

Chapter 2

Intermediary Metabolism of Carbohydrate, Protein, and Fat

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Introduction

The cause of obesity in an individual may involve many factors, both genetic and environmental, including fat cell production and development, appetite and energy regulation. However, the excessive accumulation of triglyceride (triacylglycerol) that characterizes obesity and its effects on the use and storage of various fuels (glucose, fatty acids, and amino acids) are clearly the result of abnormalities in metabolism. The three fatty acyl chains in a triglyceride molecule can be derived either from fats in the diet or de novo fatty acid biosynthesis from acetyl-CoA originating from carbohydrate or protein/amino acid metabolism (though de novo fatty acid synthesis is considerably less important in humans than in rodent models). The glycerol component of the triglyceride generally is derived from carbohydrate metabolism, though potentially it could also come from glucogenic amino acids. Triglyceride stores are also broken down as needed for energy production, depending in part on the availability of the other fuels; however, adipose tissue triglyceride is normally the largest energy reserve of the body. In addition, although fatty acids cannot be converted to glucose, the glycerol portion of triglyceride is an important gluconeogenic substrate during a prolonged fast, as it diminishes the need for breakdown of muscle protein for this purpose. Thus, triglyceride storage is intimately related to the whole of intermediary metabolism. The objective of this chapter is to present a brief description of the pathways of carbohydrate, protein, and fat metabolism and their interactions and regulatory mechanisms. The latter part of the chapter will focus on new insights that have been obtained from studies of genetic models with ablation or modification of particular enzymes or hormone receptors often in a tissue-specific manner. In addition, we will discuss the enzyme AMP-activated protein kinase (AMPK), which has recently been identified as a cellular mediator of many events in intermediary metabolism and whose dysregulation may be a cause of disorders associated with the metabolic syndrome and a target for their therapy.

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Principles of Metabolic Regulation

Regulation of metabolism is ultimately regulation of the enzyme catalysts in pathways. There are various kinds of regulation to be considered, all of which are important and often interact in intermediary metabolism. First, the amount of an enzyme can be increased or decreased, by changing its rate of synthesis at the transcriptional, translational, or post-translational stage, or its rate of degradation. Second, changes in the concentration of the substrate (provided it is at or below the K_M) can affect the rate of the reaction. Third, an enzyme can be regulated by metabolites that are inhibitors or activators binding to its catalytic or allosteric/regulatory sites. Fourth, an enzyme can be inhibited or activated by covalent modification, in particular by phosphorylation by protein kinases, some of which mediate hormonal actions. In addition, the importance of other types of covalent modification, such as acetylation, acylation, adenylation, and methylation, is increasingly recognized. Fifth, an enzyme can be inhibited or activated by protein–protein interactions with specific protein regulators. Sixth, an enzyme's functional activity can be affected by compartmentation within the cell and thus controlled by translocation from one area to another. Finally, different tissues may exhibit differences in metabolism despite identical or nearly identical pathways, because of the presence of isozymes, that is, enzymes that catalyze the same reaction but are different proteins and thus can have different kinetic and regulatory properties, due to differences in the catalytic site and in regulatory sites for noncovalent and covalent regulation.

