sugar formed in the liver by muscle and fat tissue.

# How do these hormones work?

## The basics: metabolic effects

I doubt that there is another hormone that has so many and wide-ranging actions as insulin. It is the major actor in control of carbohydrate, lipid and protein metabolism. These aspects are well discussed in all good medical biochemistry texts, and are summarized in the following figure, modified from Clinical Biochemistry, Gaw et al. Insulin signals a state of energy abundance, and activates glucose uptake, metabolism and storage as glycogen in muscle and fat tissue. These organs make up most of the body's mass. At the same time, insulin restrains processes that release stored energy; lipolysis and ketogenesis, glycogenolysis, proteolysis and gluconeogenesis. Insulin is necessary for uptake of amino acids to tissues and for protein synthesis. Insulin is THE central actor in homeostasis; the stabilization of the internal milieu.

## Actions of Insulin
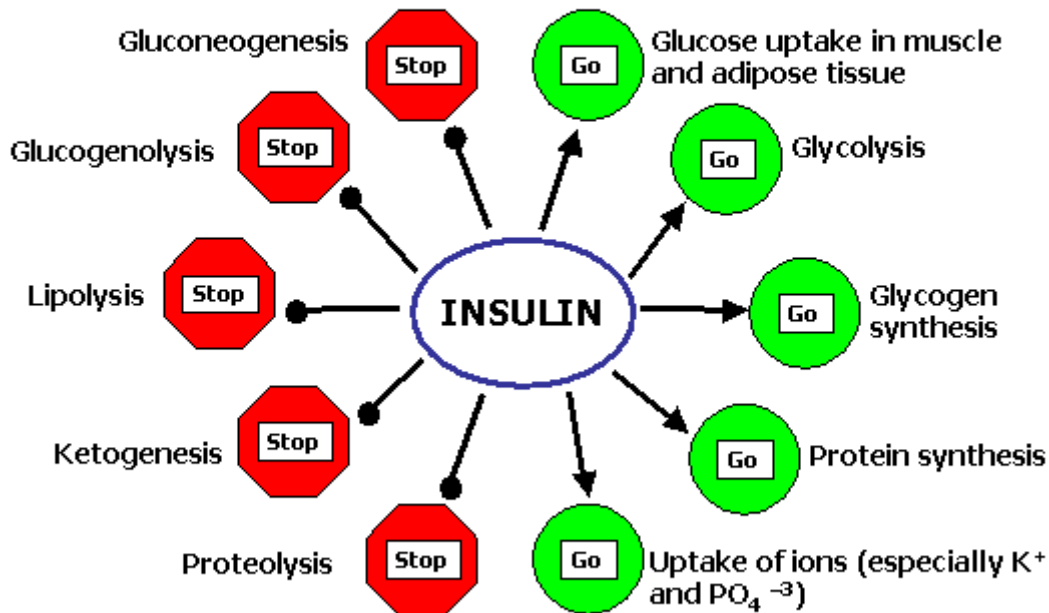


Modified from Clinical Biochemistry, A. Gaw et al, Churchill Livingstone, Edinburgh, 1995.

I have previously pointed out that insulin and glucagon act together to balance metabolism  In general we can say that insulin favors anabolic reactions; glucagon, catabolic reactions.  Put more simply, insulin favors storing energy and production of proteins while glucagon activates release of stored energy in the form of glucose or fatty acids.  The actions of these two hormones on individual metabolic processes are summarized in the following table.

| Metabolic Actions of Insulin and Glucagon | | |
|---|---|---|
| Fatty acid uptake and release  in fat. | Insulin | Stimulates synthesis of triglycerides (TG) from free fatty acids (FFA); inhibits release of FFA from TG. |
| | Glucagon | Stimulates release of FFA from TG. |
| Liver glycogen | Insulin | Increases synthesis and thereby glucose uptake and storage. |
| | Glucagon | Stimulates glycogenolysis and glucose release. |
| Liver gluconeogenesis | Insulin | Inhibits, saves amino acids. |
| | Glucagon | Stimulates, glucose synthesized and released. |
| Glucose uptake, skeletal muscle | Insulin | Stimulates uptake, storage as glycogen and use in energy metabolism. |
| | Glucagon | No receptors, no effect. |
| Glycogen, skeletal muscle | Insulin | Stimulates synthesis. |
| | Glucagon | No receptors, no effect. |
| Amino acid uptake | Insulin | Stimulates and is necessary for protein synthesis. |
| | Glucagon | No receptors, no effect. |
| Brain (hypothalamus) | Insulin | Reduces hunger through hypothalamic regulation. |
| | Glucagon | No effect. |

# Insulin action in fat cells

One of the primary actions of insulin is to control storage and release of fatty acids in and out of lipid depots .  It does this through two mechanisms; regulation of several lipase enzymes and activation of glucose transport into the fat cell via recruitment of glucose-transport protein 4 (GLUT4).

## Fat storage and synthesis of glycerol phosphate.

Splitting of triglycerides produces free fatty acids and glycerol.  One might expect that the body would use this glycerol to aid in storage of fatty acids when required.  However, that just does not occur.  Adipocytes lack glycerokinase that is essential for synthesis of α-glycerol phosphate from glycerol.   The glycerol released by lipolysis must be transported to the liver for further metabolism.  Where does the glycerol phosphate that is needed to esterify fatty acids come from?  How do adipocytes take up and store fat from the diet?



