Integration of Metabolism

Our bodies are an integrated system of organs, each with its own requirements for nourishment and energy utilization. In spite of this, our tissues share a common circulation system. Strict limits on the blood levels of ions, lipids and sugars must be upheld if a healthy situation is to be maintained. These restrictions are valid at rest, while we work and after meals. How do we organize our bodies and survive under differing situations? The question is extremely difficult to answer. Physical activity and meals greatly alter influx to and uptake from the circulation. And yet, feedback and feed-forward control mechanisms on the enzymatic level, central nuclear control of protein synthesis and hormonal messaging and signaling all play a part in the integration of metabolism which those of us who are healthy manage so well.

Here comes my effort to clarify this jungle. Have patience and please remember my "closing remarks" (click here if you have forgotten them).

Integration of metabolism is essential on both short-term and long-term bases. Perhaps the most crucial short-term element is maintenance of a stable blood glucose level. The table below has been presented earlier but I will use it here to emphasize the fact that exercise can quickly reduce blood sugar levels. Maintenance of blood glucose levels over 2.5-3 mmol/l is essential for brain function. One might expect, therefore, that nature had equipped us with a sizable glucose reserve. Surprisingly, the total amount of glucose in the blood and liver is so small that it can be exhausted in minutes. The same result, a rapid reduction of blood glucose levels and ensuing loss of consciousness, follows administration of large doses of insulin (insulin-shock therapy). Stated more clearly, the body's metabolic balance can quickly be disrupted through excessive activity or hormonal derangement. And yet, this does not normally occur. Physiological processes adjust carbohydrate and fat metabolism such that blood glucose values do not fall markedly. If we have a shortage of sugar, fat metabolism takes over. Integration of metabolism protects us against metabolic catastrophes!

Energy Stores in Man				
Tissue Fuel	Provides fuel for			
	Reserve, grams	Starvation	Walking	Marathon
Fat	9000-15000	34 days	11 days	3 days
Muscle Glycogen	350	14 hours	5 hours	70 minutes
Liver Glycogen	80	3.5 hours	70 minutes	18 minutes
Blood/Extracellular Glucose	20	40 minutes	15 minutes	4 minutes
Body Protein	6000	15 days	5 days	1.3 days

Integration of metabolism is important on a long-term basis too. Blood sugar, glucose, is not an "inert and gentle" component of our diet. Glucose is toxic! Chronic high blood levels of glucose lead to protein denaturing and the development of blindness, neuropathy and the kidney damage seen in diabetes. High blood sugar levels lead to increased circulating triglycerides and are responsible for development of cardiovascular disease. Again, integration of metabolism and control by hormones and metabolites normally prevent these adverse effects of sugar. Let us look at the integration process.

What is "Energy"?

We often speak of "body energy", of being energetic or exhausted. What do we really mean by this. What is the physiological basis for movement, growth, speech and even reproduction? What drives our bodies?

Our physical and mental activity is powered by the energy we capture from our food and our body's fat and sugar reserves. However, we do not function by using these pools of substrates directly. The energy obtained by "burning" food must first be captured as "high energy" phosphate bonds in adenosinetriphosphate (ATP) before it can be utilized. Energy to perform "work" comes from splitting off one or two of these, forming P_i, ADP and AMP. This splitting of high-energy phosphate bonds is discussed below and elsewhere in Medbio.info click here. The point to remember now is that ATP has a rapid turnover rate. About 50 % of our total ATP reserve is renewed hourly when we are at rest. In fact, all of the ATP in working skeletal muscles can be used and regenerated in just a few minutes.

The amount of ATP required to provide energy for normal activity is striking. A sedentary male weighing 70 kg uses about 2000 kcal per day. Approximately 70 and 80 kilograms of ATP daily are required to provide so much energy. The whole body content of ATP at any one time is in the neighborhood of 250 grams. Thus, an enormous cycling of ADP back to ATP is required to balance ATP consumption. Amazingly, ATP concentrations are quite stable in spite of this extremely rapid turnover.

Sources of ATP.

ATP levels are maintained through several processes:

1. Adenylate kinase.

ATP has two "high-energy" phosphate groups. Splitting off the outer or gamma-phosphate group of ATP yields ADP and inorganic phosphate. Splitting off both high-energy groups in one step yields AMP and inorganic pyrophosphate (pp_i). Adenylate kinase, an enzyme found in all tissues, catalyzes a transfer of the energy-rich phosphate bond from one ADP molecule to another, giving ATP and AMP. The conversion is very rapid in muscle and liver. The data below are from skeletal muscle. Note that muscle activity gives relatively small changes in both ATP and ADP concentrations. However, through the action of adenylate kinase, adenosine monophosphate (AMP) levels increase markedly in the working situation. There is a 4-fold rise in AMP levels in this example of a work situation. AMP levels are crucial in

Adenylate Kinase

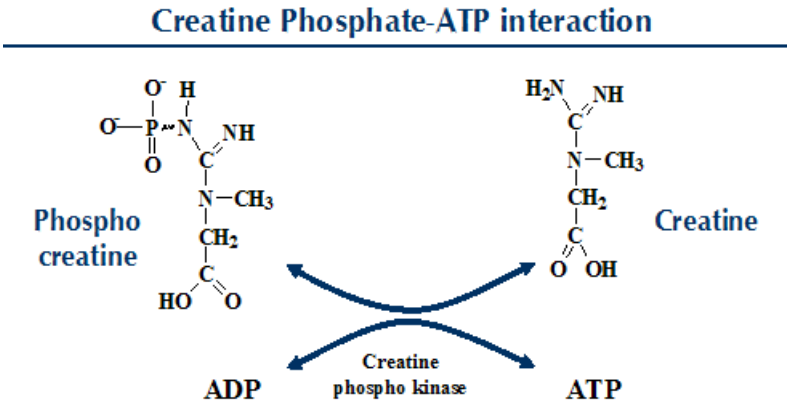
$$2\,ADP \rightleftharpoons ATP + AMP$$

At rest:	1.0 mM	5mM	0.2mM
Work :	1.5 mM	4mM	0.8Mm

adjusting the balance between carbohydrate and fatty acid metabolism in varying physiological situations. AMP is an active intracellular signal substance. We shall see that this "normal" AMP is coupled to a kinase which controls, among other things, uptake and metabolism of fatty acids. AMP is also an activator of glycogen mobilization and, therefore, sugar metabolism.

2. Creatine Phosphokinase/Phosphocreatine.

Most of our body tissues contain phosphocreatine at concentrations approximately three times that of ATP. Phosphocreatine is a reserve source of high-energy phosphate. This reserve can be transferred to ADP, thus regenerating ATP to replace that used by our various metabolic processes. While the creatine phosphokinase reaction is our most rapid ATP-yielding reaction, the amount of ATP which is produced is quite small. Muscle tissues have about 5 mmol/l ATP and approximately 17-20 mmol/l of creatine phosphate. Under extreme work (sprinting, for example) the phosphocreatine reserves are used up in about 30-40 seconds. However, "seconds do count" in sport. During those few seconds muscles can and do work with "explosive force". Olympic sprinters are very large, very muscular persons, not the thin and light runners I used to imagine. They are capable of running 100 meters almost without breathing. During a 100 meter sprint around 1/2 of the energy they use comes from high-energy phosphate stored in their muscles as creatine phosphate.



While phosphocreatine is important for maximal performance, other sources of energy production must come into play after the first 30 seconds of a sprint.

3. Anaerobic Metabolism.

In "second place" in the ATP-synthesis race (after phosphocreatine) comes ATP synthesis coupled to anaerobic metabolism. This is the cytosolic formation of ATP driven by oxidation of glucose (or glucosyl groups from glycogen) to pyruvate and lactate. ATP formation through cytosolic glycolysis proceeds with a speed equal to about 50% of that we see using creatine phosphate and creatine phosphokinase. Rapid, yes, but how much ATP can we make when the oxidation process is limited to formation of pyruvate and lactate from glucose or glycogen? Only two ATP molecules result for each glucose molecule that is processed. Three ATPs are formed for each glucosyl group that derived from glycogen. The secret here is that anaerobic glycolysis is very rapid. While it is relatively ineffective measured by energy production per glucose molecule consumed, glycolysis does turn out a lot of ATP in a short time. The big (and painful) disadvantage is that a lot of lactic acid is produced and accumulates in the working muscle. Furthermore, lipids cannot be used as substrates for anaerobic metabolism. Only glucose or

glycogen work here. If we press anaerobic glycolysis to the limits, muscles exhaust their stored glycogen and take up so much glucose from the blood that hypoglycemia and CNS malfunction result. You can click here for more information.

4. Aerobic Metabolism.

How do we manage this ATP-balancing? Where does the ATP synthesis take place? The obvious answer is that portion of our cells which is coupled to use of oxygen; to the air upon which we are so very dependent. All of our cells, with the important exception of blood cells, contain mitochondria. These organelles (mitochondria), probably originally derived from invading bacteria, can completely burn carbohydrates, fats and some amino acids to carbon dioxide and water. It is the mitochondria that use oxygen and form water while oxidizing our "food". Their actual substrate is acetyl-CoA. All food that can be reduced to 2-carbon fragments can serve as a substrate for mitochondrial ATP production. The combustion process is coupled to reduction of oxygen giving water as a byproduct. Approximately 30% of the energy released in this process is trapped in the terminal phosphate group in ATP. The rest of the energy in acetyl-CoA escapes as heat, keeping us nice and warm! We produce around 15 moles of ATP for each mole of acetyl-CoA that is processed.