Nutritional and hormonal states are intertwined in affecting intermediary metabolism. Food intake raises the level of the key peptide hormone insulin, which is synthesized in and secreted from the β -cells of the pancreatic islets primarily in response to glucose. However, fatty acids and some amino acids can potentiate the secretory response, as can certain gut hormones such as glucagon-like peptide (GLP)-1. Insulin is the primary regulator of whole body carbohydrate metabolism. Increases in its concentration activate glucose uptake in muscle and fat cells, inhibit glucose synthesis (gluconeogenesis) and glucose output by the liver, and stimulate glucose storage into glycogen, whereas decreases in its concentration have the opposite effect. In addition, insulin promotes other kinds of fuel storage, by stimulating triglyceride synthesis and inhibiting lipolysis (triglyceride breakdown) and by similar effects on protein synthesis and degradation. A number of counter-regulatory hormones oppose the action of insulin, including the peptide hormone glucagon, which is secreted from the α -cells of the pancreatic islets in response to low blood glucose and promotes hepatic glycogen breakdown and gluconeogenesis as well as adipose tissue lipolysis, and the catecholamine epinephrine (adrenaline), which is secreted from the adrenal glands in response to various excitatory stimuli and promotes glycogen breakdown and lipolysis. In subjects with diabetes, a lack of insulin or resistance to its action leads to high blood glucose levels due to impaired glucose disposal (primarily into muscle glycogen) and unrestrained hepatic glucose output. Also contributing to these abnormalities are excessive lipolysis and hence circulating fatty acid levels and increased protein

breakdown. Obesity, which is often although not always associated with elevations in circulating free fatty acids as well as inflammatory cytokines, is thought to contribute to the development of diabetes by causing insulin resistance, thus increasing the amount of insulin necessary for glucose homeostasis.

Carbohydrate and Energy Metabolism

Glucose Transport and Phosphorylation

Glucose enters most cells through glucose transporters that allow passive diffusion down the concentration gradient from the blood. Glut1 is present in most cells. Glut4 is the dominant transporter in muscle and fat cells. It is stimulated by insulin and also by anoxia or low energy state in a process that involves translocation of Glut4 in intracellular vesicles to the plasma membrane. In liver and pancreatic β -cells, the dominant isoform is Glut2, a high capacity transporter that essentially equilibrates glucose, so that its cytoplasmic concentration is close to that in plasma. Liver also releases glucose, derived from breakdown of the storage polymer glycogen and from synthesis by the pathway of gluconeogenesis. Thus, movement of glucose across the hepatocyte plasma membrane is functionally bidirectional. Energy-linked glucose transporters are found in intestinal and kidney cells where glucose must be taken up against its concentration gradient. For this purpose, these cells utilize the sodium gradient across the plasma membrane, which is established by expulsion of sodium by the sodium–potassium ATPase.

Once glucose enters the cell, it can be trapped by phosphorylation by hexokinase, an enzyme that uses ATP and produces glucose 6-phosphate and ADP. In liver, the dominant hexokinase isoform is glucokinase, or Type 4 hexokinase, which has a high K_M for glucose of about 10 mM, in comparison to basal blood (plasma) glucose levels of about 5 mM. This is of especial note because the portal vein brings nutrients directly to the liver from the intestine; therefore, a rise in portal vein glucose resulting from carbohydrate ingestion readily increases its metabolism in liver. A glucokinase variant is also the major hexokinase isozyme in pancreatic β -cells where it serves as the “glucose sensor” that promotes the increase in glucose metabolism that causes increased insulin release and synthesis [1].

Glucose 6-phosphate is a central branch point in carbohydrate metabolism (Fig. 1). It can be further metabolized in the glycolytic pathway to pyruvate, which in turn can be converted to lactate or alanine or oxidized to acetyl-CoA which can enter the citric acid cycle or be used for fatty acid synthesis. Glycolysis also supplies the glycerol portion of the triglyceride molecule via conversion of the glycolytic intermediate dihydroxyacetone phosphate to glycerol 3-phosphate. Alternatively, glucose 6-phosphate can be converted to glucose 1-phosphate for glycogen synthesis or metabolized in the pentose phosphate pathway, to generate the ribose 5-phosphate needed for nucleotide/nucleic acid synthesis and the NADPH needed for many