Insulin action in adipocytes and ketogenese in liver
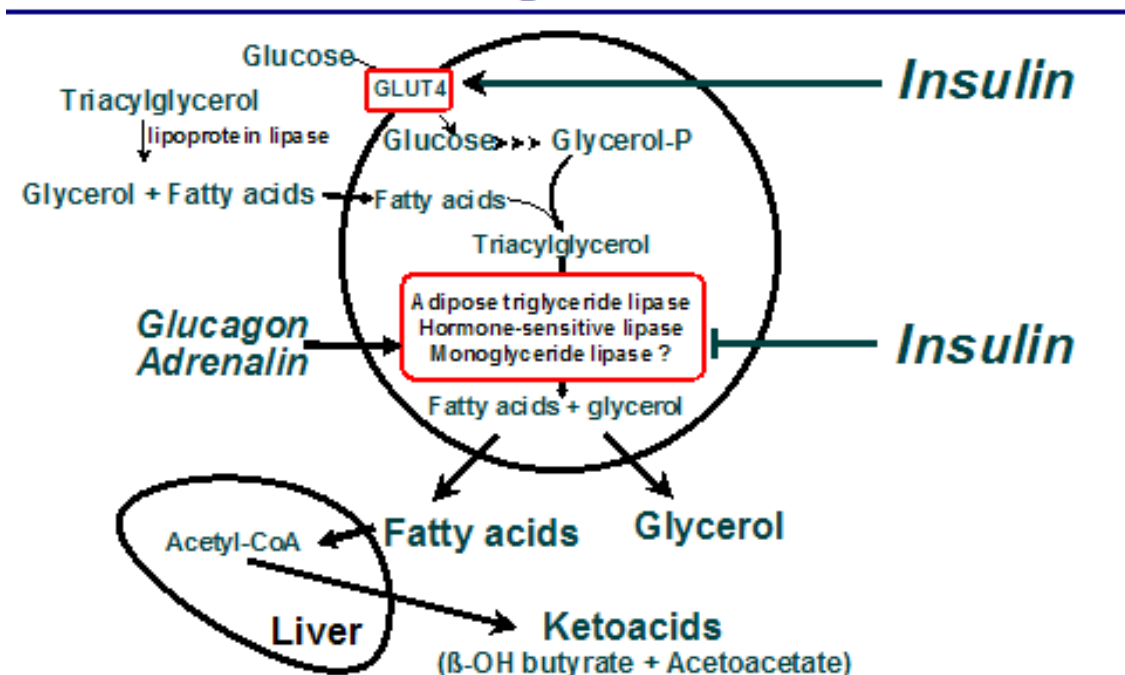
The "backbone" of triglycerides is α-glycerol phosphate produced from glucose in fat cells. Storage of triglycerides after a meal is, therefore, dependent upon insulin-stimulated glucose uptake and glycolysis.  Normally, fat cells take up fatty acids and glucose simultaneously.  The fatty acids come from the action of lipoprotein lipase at the capillary wall.  Glucose uptake is stimulated by insulin and occurs through the insulin-sensitive glucose transport protein GLUT4.  The high blood lipid levels seen in diabetes are , at least partially, due to reduced insulin-stimulation of glucose uptake at the fat cell membrane.

Splitting of triglycerides back to glycerol and fatty acids follows the actions of several lipases. Present (2008) knowledge suggests that triglycerides are split to diglycerides by adipose triglyceride lipase (ATGL) at the fat droplet-cytosol border*.  Diglycerides are thereafter converted to monoglycerides by hormone-sensitive lipase (HSL).   The monoglycerides are hydrolyzed by a cytosolic monoglyceride lipase.  These three enzymes and their control elements (also proteins, especially perillipin A) are extensively phosphorylated, apparently by protein kinase A. Phosphorylation follows activation of adenyl cyclase, mainly through stimulation by glucagon and adrenalin.  The cyclic AMP formed by the activated cyclase stimulates phosphorylation by protein kinase A (PKA) and activation of the various lipases.  The important point to remember here is that the combined lipase activity increases and decreases in tact with cyclic AMP levels.  Cyclic AMP synthesis and degradation is under strong hormonal control.  Adrenalin and glucagon are the most important stimulating agents while insulin rapidly lowers cAMP levels through activation of phosphodiesterase.   In effect, glucagon and adrenalin "turn on" lipolysis while insulin "turns off" breakdown of triglycerides in fat cells.  This process is of major importance in our physiology and, in fact, is much more complex than I have described here.  Please go to the following publications for more information:

1.  Regulation of Lipolysis in Adipocytes, R. E. Duncan et. al., Annu Rev. Nutr. 2007. 27:79-101.

2.  Adipose Triglyceride Lipase, function, regulation by insulin and comparison with adiponutrin,
 E. E. Kershaw et. al., Diabetes 2006, 55, 148-157.

Click on the titles if you have library connections.

*  NB: While this triglyceride lipase is called "adipose triglyceride lipase" or ATGL, it is found at considerable levels in many other tissues.

# Eating and working; how is blood glucose stabilized?

Mental awareness and the feeling of well-being is dependent upon a steady stream of fuel to the brain. This organ is normally entirely dependent upon glucose as its energy substrate. A twenty percent decline in blood sugar can lead to nausea, restlessness and other neurological symptoms. How are blood sugar levels controlled?