While aerobic synthesis of ATP is the most effective way to produce "useable energy", it is a relatively slow process. Please go to the section describing muscle metabolism if you will go through the details of this process (Click here).

How do I Choose a Substrate for Mitochondrial Metabolism?

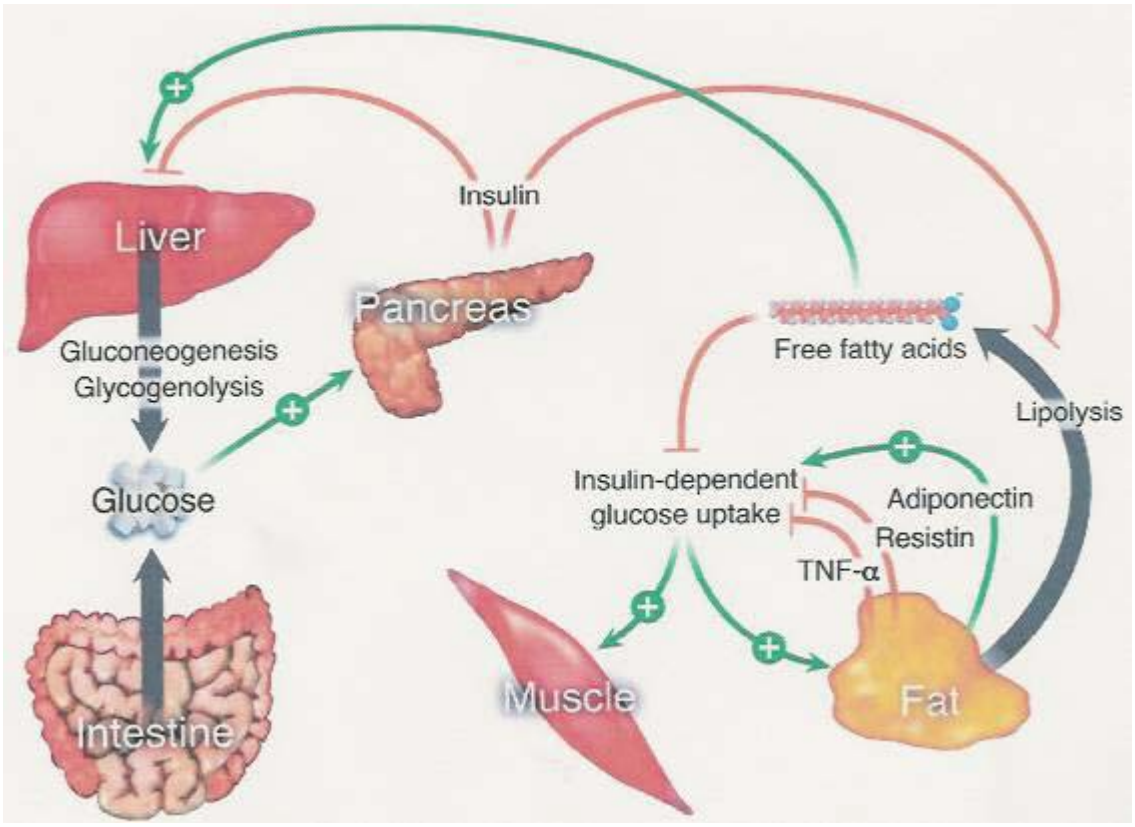
As stated above, acetyl-CoA is the actual substrate for mitochondrial metabolism. This is formed by ß-oxidation of fatty acids, decarboxylation of pyruvate, or from amino acids.

Overweight has become a major and global threat to our health. Accumulation of fat, especially centrally, is coupled to hypertension, diabetes type 2 and CVD. Wouldn't it be nice to "decide" to stop using carbohydrates and just burn away that fat? Unfortunately, things just do not work that way. Our tissues have strong demands as to which substrate they can use. Brain metabolism is normally completely dependent upon blood glucose as substrate; fatty acids do not cross the blood-brain barrier. Blood cells, which do not have mitochondria, are also completely dependent upon anaerobic metabolism and, therefore, blood sugar. Blood sugar levels must also be carefully controlled; too much glucose is toxic and too little leads to CNS disturbances. Blood glucose levels must be held in the range of 4-5 mmoles/l between meals and under 10 mmoles/l after meals. This kind of management requires hormonal regulation of many processes. The main actors here are insulin, glucagon, adrenaline and growth hormone. Many other hormones control appetite and secretion of these "key" hormones. Comprehension of the mechanisms at work is difficult because these enzymatic processes and hormonal control are tightly integrated. Furthermore, our different organs have their own complicated steering systems. What is true for the liver may not be applicable to muscles. An additional factor is the frequent discovery of new hormones and other elements in these so very complicated processes. One thing is sure; new discoveries are altering possibilities for medical treatment of metabolic and endocrinologic imbalances. New approaches to type 2 diabetes, overweight, and cardiovascular diseases are frequently reported in the literature.

Turning Metabolism Off and On.

Insulin Affects both Glucose and Lipid Metabolism.

A good starting point for understanding control of metabolism is a figure recently published in Nature Medicine 10, 355-361 (2004) by R. M. Evans, G. D. Barish and Yong-Xu Wang. The



authors present information about "cross-talk" between various organs. Here, the signal initiating "cross-talk" is either an increase in blood sugar or fatty acids levels. This can occur following a meal and uptake from the small intestine or as a result of stimulation of glucose release from the liver. The figure is simplified to aid understanding, but remember, changes in glucagon usually oppose alterations in insulin levels. Thus, gluconeogenesis and glycogenolysis are often initiated by rising glucagon and falling insulin levels.

Increased circulating glucose levels stimulate pancreatic secretion of insulin. This has several immediate effects:

1. Increased skeletal muscle glucose uptake.

2. Inhibition of hepatic gluconeogenesis and glycogenolysis and stimulation of glucose uptake in the liver (not shown).

3. Inhibition of lipolysis in fat tissue.

Muscle tissue and liver do not just take up glucose. They must "do something with it" or it will diffuse over the cell membrane and return to the circulation. Both tissues have glycogen reserves

and these will be filled when glucose is taken up. Further, skeletal muscle, which makes up over 50% of the body, will use glucose as a substrate for "aerobic glycolysis", that is, complete glucose "burning" from the sugar phosphate and mitochondrial metabolism to CO_2 and water. Approximately 25% of the carbohydrate content of a meal will normally be used as an energy source in skeletal muscles. You can click here for more information about carbohydrate metabolism after meals.

What happens after a meal? Insulin stimulates muscular glucose uptake and metabolism, and forces the tissue to reduce the use of fat as an energy substrate. After all, acetyl-CoA, the common substrate formed from both sugars and fats for entry into mitochondrial metabolism is used at a constant rate as long as the work load does not change. I will come back to control of fatty acid use soon but will point out here that insulin inhibits release of fatty acids from fat cells (inhibits lipolysis as shown in the figure above). Muscle takes up and uses fatty acids in proportion to the concentration of fatty acids in blood. Thus, insulin speeds up glucose uptake and metabolism, while setting down the rate of lipolysis and release of fatty acids from fat cells to the circulation. This sounds like a simple rule, but things are not so simple. Remember, insulin swings markedly after a meal, increasing from basal to maximal concentrations during the first hour after eating, then falls rapidly. At the same time, glucagon levels swing in the opposite direction and have effects opposing insulin. The united effect on metabolism is always the result of the balance between these two hormones. Metabolism and the associated choice of energy substrate follow an integrated response to the hormonal picture.

We can also see from the figure that free fatty acids reduce insulin's effect on glucose uptake. Free fatty acids are involved in "insulin resistance", that is, the concentration of fatty acids in the blood is inversely correlated with tissue responsiveness to insulin. Chronically increased serum fatty acids as seen in overweight and obesity are implicated in the development of diabetes type 2, where we see a decreased response to insulin, often counteracted by markedly increased insulin levels.

The figure above also depicts the very important role of adipocytes as endocrine cells. Fat cells produce a number of peptide hormones (adipokines) that have been identified during the past five to ten years. These are involved in regulation of tissue response to hormones. Resistin appears to dampen muscle, liver and fat cell responses to insulin as does TNF-α. Adiponectin sensitizes receptor-cells to insulin. Leptin regulates metabolism and appetite.

Control of Lipolysis in Fat Cells.

The "stress" hormones adrenalin, noradrenalin and growth hormone activate lipolysis through a common mechanism. Glucagon, the "hunger" hormone, shares this mechanism. All of these, by combining with their specific receptors, activate adenyl cyclase and increase the adipocytes content of 5-cyclic AMP (cAMP). This in turn activates protein kinase A (PKA). The following phosphorylation of hormone-sensitive lipase (HSL) was thought to initiate splitting of triglycerides and efflux of free fatty acids to the circulation. Insulin opposes this phosphorylation by down-regulating formation of cAMP and by activating a protein phosphatase which dephosphorylates hormone-sensitive lipase.