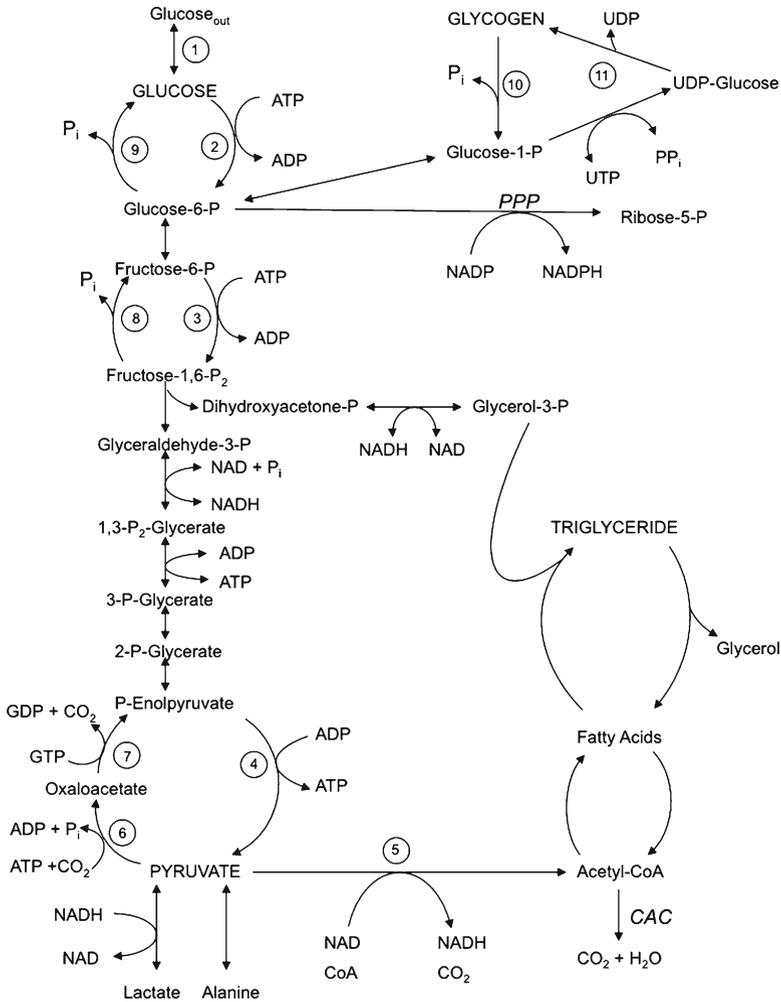


Fig. 1 Glucose and glycogen metabolism and the connections to the pentose phosphate pathway (PPP), the citric acid cycle (CAC), and fatty acid and triglyceride metabolism. Important regulatory steps are 1, glucose transport, notably by insulin-stimulated Glut 4 in muscle and fat; 2, hexokinase (glucokinase in liver and pancreatic β -cells); 3, phosphofructokinase; 4, pyruvate kinase; 5, pyruvate dehydrogenase; 6, pyruvate carboxylase; 7, phosphoenolpyruvate carboxykinase; 8, fructose 1,6-bisphosphatase; 9, glucose 6-phosphatase; 10, phosphorylase; 11, glycogen synthase

purposes including the synthesis of fatty acids. In addition to its formation by the hexokinase/glucokinase reaction, glucose 6-phosphate can be produced via glucose 1-phosphate following glycogen breakdown and from pyruvate, lactate, alanine, or citric acid cycle intermediates by the process of gluconeogenesis. Glucose 6-phosphatase, present in significant amounts only in the fully gluconeogenic tissues of liver and kidney cortex, can hydrolyze glucose 6-phosphate to yield free glucose.