## Resting conditions:

Let us first examine basal conditions, that is, the post-absorptive state in resting persons. Glucose uptake from the small intestine is not active and yet the level of blood glucose is held
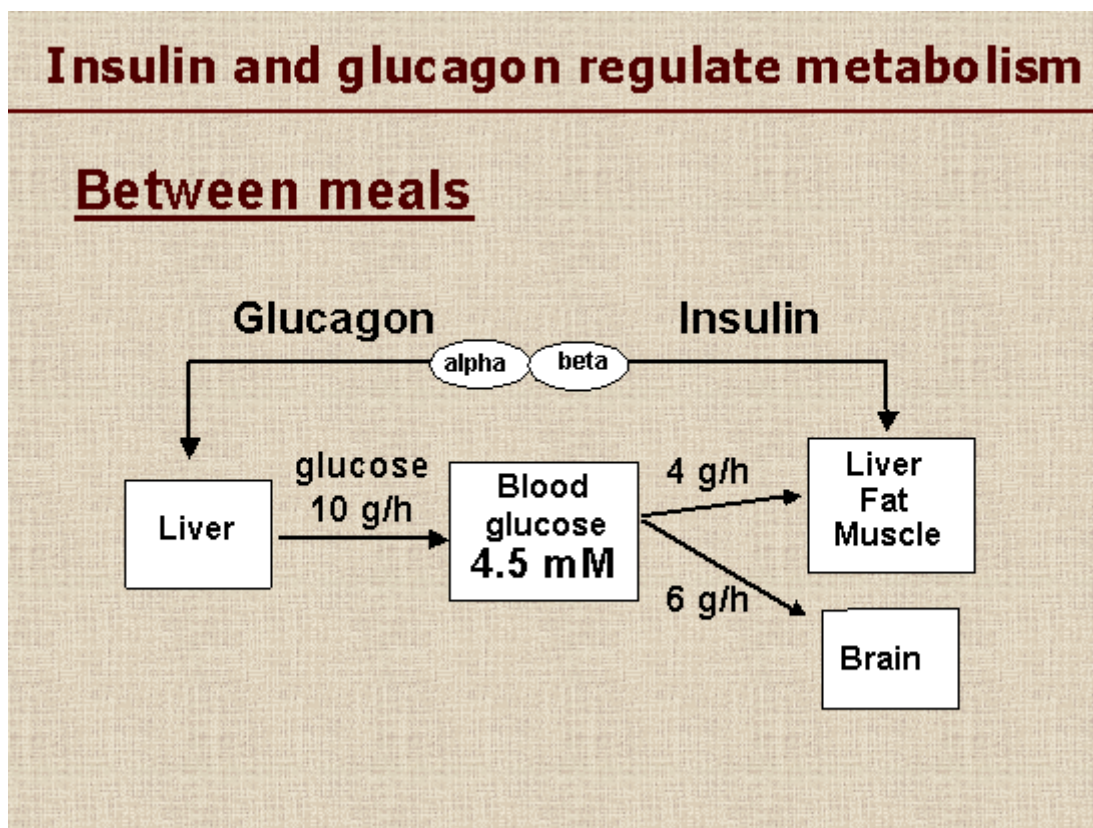


relatively constant. This occurs in spite of the fact that the resting body uses about 10 grams of glucose per hour. More than half of this goes to drive energy production in the brain. The brain uses 5-6 grams of glucose constantly; while sleeping, going for a walk or taking an examination in medical biochemistry! MR imaging has clearly demonstrated that various areas of the brain are specifically activated by specific tasks; some areas are quiescent, others active at various times. Never the less, the brain's total energy use is relatively constant, thus explaining the unvarying need for glucose.

Between meals, the glucose that is taken up by the body's various tissues is replaced by the liver, either through glycogenolysis (use of stored glycogen reserves) or gluconeogenesis (synthesis of glucose from smaller metabolites).  Regulation of these two processes requires the coordinated effects of insulin and glucagon.  The molar ratio between the concentrations of these hormones controls metabolism.  Secretion of hormones from the Islets of Langerhans is precisely adjusted to coordinate the various functions of insulin and glucagon and to stabilize blood sugar levels.

# At work:

Skeletal muscle can increase its energy turnover 18-20 fold under exhaustive work loads, using fatty acids and glucose as substrates for aerobic metabolism and ATP production.  However, hard-working muscles are dependent upon glucose as a substrate for anaerobic energy
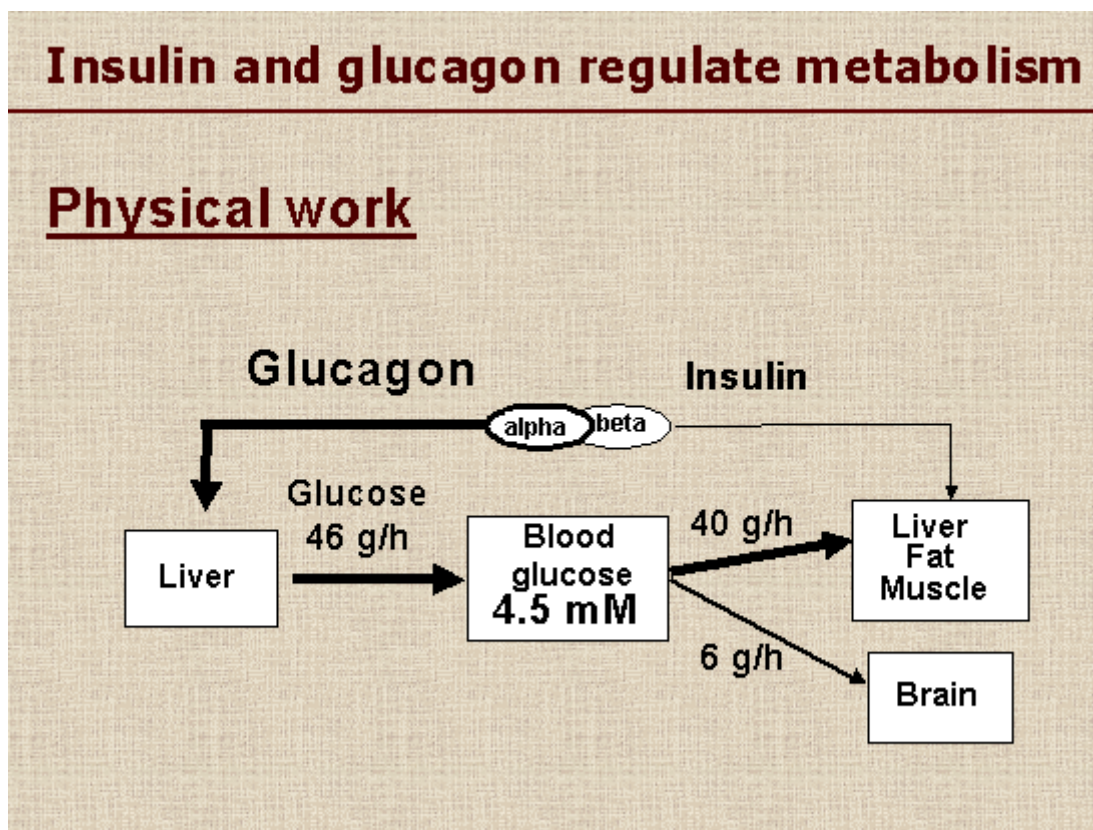