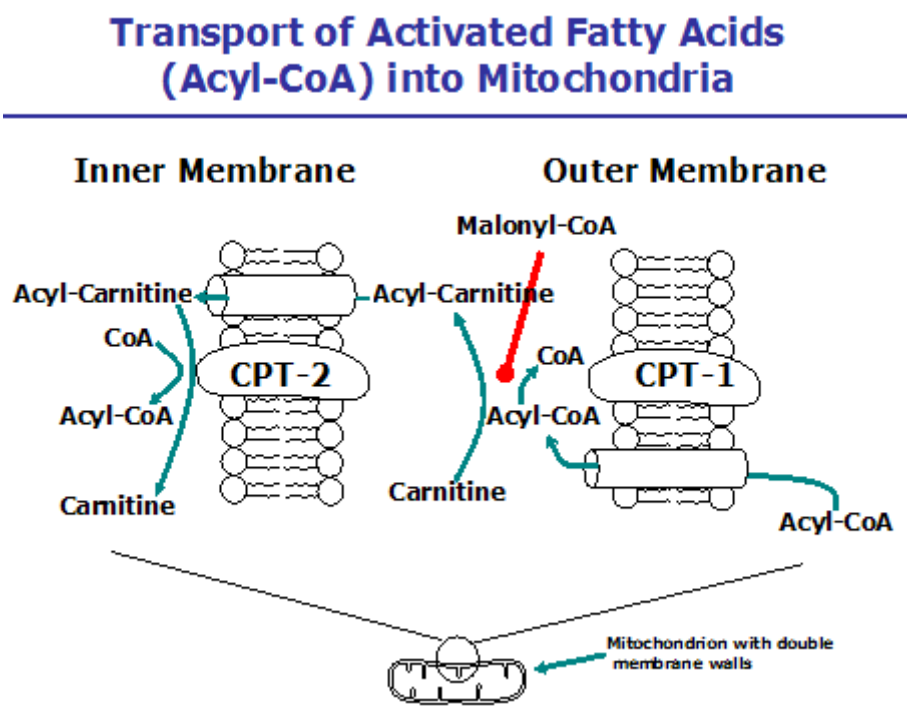
This story has become far more complex during the last year or two. Activation of HSL alone is insufficient to start up lipolysis. PKA must phosphorylate at least two important proteins before triglyceride degradation can begin. The lipase is not found at the fat droplet surface but must be transferred there by perilipin, another cytosolic protein. Perilipin is activated through phosphorylation by PKA. Both perilipin and HSL must be phosphorylated to activate lipolysis.

Current work indicates that several other enzymes are involved in lipolysis. It appears that triglyceride catabolism is under control of three lipases. The initial deacylation of triglycerides is catalyzed by adipose triglyceride lipase found in the fat droplet (see Fat Mobilization in Adipose Tissue is Promoted by Adipose Triglyceride Lipase, Science 306, 1383-86, 2004 (click here if you have access to Science). Hormone-sensitive lipase (HSL) binds to the fat droplet's membrane and uses diacylglycerides as its substrate. A cytosolic monoglyceride lipase catalyses splitting of the third fatty acid from the glycerol "backbone". Thus, it presently appears that catalysis of triglycerides is a three or four-step process, where adipose triglyceride lipase removes the first fatty acid chain, hormone-sensitive lipase the second and monoglyceride lipase takes the third fatty acid chain. Perilipin phosphorylation is required to transfer HSL to the lipid droplet surface. Together, these enzymes determine the minute to minute level of free fatty acids bound to serum albumin. The rate of fatty acid metabolism is a direct function of the level of the free fatty acid-albumin complex in the circulation.

What Controls Uptake and Oxidation of Fatty Acids?

Metabolism of fatty acids differs from tissue to tissue. Our major bodily "chemical factory", the liver, can both synthesize and oxidize fatty acids. Skeletal muscle does not produce fatty acids but has an active oxidizing system for these.

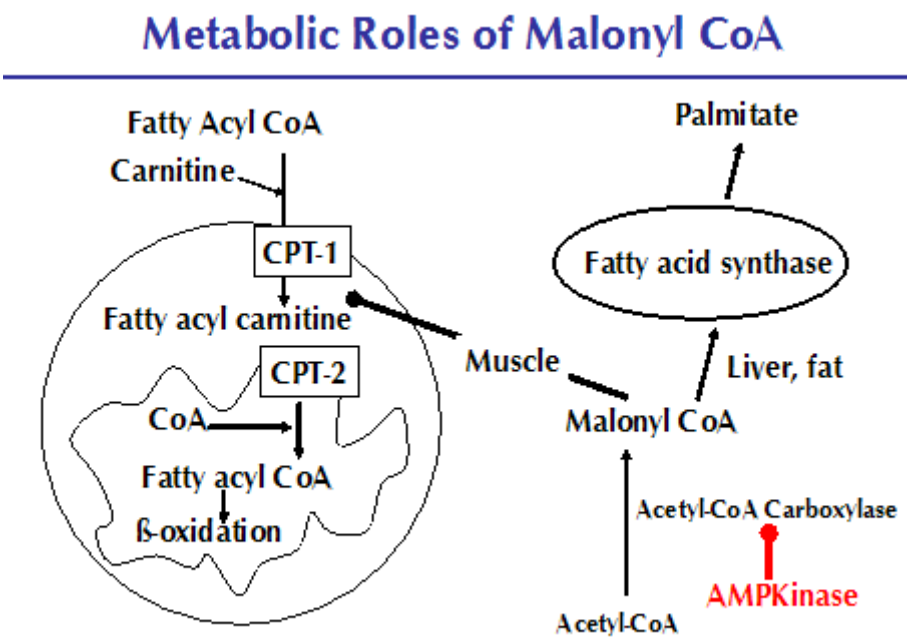
Fatty acid metabolism is divided between two compartments. Initial activation of fatty acids taken up from the circulation is carried out in the cytosol with acyl-CoA being the final product. This must be taken up into mitochondria before beta-oxidation can reduce these long carbon chains to acetyl-CoA and send them further for oxidation. The "catch" here is that acyl-CoA molecules cannot cross the inner mitochondrial membrane. They must be converted to carnitine derivatives in the area between the inner and outer membrane,, moved across the inner membrane as acyl-carnitine, and resynthesised as acyl-CoA within the mitochondrial matrix. This transport of fatty acids is dependent upon the two-stage carnitine-palmitoyl-transferase system (CPT1 and CPT2), found in the two mitochondrial membranes. The carnitine transport system for fatty acids gives the key to understanding control of oxidation of these most important substrates for energy production. The secret to understanding this transport is that it is strongly inhibited by malonyl-CoA and that synthesis of malonyl-CoA is in turn regulated by AMP (not the cyclic derivative, but old-fashioned 5-AMP).



Synthesis of Malonyl-CoA; Acetyl-CoA Carboxylase.

Malonyl-CoA is the main regulator of uptake and oxidation of fatty acids in mitochondria. And, mitochondria are our major building site for effective synthesis of metabolic energy in the form of ATP. What are the physiological functions of malonyl-CoA and what controls its level?

Malonyl-CoA is a 3-carbon derivative of acetyl-CoA, the major break-down product of fatty acids and an intermediate in the synthesis of these long-chain lipids. It is formed through the action of the enzyme acetyl-CoA carboxylase in the presence of the vitamin biotin by coupling a activated CO_2 with acetyl-CoA. Malonyl-CoA has two important roles in metabolism.



1. It's formation is the "opening step" in the synthesis of fatty acids. Conversion of carbohydrates to fatty acids is a function of the liver and, to a lesser degree, of fat tissue.

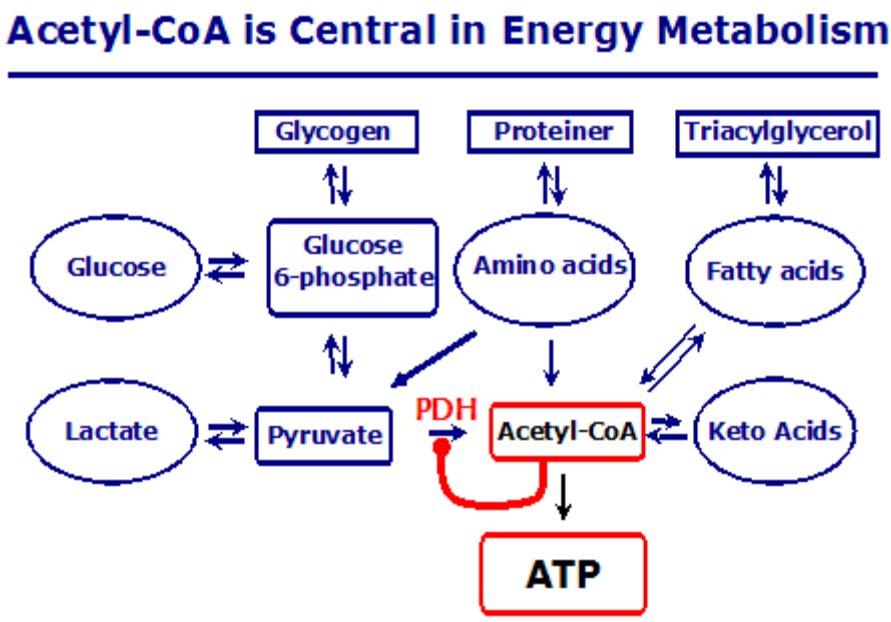
2. Malonyl-CoA coordinates oxidation of fatty acids with energy need, especially in skeletal muscle.