Glycolysis

The further metabolism of glucose 6-phosphate in the glycolytic pathway begins with its conversion to fructose 6-phosphate by phosphoglucose isomerase (Fig. 1). Phosphofructokinase then catalyzes the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Fructose 1,6-bisphosphate is cleaved by aldolase into the two triose phosphates glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The latter are interconverted by triose phosphate isomerase. Glycolysis continues from glyceraldehyde 3-phosphate with its conversion to the high energy phosphate donor 1,3-bisphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase, using NAD and P_i . 1,3-Bisphosphoglycerate is then used to phosphorylate ADP to ATP by phosphoglycerate kinase (named for the reverse reaction). The resulting 3-phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase and then to the second high energy phosphate donor phosphoenolpyruvate by enolase. Phosphoenolpyruvate is then used to phosphorylate ADP to ATP by pyruvate kinase (also named for the reverse reaction). This makes for a net of 2 ATP produced in glycolysis per glucose molecule, since 1 ATP is used in the hexokinase reaction and 1 in the phosphofructokinase reaction, but four are synthesized in the lower glycolytic pathway from the two triose phosphates. Pyruvate is converted to lactate in the lactate dehydrogenase reaction, if it must be used to reoxidize the NADH produced in the glyceraldehyde-3-phosphate dehydrogenase reaction; however, if the cytosolic NADH can be reoxidized by shuttles transferring the reducing equivalents to the mitochondrial electron transport chain, then pyruvate is available to be further oxidized in the pyruvate dehydrogenase (PDH) reaction.

Hexokinase is usually considered the first enzyme in glycolysis. However, as indicated above, its product glucose 6-phosphate can be used in other pathways, notably glycogen synthesis and the pentose phosphate pathway. Therefore, phosphofructokinase is the first non-equilibrium step that is purely glycolytic; it is an important control point, regulated by a number of metabolites that reflect the fuel and energy state of the cell. Phosphofructokinase is inhibited by ATP and citrate and activated by ADP, AMP, P_i , fructose 1,6-bisphosphate, and fructose 2,6-bisphosphate. ATP is a substrate for phosphofructokinase because the enzyme is a kinase; however, ATP is also an allosteric inhibitor binding at a separate regulatory site. This is an example of classic feedback inhibition of an early step in a pathway by an ultimate end product, as one of the major functions of glycolysis is to produce ATP. Muscular contraction hydrolyzes ATP to ADP and P_i ; thus these rise and activate as ATP falls and inhibits less. ADP is a more sensitive indicator of ATP usage than ATP itself, as ATP levels in muscle are ten times that of ADP. Thus if 10% of ATP is used, the concentration of ADP doubles. AMP is an even more sensitive indicator of ATP usage, since the AMP concentration varies as the square of the ADP concentration because of equilibration of the adenine nucleotides in the myokinase (or adenylate kinase) reaction ($AMP + ATP \leftrightarrow 2 ADP$), and the principal emphasis has been on AMP as the indicator of the energy state. AMP is also a major regulator of other pathways via AMPK, in particular fatty acid oxidation (see below).

Citrate inhibition of phosphofructokinase is rationalized as mediating the effect of an alternative fuel, fatty acids or ketone bodies, to spare glucose usage, as part of a glucose-fatty acid cycle. This was originally proposed by Randle in heart but may also function in some circumstances in skeletal muscle and brain [2]. β -Oxidation of fatty acids produces acetyl-CoA inside the mitochondrion, where it is converted to citrate, which can then be transported out to the cytoplasm to inhibit phosphofructokinase.

The phosphofructokinase activator, fructose 2,6-bisphosphate is particularly important in regulation of liver glycolysis/gluconeogenesis. It is made, and also degraded, by the bifunctional enzyme phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB). The liver isoform is regulated by phosphorylation at a single site that inhibits the kinase activity and activates the phosphatase activity. This phosphorylation, by the cyclic-AMP dependent protein kinase (PKA) in response to glucagon, thus causes a decrease in fructose 2,6-bisphosphate and hence decreased activity of the glycolytic enzyme phosphofructokinase, as well as decreased inhibition of the opposing gluconeogenic enzyme fructose 1,6-bisphosphatase, thus promoting net gluconeogenesis. The muscle isoform is a splice variant lacking the phosphorylation site and therefore is not inhibited when PKA is activated to promote glycogenolysis, say in response to epinephrine. In contrast, the heart isoform is activated by phosphorylation, in response to insulin or by AMPK. Recent work has proposed an important role for the inducible isoform PFKFB3 in fat cells, to promote glycerol 3-phosphate production for triglyceride synthesis [3, 4]. Like the heart isoform, PFKFB3 is activated by insulin.