production.  As we can see from the figure below, despite the huge increase in the amount of glucose taken up from blood by working muscles, the level of blood glucose is not altered. Decreased secretion of insulin and a marked increase in glucagon secretion prompt the liver to break down glycogen  and start gluconeogenesis.  These actions provide the glucose required to balance glucose uptake by muscles.  One might protest, " but we need insulin to activate glucose uptake by the working muscle fibers".  The answer to this is that glucose uptake through GLUT4 is activated by glucose consumption as well as insulin in muscle cells.  A feedback control mechanism coordinates glucose metabolism and glucose uptake in muscle.

An often-ignored point is that insulin inhibits hepatic gluconeogenesis.  The reduction in insulin secretion while working is a significant factor in the promotion of gluconeogenesis.  The

importance of this is obvious when we consider diabetes.  Diabetic hyperglycemia is partially due to glucagon-stimulated hepatic glucose synthesis which occurs in spite of the high blood glucose levels that follow the loss of insulin or insulin resistance.  Inhibition of hepatic gluconeogenesis with metformin is an important component of treatment for type 2 diabetes.

Note again that the brain takes up glucose at the same rate as that seen under basal conditions.

Extremely hard and prolonged physical work can lead to a pronounced fall in blood sugar. Marathon runners, skiers and others that press their bodies to maximal performance sometimes do collapse before the finish line. Why?  The answer lies in the fact that hepatic gluconeogenesis alone cannot provide glucose as quickly as skeletal muscles can remove it from the blood.  When glycogen stores are exhausted one of two things must happen:  (1) the individual must reduce speed and energy output so that the blood sugar level can remain within normal boundaries or (2) with time a hypoglycemic episode will occur and collapse <u>will</u> follow.

## After a good meal:

One act does alter blood glucose levels; eating a good meal.  A normally balanced meal provides about 90 grams of glucose, mostly as polysaccharides.  These are usually absorbed over a period of about 120 minutes.   The glucose released from food is used as an immediate energy
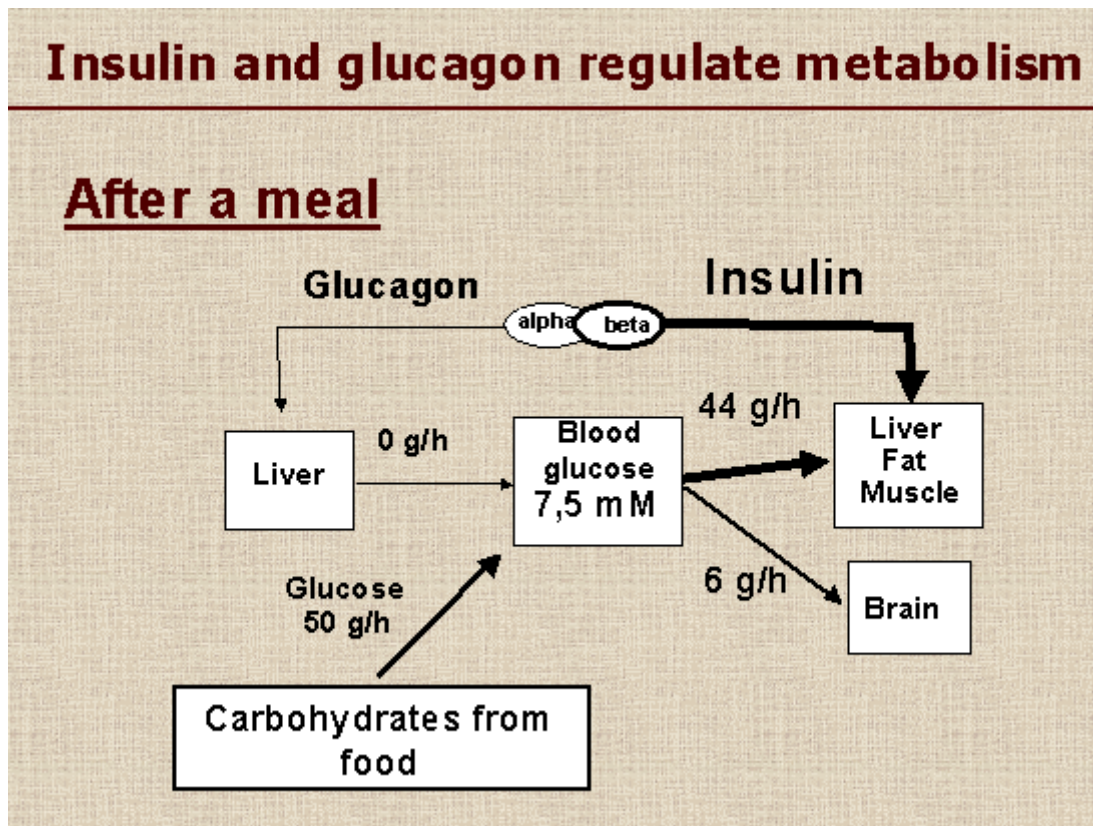