The key to this is the fact that acetyl-CoA carboxylase, the enzyme that catalyzes malonyl-CoA formation is inhibited strongly through phosphorylation by AMPK (AMP kinase). An increased level of AMP can therefore turn on fatty acid oxidation and mitochondrial synthesis of ATP. At the same time, the increased oxidation of fatty acids tends to turn off carbohydrate oxidation.

We are now back to the beginning of this discussion of coordination of energy metabolism. Remember, AMP is that nucleotide (or signal) which varies most when we use ATP. When energy utilization exceeds ATP synthesis AMP concentration will begin to rise. In muscle, this initiates activation of AMP kinase, stops acetyl-CoA carboxylase activity, reduces malonyl-CoA levels and accelerates fatty acid oxidation in mitochondria. And, these are "effective" ATP synthesizers which restore our energy balance. This takes time, but works well as long as we do not try to "sprint forever"! (Click here for details of energy use in working muscle).

The Carbohydrate-Sparing Effect of Fatty Acid Oxidation.

A basic problem we all face is that carbohydrate reserves in the body are limited. We can, in fact, use up glycogen in muscle and the liver in a matter of minutes. Running for about 15 minutes at maximal speed for that period of time can bring us to the "hypoglycemic brink". More clearly defined; we can come to experience a "red-out" or "black-out" because of falling blood glucose concentrations during extreme exercise. Just how do we manage to brake carbohydrate oxidation when we physically work over longer periods? This is a most complex situation, governed by hormones and allosteric controls but, stated simply, we turn on oxidation of fatty acids and turn off carbohydrate entry into mitochondrial metabolism.

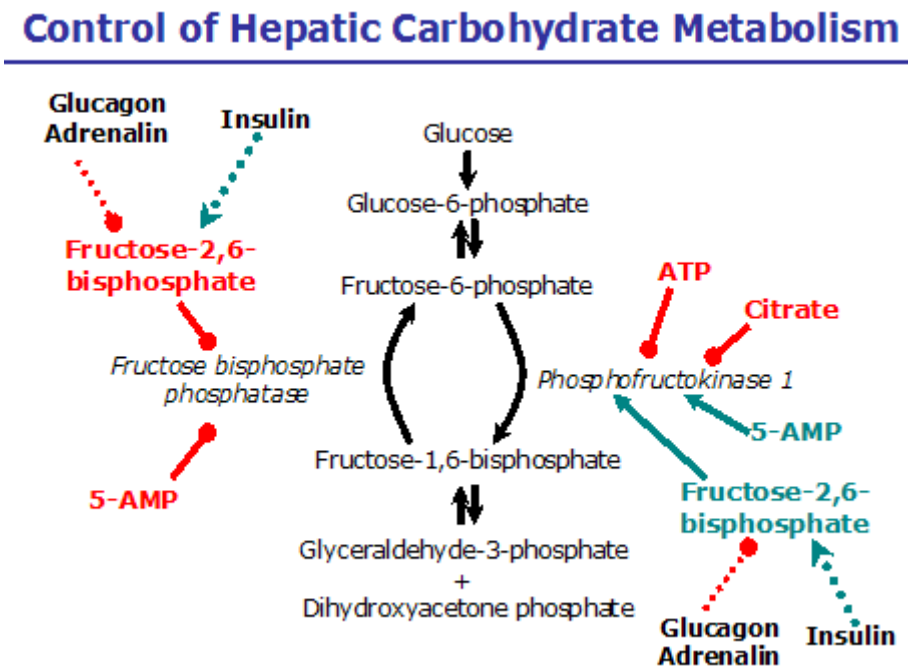


The key to this is a combination of hormonal and feedback control of pyruvate dehydrogenase (PDH). PDH is actually a complex comprised of three enzymes and five cofactors. Many factors play in here, but we should note that PDH is product-inhibited. That is, acetyl-CoA, the final result of PDH action on pyruvate, inhibits mitochondrial oxidation of pyruvate. Carbohydrate and fat metabolism are thereby coupled together. As long as circulating fatty acids cover the mitochondrion's requirement for acetyl-CoA, they do not utilize pyruvate derived from glucose or glycogen.

Insulin also plays an important role here. Insulin controls the state of phosphorylation of PDH. The enzyme is activated by phosphorylation. Insulin activates a protein phosphatase and triggers dephosphorylation of PDH. Therefore, PDH is controlled by factors arising from physical work (acetyl-CoA), and hormones that swing following meals.

Control of Carbohydrate Metabolism; the "Phosphofructokinase-Fructose bisphosphate phosphatase Couple".

Blood sugar, or glucose, is the major source of energy for many tissues. Blood cells and the brain are normally completely dependent upon blood sugar. Their metabolism is locked to this substrate and they have no reserve carbohydrate. Glycogen stores are not found in these tissues. And, while skeletal muscle can cover much of its energy requirement through oxidation of fats, hard working muscle uses carbohydrates. Muscle tissue can, in fact, take up so much glucose from the circulation that hypoglycemia and loss of consciousness results.



We can get an overview of regulation of carbohydrate by studying hepatic metabolism. We find all of the hormone and enzyme functions that control carbohydrate metabolism there. The major control points in glycolysis and gluconeogenesis are the enzymes which catalyze the reactions between fructose-6-phosphate and fructose-1,6-bisphosphate. Phosphofructokinase-1 (PFK-1) and fructose bisphosphate phosphatase are regulated by allosteric "feedback" mechanisms and by hormones. They are regulated by common signal substances. However, these have opposite effects on these two enzymes and, therefore, upon metabolism.

Let us look at PFK-1 first. The PFK-1 step is the slowest in glucose metabolism (glycolysis). It is, therefore, very well suited as THE primary controlling point in this process. PFK-1 is inhibited by ATP and stimulated by its breakdown product, 5´-AMP. We have previously seem that ATP levels are surprisingly stabile while AMP swings markedly during energy utilization. PFK-1 is sensitive to the physiological concentrations of these nucleotides and its activity increases as AMP levels increase.

PFK-1 is also sensitive to citrate which is released from the mitochondria to the cytosol when the liver uses fatty acids. This occurs between meals and is a part of the "fatty acids spare carbohydrate" business. Not only does fatty acid oxidation turn off pyruvate dehydrogenase and pyruvate uptake to the mitochondria; it also turns off the source of pyruvate.

Both PFK-1 and fructose-1,6-bisphosphate phosphatase are regulated by another of those "fructose-diphosphate" things. A hormone sensitive kinase, phosphofructokinase-2, produces the 2,6 bisphosphate from fructose-6-phosphate. This kinase is subject to cyclic AMP-stimulated

phosphorylation. The phosphorylated form has phosphatase activity, not kinase activity. The phosphorylated form uses fructose-2,6-bisphosphate as its substrate, thus reversing the effects of the non-phosphorylated PFK-2.

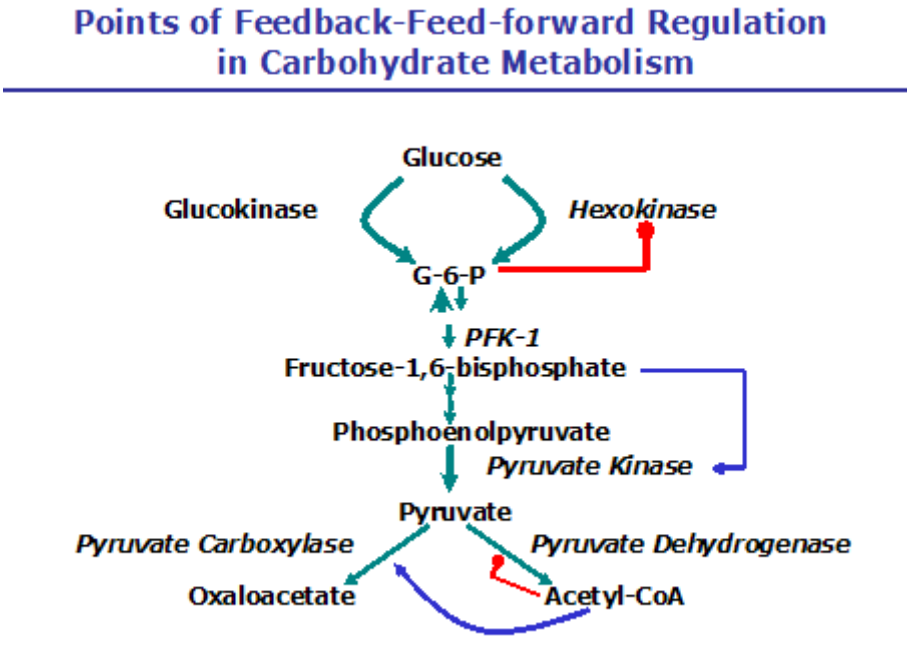
Fructose-2,6-bisphosphatase controls carbohydrate metabolism by regulating the activities of PFK-1 and fructose bisphosphate phosphatase. Hormones that increase the rate of glycolysis increase the level of fructose-2,6-bisphosphate. Hormones that phosphorylate PFK-2 reduce the levels of fructose-2,6-bisphosphate and favor gluconeogenesis.