Fructose 1,6-bisphosphate was early recognized as an activator of phosphofructokinase, a somewhat puzzling property because it is a product of the reaction. Once the more potent fructose 2,6-bisphosphate was discovered, it was thought that fructose 1,6-bisphosphate activation was not so important, that perhaps the hexose bisphosphate binding site just could not be made that specific. However, whereas there is only about tenfold difference in sensitivity for muscle type phosphofructokinase, there is a 1,000-fold difference or more for the other phosphofructokinase isoforms. This suggests that the product activation of muscle type phosphofructokinase, which can lead to oscillatory behavior of glycolysis, might have some special role. It has been suggested that this may underlie the normal oscillatory secretion of insulin in the pancreatic β -cell [5]. More recently, it has been found that phosphofructokinase-M deficient mice have greatly reduced fat stores, despite the presence of the other two isoforms in fat, suggesting the possible importance of glycolytic oscillations for glycerol 3-phosphate generation [6].

Hexokinase can follow the lead of phosphofructokinase, because hexokinase is inhibited by glucose 6-phosphate. This is not product inhibition at the active site, but rather is mediated by binding to a separate regulatory site, apparently created by gene duplication. (Glucokinase is not sensitive to glucose 6-phosphate inhibition and is half the size of hexokinase, because it lacks this duplicated portion.) This allows hexokinase to be responsive to the demand for glucose 6-phosphate. Thus, if phosphofructokinase is inhibited, then the concentration of glucose 6-phosphate (in equilibrium with fructose 6-phosphate) will rise and inhibit hexokinase; on the

other hand, if phosphofructokinase is activated, such as by muscular contraction, and uses fructose 6-phosphate, then the concentration of glucose 6-phosphate will also drop and hexokinase will be deinhibited. Use of glucose 6-phosphate for glycogen synthesis could also deinhibit hexokinase.

Fructose

Fructose metabolism is of increasing interest because of the now widespread incorporation of high fructose corn syrup in beverages and other foodstuffs. The advantage to the food industry is that free fructose is even more potent as a sweetener than common sugar (sucrose, a glucose–fructose disaccharide). Fructose is largely metabolized in the liver, where it bypasses the limiting glycolytic steps of glucokinase/hexokinase and phosphofructokinase. It is phosphorylated by a specific fructokinase to fructose 1-phosphate, which is then cleaved by liver aldolase to dihydroxyacetone phosphate and glyceraldehyde. (Muscle aldolase is relatively specific for fructose 1,6-bisphosphate, in contrast to the liver isoform.) The glyceraldehyde is then phosphorylated by triokinase to enter the glycolytic pathway as well.

Fructose does not compete with glucose for metabolism, but rather fructose increases glucose metabolism in liver. This surprising effect has been explained as follows: There is a glucokinase inhibitory protein that binds to and sequesters glucokinase in the nucleus. Fructose 1-phosphate prevents that binding and promotes glucokinase translocation to the cytoplasm. Fructose 6-phosphate counters the action of fructose 1-phosphate.

Whether fructose consumption is contributory to obesity and the metabolic syndrome and associated diseases as a result of its distinct metabolism or simply by increasing caloric consumption is not established. Under some conditions fructose metabolism can cause excessive ATP use, leading to purine degradation and elevated uric acid levels. This has recently been proposed to be responsible for increases in hypertension and other facets of metabolic syndrome [7].

Fructose not removed from the blood stream by the liver could potentially be readily taken up and metabolized in adipose tissue, by its own transporter Glut5 and then presumably via hexokinase and phosphofructokinase. Hence, it could serve as another source of glycerol 3-phosphate for triglyceride synthesis, in addition to glucose passing through the insulin-regulated Glut4.