substrate and any excess will be stored as glycogen (and fat in the case of over-nutrition).

A prerequisite for oxidation or storage of glucose is that it is transported into cells over their plasma membrane.  Uptake of larger molecules requires the presence of specific carriers.  In the

case of glucose we find a family of at least five glucose transport proteins with varying characteristics.  Click here for more information.  The important point here is that the body's largest tissue, skeletal muscle, is dependent upon GLUT4 for uptake of glucose and that this transport must be activated by insulin or muscle activity.  In adipose tissue, insulin is required for storage of lipids from the diet and for control of lipolysis.

Blood glucose levels after a meal must be held below the renal threshold for recovery of glucose from the glomerular filtrate (8-10 mmol/l).  If this is exceeded, glucose is lost to the urine as is seen in diabetes.

So, after a meal insulin secretion is activated, glucagon secretion is minimized, and the liver takes up glucose which is then stored as glycogen to be used to buffer blood glucose at a later time.  Insulin also stimulates glucose uptake and glycogen synthesis in muscles.  Note that muscle glycogen cannot be released to the circulation.  Muscle glycogen is used exclusively as a substrate for muscle activity.

Note once more that the brain's glucose uptake is constant.

# Tissue distribution of glucose after a meal.

If one is to understand the actions of insulin and the pathology that follows insulin deficiency or resistance, one must know where this hormone acts.  We can gain insight in this by following the fate of glucose after a meal.  An average meal contains about 90 grams of glucose.  This glucose either will be utilized as an immediate energy substrate for ATP synthesis or will be stored as glycogen or fat.

Let us consider a person who has a good control over his/her weight and where food consumption and the energy used are in balance.



Distribution of glucose after a meal
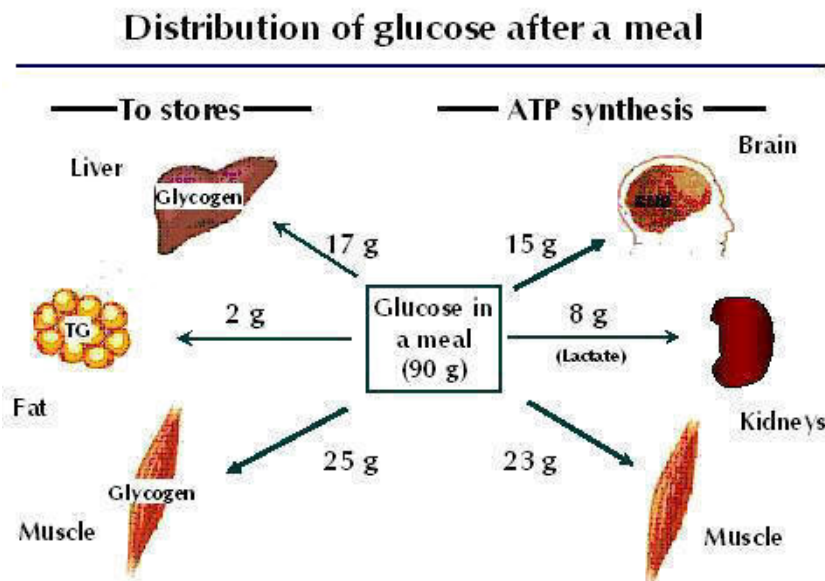
Around 15-18% of the ingested glucose goes to nourish the brain during the absorptive period.  Note that there is no storage form of glucose in the CNS; all of the glucose that is taken up is "burned".  The brain is, therefore, extremely sensitive to reduced blood glucose levels.

The liver stores excess glucose as glycogen, readying a buffer for blood glucose to meet the coming post-absorptive period.  Little glucose is normally converted to fat.  Note that over-eating carbohydrates (sucrose and fructose) can and does lead

to fat production and storage.

The kidneys take up about 9-10% of the consumed glucose as lactate which is excreted from red blood cells.  RBCs lack mitochondria and, therefore, must release the anaerobically oxidized glucose they use as pyruvate and lactate.

Skeletal muscles dominate in the fight for blood sugar after a meal, accounting for about 50% of the total glucose uptake.  Approximately half of this is stored as glycogen, the rest is used as an immediate energy substrate.  If you recall that insulin is needed to activate glucose uptake in muscle (GLUT4 is the carrier here) you will acknowledge that skeletal muscle must be the major target organ for insulin.  A reduction in the effect of insulin in skeletal muscle (insulin resistance) is the key mechanism leading to impaired glucose tolerance (IGT) and diabetes type 2.