The liver is sensitive to several hormones that increase cyclic AMP. These are glucagon, adrenalin and noradrenalin. These inhibit glycolysis by reducing the concentrations of an activator of PFK-1 (fructose-2,6-bisphosphate). The same hormones stimulate gluconeogenesis by removing an inhibitor of the key enzyme (by inhibiting the action of an inhibitor).

The liver is also responsive to insulin which increases breakdown of cyclic AMP through activation of phosphodiesterase. Thus, insulin activates glycolysis by increasing the activity of PFK-2 and synthesis of fructose-2,6-bisphosphate. This is coordinated with inhibition of gluconeogenesis at the fructose bisphosphate phosphatase step by the same signaling substance.

In summary, it is fructose-2,6-bisphosphate levels that are a major regulator of carbohydrate metabolism. This is a control substance synthesized in answer to stress or hunger and geared towards stabilizing blood glucose levels. Reducing synthesis of fructose-2,6-bisphosphate turns off carbohydrate "burning" and starts up glucose production from smaller substances.

A Short Overview of "Secondary" Sites of Allosteric Regulation of Carbohydrate Metabolism.



The constant adjustment in the rates of PFK-1 and fructose bisphosphate phosphatase lead to fluctuations in the concentrations of metabolites before and after these reaction steps. In most tissues, hexokinase is responsible for the initial reaction in glycolysis, phosphorylation of glucose and formation of G-6-P. Hexokinase is inhibited by physiological concentrations of this intermediate. Thus, a reduction

of PFK-1 activity will be reflected in an increase in G-6-P. This markedly inhibits hexokinase activity and reduces uptake of glucose to most cells.

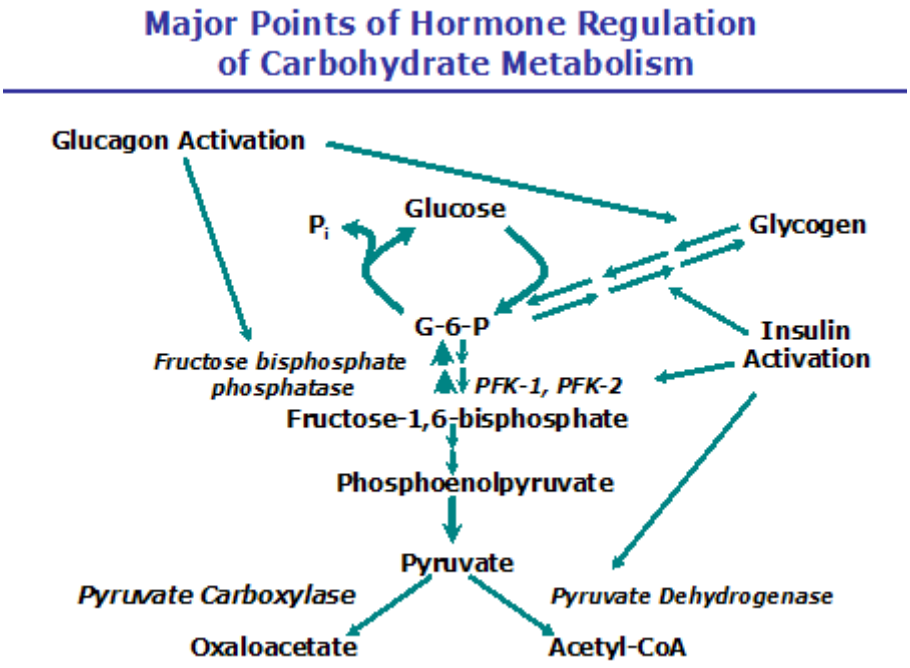
Fructose-1,6-bisphosphate activates pyruvate kinase in a "feed-forward" manner, assuring that glycolysis will get as far as pyruvate. In most tissues there follows a control point which was explained earlier in respect to "the carbohydrate-sparing effect of fatty acids". If there is an acetyl-CoA excess in mitochondria, this will "turn off" conversion of pyruvate to acetyl-CoA. A "backup" in glycolysis results, turning off glucose metabolism.

Liver metabolism.

Hepatic energy metabolism quite generalized and most of the possible metabolic pathways operate in the liver. However, some significant differences are found. The major glucose-phosphorylating enzyme in the liver is glucokinase. This enzyme is not product-inhibited and the glucokinase reaction proceeds rapidly even when PFK-1 is overwhelmed with substrate. This ensures uptake and storage of sugar in the liver after meals. The liver has several means of storing glucose. It can be stored as glycogen to be used to rapidly stabilize blood sugar levels in postprandial periods and during exercise. Glucose in excess of that required for energy metabolism and glycogen storage is converted to fatty acids and triglycerides. These are then sent out into the circulation as VLDL for transport to and storage in adipose tissue. Excessively high blood sugar levels lead to increased blood triglycerides through this mechanism.

Hormonal Control of Hepatic Carbohydrate Metabolism.

Hepatic carbohydrate metabolism is strongly influenced by insulin and glucagon. These hormones stabilize blood sugar levels through regulation of glycolysis and gluconeogenesis.



Insulin acts at three major points:

1. Glycogen Syntase.

2. PFK-2 and, therefore, PFK-1.

3. Pyruvate dehydrogenase.

Glucagon and adrenalin activate glucose formation and release from the liver to stabilize blood glucose between meals and under physical work. They do this through activation of

the cyclic AMP/protein kinase A system. The protein phosphorylation which results activates glycogen phosphorylase and fructose bisphosphate phosphatase. (The latter through the PFK-2 - fructose-2,6-bisphosphatase system).

Metabolic Control is Organ Specific

Differing enzymatic makeup gives differing metabolic patterns.

The mechanisms of metabolic integration would be much easier to understand if they were common for our various organs. Unfortunately (for ease of understanding, not function) this is not the case. All of our cells are equipped with the same genetic information. In spite of this, the various cell types express or suppress differing genes. Tissues differ, therefore, in their enzymatic makeup, in their hormone responsiveness and the possibilities for transport of various substances over cell membranes.

There are countless examples of differing enzymatic activities in our various tissues. I will pinpoint just a few examples here.

We can use hepatic metabolism as a "reference", since the liver carries out most of the steps in carbohydrate and lipid metabolism. Here we have both active glycolysis and gluconeogenesis, deamination of amino acids and ureogenesis and lipid synthesis.

Skeletal Muscle.

Skeletal muscle normally makes up about one half of the body's mass and, therefore, dominates energy metabolism. In spite of the fact that skeletal muscle uses much of the glucose we consume or produce daily, muscle does not carry out gluconeogenesis, cannot dephosphorylate G-6-P and, therefore, cannot generate glucose and stabilize blood sugar levels. Muscle lacks receptors for glucagon and does not react to the increases in glucagon levels seen postprandial. The relatively large glycogen reserves in skeletal muscle cannot be mobilized to buffer blood sugar but are important for energy metabolism in muscle. These are activated through the adrenergic nervous system and adrenalin.

The energy stored as muscle glycogen can only be utilized in the muscle cells where it is found. However, if it is used in anaerobic metabolism (that is, from glycogen to pyruvate and lactate) the lactic acid formed can be transported to other tissues. Both the heart and kidneys use quantities of lactate produced in other tissues.

Unlike the liver, skeletal muscle lacks fatty acid synthetase and cannot synthesize fatty acids and triglycerides. In spite of this, the initial step in fatty acid synthesis, acetyl-CoA carboxylase, is active and is subject to control by AMP-kinase. As previously described, the synthesis of malonyl-CoA regulates transport of fatty acids over the mitochondrial membrane and, therefore, the rate of fatty acid oxidation in skeletal muscle.

Brain.

Energy metabolism in the brain is normally based wholly on glucose. This organ uses six grams of glucose hourly, corresponding to around 15 % of the carbohydrate content of a normal meal. The brain has no glycogen reserve. Glucose, which the brain is normally completely dependent

upon, must come from the circulation. The "K_m" for glucose uptake over the brain's outer membrane is approximately 1.0 mmolar. If blood glucose levels fall below 2.5-3 mmol/l uptake rates fall off and dizziness and loss of consciousness can quickly result.

One might think that nature was so clever that provision for fat-burning would be built into the brain's metabolism, but this is not the case. The so-called blood-brain-barrier prevents uptake of fatty acids into the brain. The ketone bodies, acetoacetate and ß-hydroxybutyrate are produced from fatty acids in the liver. These can partially replace glucose as they are transported over the plasma membrane into the brain.