Pyruvate Dehydrogenase and the Citric Acid Cycle

Pyruvate dehydrogenase is an enzyme complex that oxidizes pyruvate to acetyl-CoA, using NAD and CoA and producing NADH and CO₂. Regulation of this step is very important, because although acetyl-CoA can be incorporated into fatty acids

or made from fatty acids, carbon at that stage cannot be converted back to glucose. The regulation of PDH is on two levels. First, acetyl-CoA and NADH inhibit the enzyme as products at the active sites, and this is competed by the respective substrates CoA and NAD, so the acetyl-CoA/CoA and NADH/NAD ratios are the important inhibitory parameters. Second, the complex is inactivated by phosphorylation by PDH kinase (also bound in the complex), where the kinase is activated by high acetyl-CoA/CoA, NADH/NAD, and ATP/ADP ratios. The phosphorylation is reversed by a specific PDH phosphatase, also bound in the complex, which is activated by high pyruvate concentrations and by insulin. Thus, PDH is inhibited if there is already plenty of acetyl-CoA for the citric acid cycle, or NADH for the electron transport chain and oxidative phosphorylation, or ATP, the end product itself, so that pyruvate is spared for possible need for gluconeogenesis. On the other hand, if there is abundant pyruvate or high glucose, as indicated by high insulin levels, then there is no need to spare the pyruvate.

Acetyl-CoA can be completely oxidized to CO_2 in the reactions of the citric acid cycle, with the reducing equivalents captured in the form of NADH and FADH_2 for transfer to the electron transport chain (Fig. 2). In the first reaction (citrate synthase), acetyl-CoA is combined with oxaloacetate (four carbons) to form citrate (six carbons). Citrate is then sequentially converted to cis-aconitate and to isocitrate by aconitase. Isocitrate dehydrogenase produces α -ketoglutarate (five carbons), NADH, and CO_2 . α -Ketoglutarate dehydrogenase, an enzyme complex analogous to PDH, produces succinyl-CoA (four carbons), NADH, and CO_2 . Succinyl-CoA synthetase then converts succinyl-CoA to succinate and CoA, coupled with the synthesis of GTP from GDP and P_i or of ATP from ADP and P_i , depending on the isoform; the GTP-producing isoform is dominant in liver and may provide a connection to a GTP-requiring enzyme in gluconeogenesis, whereas the ATP-producing isoform is dominant in skeletal muscle. Succinate dehydrogenase produces fumarate and FADH_2 . Fumarate equilibrates to malate through the fumarase reaction. Finally, malate dehydrogenase produces a third equivalent of NADH and regenerates oxaloacetate for another turn of the cycle. Note that acetyl-CoA for the citric acid cycle in some tissues can also come from β -oxidation of fatty acids, from metabolism of ketone bodies (β -hydroxybutyrate and acetoacetate), and from ketogenic amino acids, as well as from carbohydrate via pyruvate.

Gluconeogenesis

Another major pathway using pyruvate is gluconeogenesis. Gluconeogenesis occurs principally in the liver and to a lesser extent in the kidney, and it is an important source of glucose for the brain and nervous system during brief and sustained fasting and prolonged exercise. Gluconeogenesis uses many of the same reactions as glycolysis in reverse. However, the hexokinase/glucokinase, phosphofructokinase, and pyruvate kinase reactions involve large changes in free energy and are not reversible under cellular conditions; therefore these three steps are reversed by specific gluconeogenic enzymes