# Energy metabolism during a fast, in starvation and uncontrolled diabetes

## Between meals and during a fast:
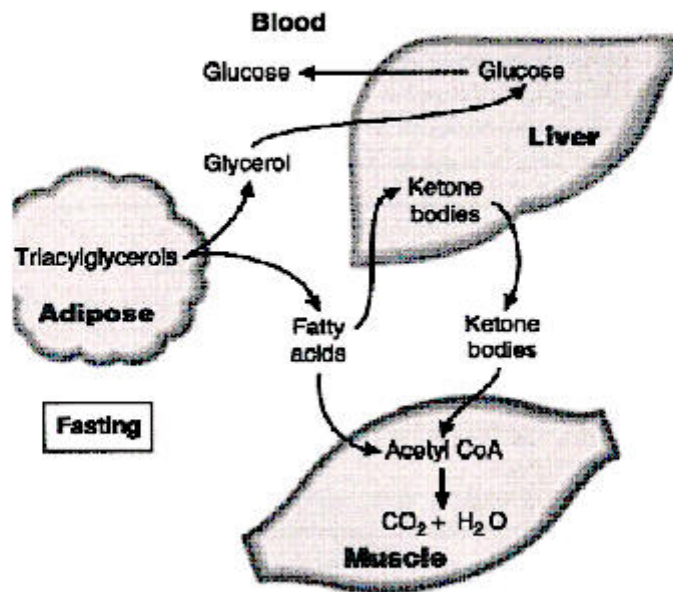


We have seen how glucose is distributed among the body's various tissues after a meal. Energy production during the absorptive period uses glucose from the diet as substrate. Furthermore, we have seen that the insulin to glucagon ratio is lowered during the post-absorptive period and during a fast. Let us now examine the effects of this alteration in hormone levels upon distribution of substrates for energy metabolism. As pointed out earlier, insulin inhibits lipolysis in adipose tissue while glucagon is a major activator of hormone-sensitive lipase. The change in the insulin/glucagon ratio seen in fasting and between meals activates adipocyte lipolysis. As we can see in the figure to the left, adipose tissue supplies fatty acids to drive aerobic metabolism in muscle, liver and other tissues (but not the brain; fatty acids are not taken up here). Excess fat can be converted to ketone bodies in the liver. The levels of these remain rather low and they are used as energy substrates in muscle. Once more, we see that glycerol arising from lipolysis cannot be reused in fat cells but is circulated to the liver where it enters gluconeogenesis.

Let's sum up.  After a meal, fat from food is stored and carbohydrates from the diet are burned. Between meals stored fat is released from fat cells and supplies fuel for most of the body's organs until the next meal.  Remember, blood cells and the brain must use glucose as their energy source.  We say that "fat spares sugar" so that an even supply of fuel is available to all of the body's tissues.  And, remember, this is possible only through the minute-to-minute adjustments in the ratio of insulin to glucagon.

# Starvation:

We have already seen that gluconeogenesis supplies glucose during fasting and starvation.

Consider the changes in blood levels of insulin and fuels shown in the next figure.  The marked fall in insulin levels seen in starvation leads to an increase in plasma levels of both free fatty acid and especially ketone body levels.  Most of the fatty acids liberated from adipose tissue are



Blood Levels of Energy Substrates in Starvation
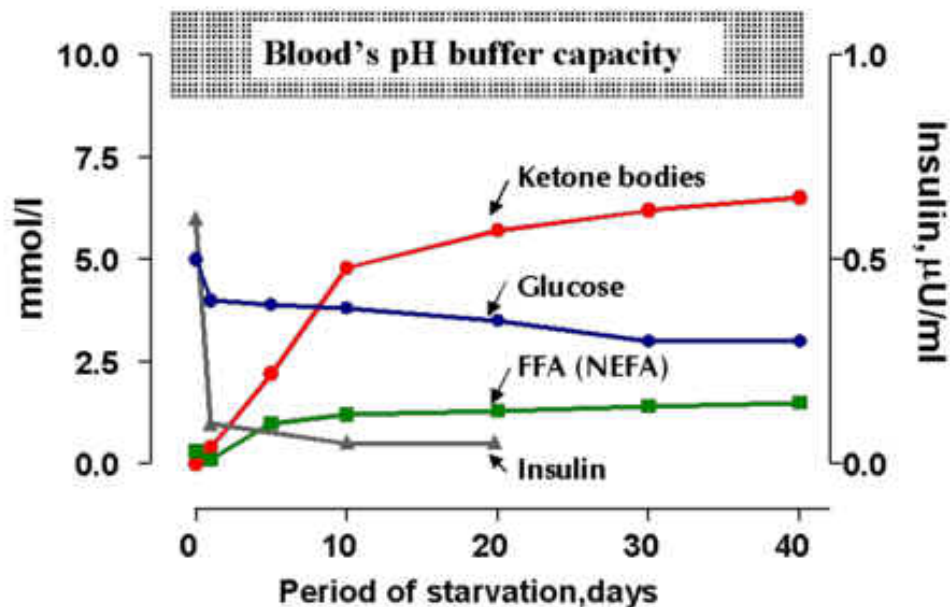
converted to ketone bodies (acetoacetate and beta-hydroxy butyrate) by the liver during starvation.  The level of these "ketone bodies" rises abruptly during the two first weeks of starvation, and then slowly increases.  When plasma ketone body levels reach about 5mmol/l they can supply the brain with around 50% of the substrate required for ATP production.  Even during starvation about 50% of the brain's energy must come from glucose.  From around the second week of starvation, blood glucose levels stabilize at approximately 3.5 mmol/l.  At this glucose concentration, the brain can extract enough ketone bodies and glucose from blood to maintain normal activity.  The conversion of fatty acids to ketone bodies allows the brain to obtain energy from the large energy reserve represented by body fat.  Remember, the brain cannot take up fatty acids from the blood.