One can ask "why can those energy-rich ketone bodies only replace about half of the glucose requirement in the brain"? The answer lies in the fact that cells and organs divide the body's "work load" and survive through a unique cooperative system. Glia cells are the brain's "outer" cells and line the blood vessels that supply the brain. These form the barrier across which fatty acids cannot cross. Glia cells take up glucose, send it through anaerobic glycolysis and export the resulting lactate and some glucose into the brain's deeper regions. There, the lactate serves as the substrate for aerobic metabolism and energy winning. The glia cells appear to be partially dependent upon anaerobic metabolism for which and ketone bodies are not substrate. This may well explain the CNS's requirement for glucose in addition to ketone bodies.

A similar system is found in the testes, where Sertoli cells form a barrier between the circulation and germ cells. Sertoli cells produce lactate and send it to germ cell where it is used in energy metabolism.

Blood cells lack mitochondria and, therefore, are unable to fully oxidize glucose, their only energy substrate. These cells also produce lactate which is largely taken up by the kidneys and used as a substrate both for energy metabolism and by gluconeogenesis.

Metabolic cooperation between cells and the various organs is is the key to healthy survival. Working together is essential even though individual cells just might not know this!

Alexandre Dumas expressed this so well in "The Tree Musketeers":

> "And now, gentlemen," said D'Artagnan, without stopping to explain his conduct to Porthos - "all for one, one for all, that is our device, is it not?"
>
> "And yet!" said Porthos.
>
> "Hold out your hand and swear!" cried Athos and Aramis at once.
>
> Overcome by example, grumbling to himself, nevertheless, Porthos stretched out his hand, and the four friends repeated with one voice the formula dictated by D'Artagnan.
>
> "All for one, one for all."

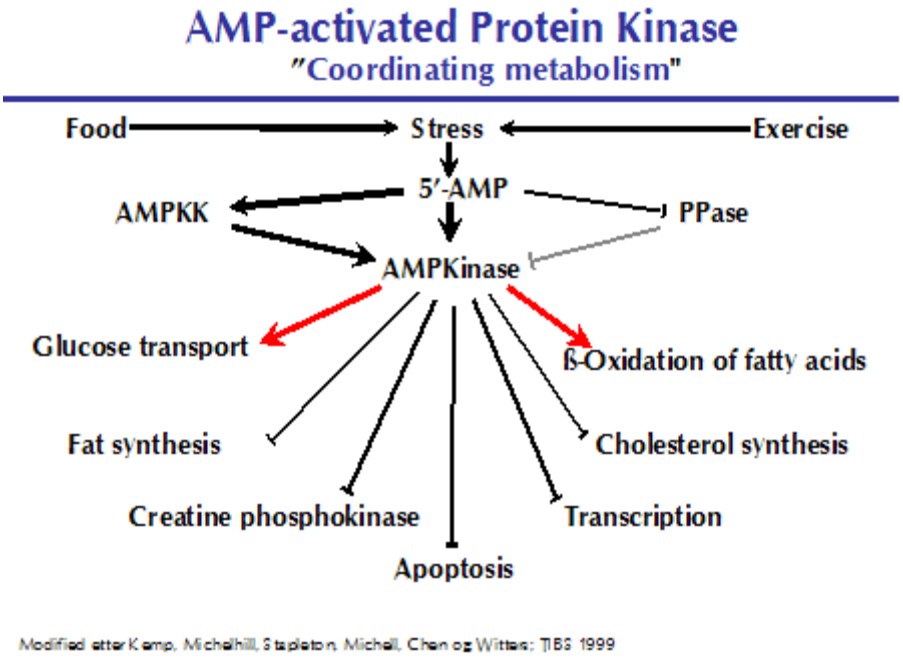
and they lived happily ever after...

This is extremely complex, but the bottom line is clear: our bodies are organized to maintain a stable milieu, using both feed-forward and feed-back allosteric signaling and hormone control of enzymatic processes.

And the big question is, what is the master key?

AMP Kinase is a major Regulator of Metabolism.

All energy-requiring bodily functions use ATP as the direct energy source. This results in relatively small changes in ATP and ADP, while AMP levels do swing rapidly. Therefore, AMP and its allosteric "target", AMP kinase, were thought to be major controllers of many metabolic processes. Some of these are listed in the next figure, modified from a paper of Kemp, Michelhill, Stapleton, Michell, Chen and Witters, TIBS 1999. AMP allosterically activates AMPK and makes the enzyme a better substrate for upstream kinases (AMPKK) and a poorer substrate for protein phosphatases. AMP kinase increases energy metabolism by increasing glucose uptake by working muscles and through activating fatty acid metabolism. It inhibits fatty acid synthesis, transfer of high-energy phosphate groups from phosphocreatine, inhibits cholesterol synthesis, DNA translation and apoptosis, or programmed cell death.



Thus, the balance between the adenine nucleotides catalyzed by adenylate kinase is tightly coupled to mitochondrial energy production as well as anaerobic carbohydrate metabolism. Rapid use of energy, that is ATP, triggers replacement through adenylate kinase, creatine phosphokinase and anaerobic metabolism. Work at lower levels which continues over time uses mitochondrial oxidation of fatty acids to generate ATP.

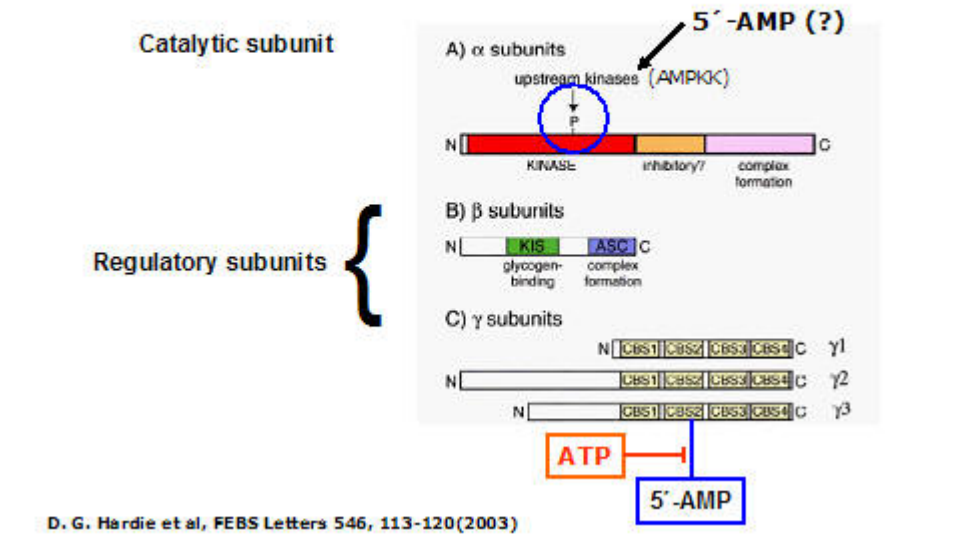
Is the AMPK (AMP-Kinase) system, THE Master Controller of Metabolism?

Research conducted during the past five years or so has revealed that the AMPK system is far more complex than previously thought. The system has also been found to be much more central in the control and integration of metabolism than earlier work had suggested.

AMPK (AMP-kinase) is an " ancient enzyme" and is found in found in all eukaryotic organisms and all tissues. The changes in AMP which follow normal ATP-turnover switch metabolism towards release of stored energy in work situations and to storage in "times of plenty". Reports from the laboratories of D. Grahame Hardie, Dario Alessi, Kei Sakamoto and Jose R. Bayascas in Dundee and Barbara B. Kahn and Thierry Alquier at Harvard and several others have demonstrated that AMPK and AMPK-related kinases control many of our most important functions. These include choice of energy substrate as mentioned above, regulation of appetite, cell polarity, division and growth and coordination of the energy state and protein synthesis. Malfunction of this primordial system appears to be involved in development of diabetes mellitus and cancer. The physiological functions of most the AMPK-related kinases are today unknown. It can be expected that these will be shown are involved in regulation of many physiological processes.

The structure of AMPK

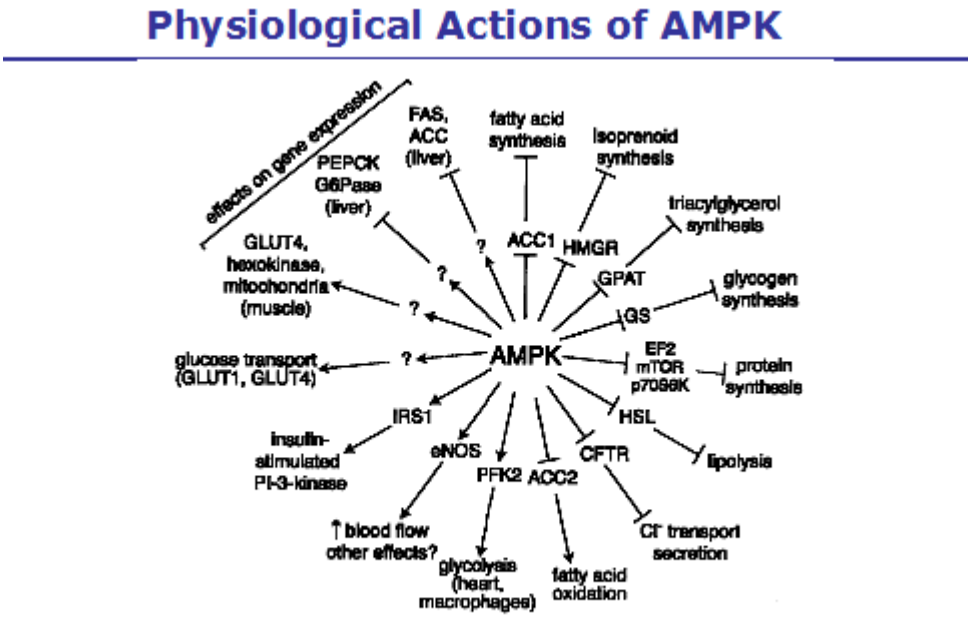
AMPK exists as conglomeration of 3 subunits; a heterotrimer to be more exact. The enzyme consists of a catalytic α-subunit, and regulatory ß- and γ-subunits. AMP binds to so-called CBS pairs in the γ-subunit and thereby activates the kinase activity in the α-subunit. Not surprisingly, ATP competes with AMP binding so that the ratio of AMP/ATP is the real controlling element here. The resulting phosphorylation of various metabolic enzymes can either activate or inactive them. The ß-subunit binds glycogen and this polysaccharide seems to inhibit AMPK. This may indicate the existence of a signaling link between AMPK and energy stores.



As shown in the figure from Hardie et al., the α-subunit is phosphorylated at threonine 172 by an "upstream" kinase. This phosphorylation point is essential for AMPK activity. AMPK lacking phosphorylation here cannot be activated by AMP. Furthermore, while AMP can allosterically increase kinase activity by a factor of five, a fifty-times activation can be elicited by complete phosphorylation at the threonine 172 site. Actually, the name, AMP Kinase is not really appropriate.

The nature of AMPKK (or LKB1) and its coupling to endocrinologic regulation has only recently been partially clarified. I will take that up soon. Note here that interference with LKB1 can eliminate phosphorylation of AMPK-α. This results in a total failure of the activating action of AMP on AMPK and loss of this essential regulation point in metabolism.

Much of the work on AMPK has come from D. Graham Hardie's group. The main points of AMPK-regulated metabolic control reported by Hardie up to 2003 are summarized in the following figure. Note that the figure only includes some of the systems under control of AMPK. Once again, we see that anabolic pathways are inhibited by AMPK while those metabolic processes aimed at release of energy from stores are activated. Further, genes responsible for production of enzymes and protein cofactors involved in these pathways are stimulated by activation of



D. G. Hardie et al, FEBS Letters 546, 113-120(2003)

AMPK. AMPK increases sugar and fatty acid oxidation, synthesis and membrane localization of GLUT4 (glucose transport protein 4), augments oxygen transport and inhibits the opposing anabolic reaction pathways. Once again, use of energy leads to increased AMP levels and these stimulate ATP production, thus maintaining balance in this so very complicated system.

An additional and very important aspect reported here is that protein synthesis is regulated by AMPK. Further studies have shown that cell polarity and division is also under control of the AMPK system. Put more clearly, protein synthesis and growth is coupled to the availability of

energy as measured and reported by the AMPK system. Loss of control of growth and protein synthesis by AMPK seems to be involved in tumor growth.

In spite of these very extensive studies, two important questions remained unanswered:

1. What was the nature of the kinase which activated AMPK through phosphorylation of threonine 172?

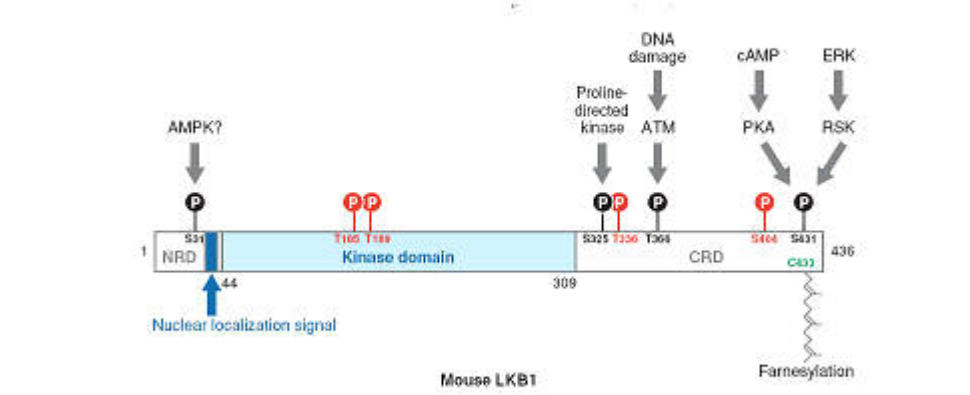
2. How do hormone-initiated signals activate AMPK?

Discovery of the nature of AMPK-Kinase (AMPKK). Peutz-Jeghers syndrome (PJS) and LKB1.

The studies by Alessi's group which led to discovery of the link between AMPK and LKB1 were carried out simultaneously with Hardie's work at the University of Dundee's School of Life Sciences. Dario Alessi had been searching for the physiological substrate for LKB1, a protein kinase shown to be defective in patients with Peutz-Jeghers syndrome (PJS). PJS is characterized by the development of benign and malignant tumors in the intestinal tract. Alessi suggested that the LKB1 that acted as a tumor suppressor and that tumor generation in PJS resulted from a deficiency in LKB1 activity. Professor D. Graham Hardie worked in the same department as Alessi. His group had studied AMPK for many years and had observed that an upstream kinase controlled its activity, but had not identified its nature. As reported here, they found that they had the answers to each other's query. AMPK was found to be the substrate through which LKB1 controlled protein synthesis and growth. Furthermore, LKB1 was, indeed, AMPKK (AMPK-kinase) and provided a link to the many diverse processes that affect AMPK activity.

The Structure of LKB1.

LKB1 has a unique structure, differing greatly from other known protein kinases. It is found in all animal tissues thus far investigated. LKB1 is phosphorylated at eight or more sites, some of these through autophosphorylation, one perhaps through AMPK and others as a follow of hormone activation. The following figure from a very recent review article by Alessi, Sakamoto and Bayascas depicts mouse LKB1. Autophosphorylation sites are marked in red. The threonine site at position 336 and the



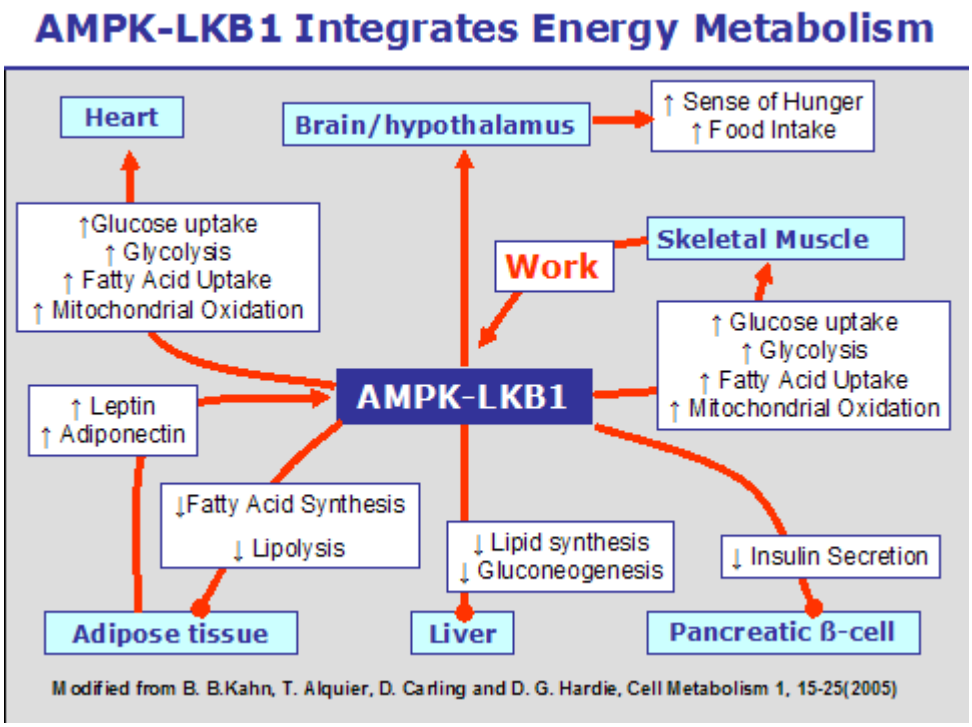
D. R. Alessi, K. Sakamoto and J. R. Bayascas, Annu. Rev. Biochem. (2006), 75; 137-163

serine at site 431 seem to be involved in control of cell growth. Modification of these prevents LKB1's modifying effects on growth of cells in culture. Alessi suggests that these sites may be involved in LKB1's tumor suppressive activity. Keep in mind that LKB1 works through AMPK. Both of these two protein kinases must be present and active to maintain growth control. A whole series of LKB1 mutations and their relationship to development of cancer are described in the cited review article (click here for that publication) [Annu. Rev. Biochem(2006), 75:137-163]._

LKB1 has recently been shown to be essential in control of blood glucose levels. Depletion of hepatic LKB1 in mice lead to loss of AMPK activity and hyperglycemia in these animals. Metformin, a key medication in treatment of type 2 diabetes, was effective only in the presence of intact LKB1. See R. J. Shaw et al, Science (2005), 310; 1642-1646 or click here for more information.