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"Ketone bodies" is a very misleading term.  They are neither particles (bodies) or ketones, but to be more precise, they are the carboxy acids acetoacetate and beta-hydroxybutyrate.  It is essential that the total level of acids in the blood does not exceed the blood's pH buffer capacity.  This is approximately 10 mmol/l.  The key to control over acid formation in starvation is the insulin/glucagon ratio.  This controls lipolysis and, therefore, the rate of lipolysis and ketogenesis.  You can see from the figure that insulin levels sink rapidly during the first day without food, but they DO NOT REACH ZERO in starvation.  (These data are from 2 studies;  I have no knowledge of studies where insulin was measured for more than twenty days of starvation).  Appropriate insulin levels are essential, also in starvation.

What limits survival time in starvation?  Blood glucose.  By switching brain metabolism to a 50-50 dependence upon glucose and ketone bodies survival time is greatly prolonged.  The body's muscles are broken down to provide amino acids that are converted to glucose by the liver and kidneys.  When the supply of amino acids becomes rate-limiting for gluconeogenesis, blood glucose levels fall and neural tissue starves and dies.

# Diabetes; metabolism in the absence of insulin.

Diabetes type I is a fatal disease in which insulin secretion totally fails following an autoimmune attack on the pancreatic beta cells.  This is in sharp contrast to starvation where insulin secretion, while reduced, is sufficient to regulate fat and carbohydrate metabolism.   Compare the following figure with those showing energy metabolism in starvation.  The total lack of insulin or lack of response to the hormone (insulin resistance) leads to two metabolic crises; a marked increase in
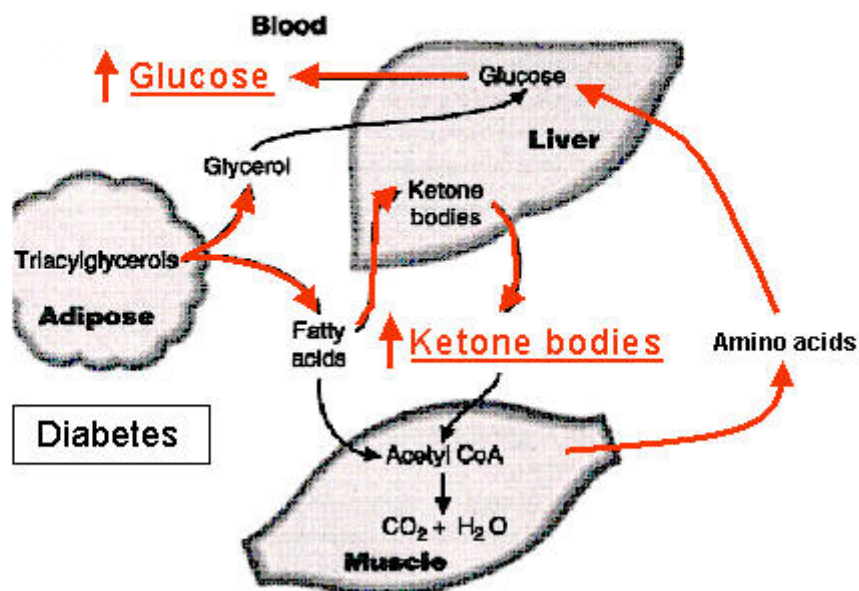
the rate of lipolysis in adipose tissue and activation of hepatic gluconeogenesis in spite of high plasma glucose levels.  The dramatically increased rate of lipolysis in adipose tissue follows the lack of insulin-inhibition of hormone-sensitive lipase.  The increase in fatty acids that results leads to a massive synthesis of ketone bodies in the liver.  These then exceed the buffer capacity of the blood, leading to ketoacidosis.  Excess acid is a potent poison for the brain.  Coma and death follow ketoacidosis.

Blood glucose levels can increase many-fold in diabetes.  In spite of this, hepatic gluconeogenesis, using amino acids as a substrate, becomes activated.  This is because insulin is a physiologically important inhibitor of glucagon secretion and hepatic gluconeogenesis. Hyperglycemia causes loss of glucose to urine and, as urine is isoosmotic with blood, loss of water and electrolytes follows.  Untreated type 1 diabetics can lose carbohydrate equivalent to two loafs of bread per day!

The high levels of glucose seen in diabetes 1 and 2 are toxic.  They can lead to formation of sorbitol in the lens of the eye, increasing osmotic pressure and disturbing protein synthesis.  This is one explanation of the development of gray star in diabetics.  The major toxic effect of glucose is probably glycation of proteins.  It is believed that much of the neurological and circulatory defects which follow diabetes are due to glycation.  Glycated hemoglobin $HbA_{1c}$ levels are used as indicators of long-term blood sugar levels.  Click here for more information.

# Hypothalamic $K_{ATP}$ Channels Regulate Hepatic Gluconeogenesis and Postpranial Blood Glucose Levels.

Blood sugar levels are dependent upon glucose uptake after meals and hepatic release of glucose between meals.   The sugar released from the liver comes either from stored glycogen or production of glucose from lactate and amino acids.  This production of glucose is largely responsible for stabilization of postprandial blood sugar levels.  The hyperglycemia noted in type
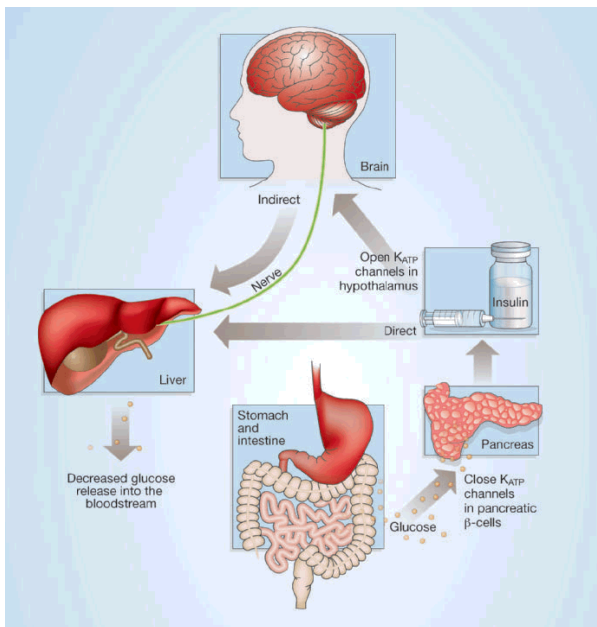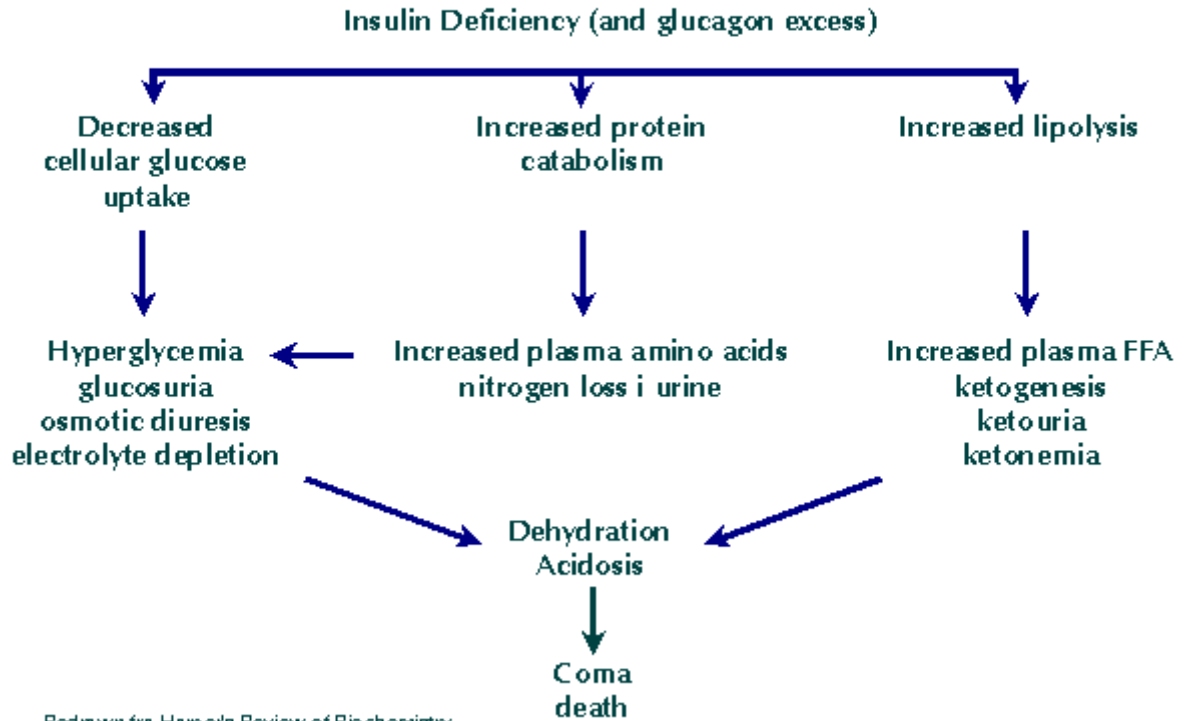


2 diabetes partially results from lack of control over hepatic glucose formation due to resistance to insulin.  It has recently become clear that part of this insulin effect occurs indirectly through insulin-sensitive receptors in the brain (more precisely, in the hypothalamus).     In a very recent article in Nature, Alessandro Pocai and coauthors presented convincing data that couples insulin-stimulation of hypothalamic $K_{ATP}$ channels with neural control of hepatic gluconeogenesis (Nature 434, 1026-1031, 2005; and an overview by Nature's editors ((click here)). Insulin stimulated opening of hypothalamic $K_{ATP}$ channels results in vagal nerve signaling to the liver and inhibition of gluconeogenesis.  This is part of the normal response to meals and following insulin release from the pancreatic ß-cells.  Thus, signaling from the brain is one of the important control mechanisms which establish correct "between-meal" blood sugar levels. Hypothalamic insulin resistance and therefore loss of control over hepatic gluconeogenesis may well be one of the important factors involved in

development of type 2 diabetes.  This model is summarized in this figure from the overview in Nature .

# Diabetic Metabolism

Insulin Deficiency (and glucagon excess)

Decreased cellular glucose uptake

Increased protein catabolism

Increased lipolysis

Hyperglycemia glucosuria osmotic diuresis electrolyte depletion

Increased plasma amino acids nitrogen loss i urine

Increased plasma FFA ketogenesis ketouria ketonemia

Dehydration Acidosis

Coma death

Redrawn fra Harper's Review of Biochemistry

A summary of metabolism in the diabetic state can be seen in the next figure. This figure applies both to uncontrolled diabetes type I and severe uncontrolled diabetes type II.