Physiological Integration by AMPK-LKB1.

In a recent review article [Cell Metabolism, 1; 15-25 (2005)] click here , Barbara B. Kahn *et al* have analyzed many of the differing actions of AMPK and LKB1 and present an integrated picture of AMPK as a master metabolic controller. Not only does this kinase regulate metabolism in our various tissues, but serves as a major integrating factor in organization of the whole body's

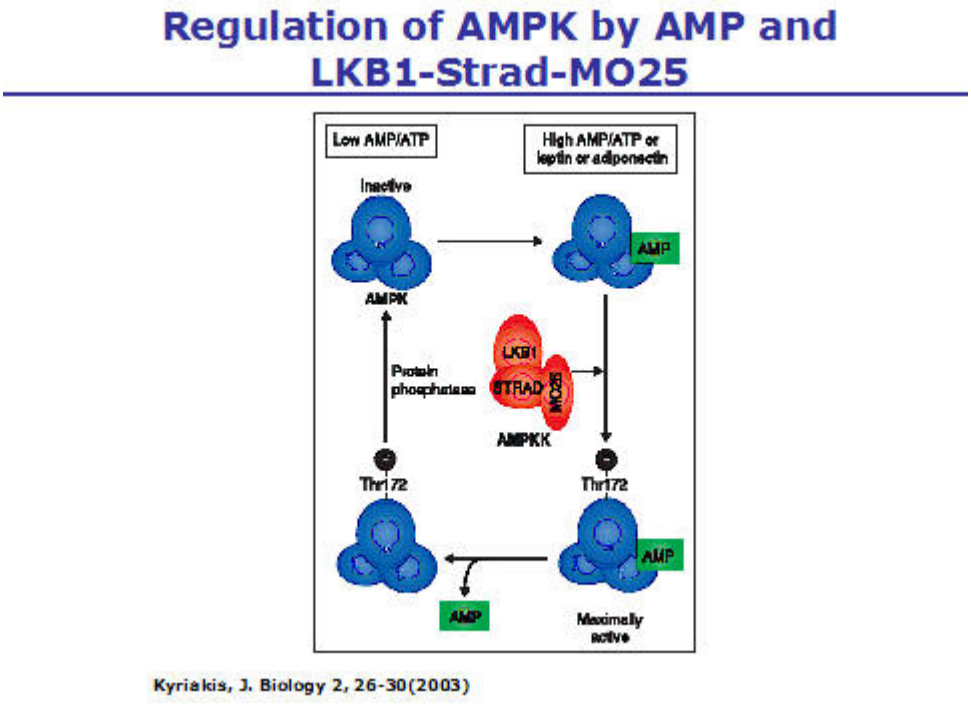


steady state. And, while 5´-AMP does activate the kinase by binding to the gamma subunit, most of the phosphorylation and control of protein phosphorylation by the α-subunit is catalyzed by upstream kinases. Thus far only LKB1 and CAMKK (calmodulin-dependent protein kinase kinase) have been identified as involved upstream kinases. I strongly suggest that interested readers go to Barbara B. Kahn's review article [Cell Metabolism, 1; 15-25 (2005)] or click here if you have library connections.

I will try to summarize our present picture of the AMPK-LKB1 system. AMPK is activated by all processes that abruptly increase energy utilization. Exercise, hypoglycemia, in fact all processes that increase energy utilization are coupled with activation of AMPK. Intake of food, signalized by changes in intestinal hormones (ghrelin, GLP-1), insulin and adipokines, alter AMPK activity and steer metabolism in the liver, skeletal muscles as well as the regulation of central control of appetite. Processes that generate ATP, that is glycolysis and oxidation of fatty acids, are stimulated by AMPK. Uptake of both glucose and fatty acids to heart and skeletal muscle is increased by AMPK. At the same time, the kinase turns off energy-using anabolic systems such as glycogen synthetase and fatty acid synthetase. AMPK is involved in the blood-glucose lowering effect of metformin, which is of major importance in treatment of type 2 diabetes. And, equally important, LKB1 appears to be a constitutively active tumor suppressor. controlling tissue growth and inhibiting tumor formation through its coordination with AMPK.

Activation of LKB1; the Roles of STRAD and MO25.

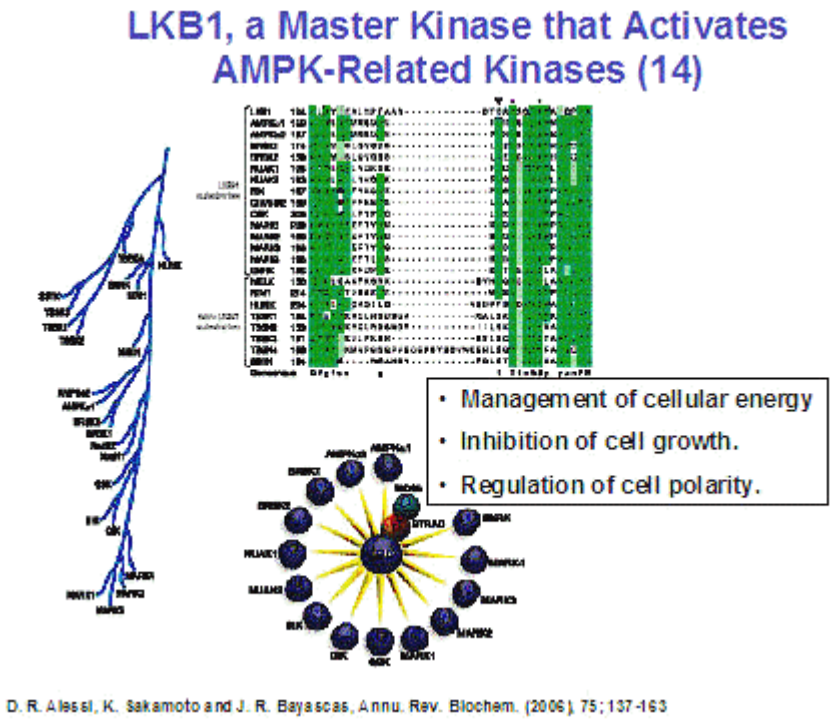
The AMPK-LKB1 story has become increasingly complex. LKB1 requires the binding of several other proteins for activation. Those which are actual today are known as STRAD (STE290-related adapter protein) and MO25 (mouse protein 25). Alessi takes this up in his coming review article (click here). John Kyriakis has presented a summary of this complex protein interaction in a recent publication in the Journal of Biology (Click Here). In the following figure from that article we can see the relationship between the actors in this play. AMP can only effectively turn on AMPK if it has been phosphorylated at threonine 172. Phosphorylation is dependent upon LKB1, which in turn must be coupled to MO25 and STRAND to exhibit activity.



Hormone interaction with LKB1 leads to phosphorylation of this kinase which is found bound to STRAD and MO25. The complex then phosphorylates AMPK at threonine 172. Binding of AMP to the phosphorylated AMPK leads continuation of this phosphorylation cascade and regulation of the several known and, probably, a number of unknown protein substrates.

The AMPK-related protein kinases.

Alessi takes up the AMPK-related kinases in his review article. These are apparent substrates for LKB1, but their physiological substrates are unknown. Fourteen such proteins have been identified to now. The next figure, taken from Alessi's review article show their similarity to AMPK and assumed major actions. To date, control of tissue growth, control of blood glucose levels and maintenance of the balance between energy use and formation seem to be under control of LKB1-AMPK. It will be exciting to follow advances in understanding the roles of the AMPK-related kinases.



D. R. Alessi, K. Sakamoto and J. R. Bayascas, Annu. Rev. Biochem. (2006) 75; 137-163

Metformin, Commonly used in Treatment of Type 2 Diabetes, Works Through the AMPK-LKB1 System!

Type 2 diabetes mellitus is characterized by high fasting levels of serum glucose. A major part of this originates in the liver, where control of gluconeogenesis is lost in diabetes. Metformin is probably the most commonly used medication for reversal of this condition. The effectiveness of metformin is however limited. With time, other medications and eventually insulin must be used to control blood glucose levels. It has been suggested that metformin also decreases insulin resistance in other tissues. The mechanism through which metformin reduces hepatic gluconeogenesis has not been identified. Quite recently R. J. Shaw *et al* (Science (2005) 310, 1642-4646) (click here if you have library connections) have shown that depletion of hepatic LKB1 in mice leads to hyperglycemia. They report further that an active AMPK-LKB1 system is essential for the action of metformin. Whether AMPK or one of the AMPK-related kinases is involved in metformin's action is unknown.

A deeper comprehension of the AMP-LKB1 system should allow development of new and specific drugs.

And Finally, a little poem by Piet Hein.

Piet Hein (a Danish scientist and author) has written a "grook" (a little poem) that might just be appropriate for those trying to understand metabolic regulation.

OMNISCIENCE

Knowing what
thou knowest not
is in a sense
omniscience.