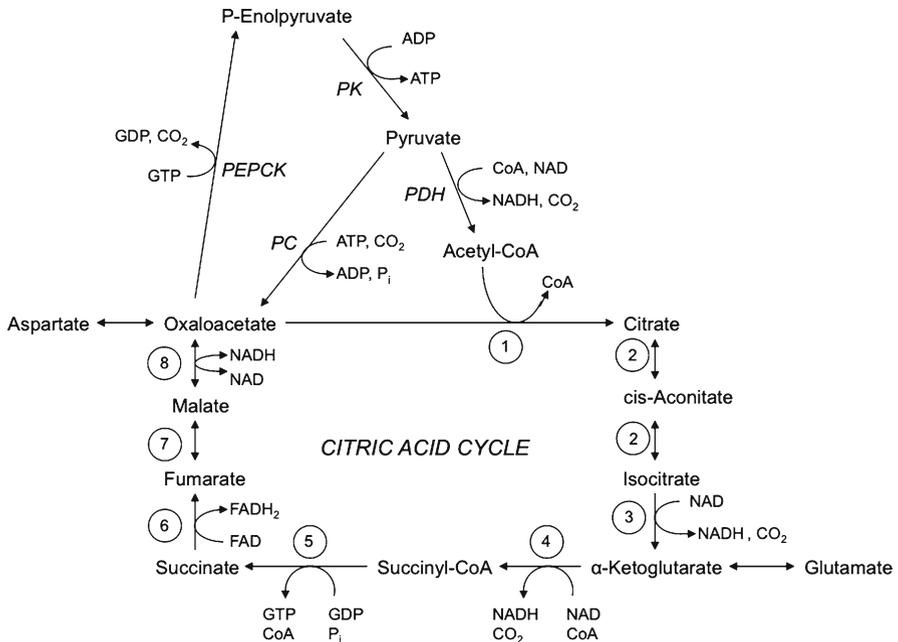


Fig. 2 The citric acid cycle and its connections to pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), and pyruvate dehydrogenase (PDH). 1, citrate synthase; 2, aconitase; 3, isocitrate dehydrogenase; 4, α -ketoglutarate dehydrogenase; 5, succinyl-CoA synthetase; 6, succinate dehydrogenase; 7, fumarase; 8, malate dehydrogenase. Aspartate equilibrates with oxaloacetate via the glutamate-oxaloacetate transaminase reaction. Glutamate equilibrates with α -ketoglutarate via various transaminases and the glutamate dehydrogenase reaction

(see Fig. 1). The synthesis of the high energy compound phosphoenolpyruvate from pyruvate involves two steps. First, pyruvate carboxylase converts pyruvate to the citric acid cycle intermediate oxaloacetate, using CO_2 and the energy of ATP hydrolysis. Then phosphoenolpyruvate carboxykinase (PEPCK) converts oxaloacetate to phosphoenolpyruvate with the release of CO_2 and more energy input via use of GTP. The reactions from phosphoenolpyruvate to fructose 1,6-bisphosphate are readily reversible in liver. Then fructose 1,6-bisphosphate is cleaved to fructose 6-phosphate and P_i by fructose-1,6-bisphosphatase. Fructose 6-phosphate equilibrates to glucose 6-phosphate, and finally glucose 6-phosphate is cleaved to glucose and P_i by glucose 6-phosphatase.

The synthesis of a glucose molecule requires the use of six high energy phosphate bonds: two each at pyruvate carboxylase, PEPCK and phosphoglycerate kinase (for the two triose phosphates that are combined to form glucose). The energy for this obviously cannot come from glycolysis. Pyruvate carboxylase has a required activator, acetyl-CoA, indicating that another source of fuel is available, in particular β -oxidation of fatty acids. The requirement of PEPCK for its substrate GTP, produced in turn by succinyl-CoA synthetase in the citric acid cycle, suggests a regulatory link between gluconeogenesis and adequate flux through the citric acid cycle.

Control of net gluconeogenesis involves regulation of the key glycolytic and the opposing gluconeogenic enzymes. Fructose-1,6-bisphosphatase is inhibited by AMP and fructose 2,6-bisphosphate, whereas these compounds activate phosphofructokinase. Glucagon, a signal of low glucose, causes phosphorylation and inhibition of liver PFKFB and thus a decrease in fructose 2,6-bisphosphate, which promotes net gluconeogenesis. There are several mechanisms for inhibiting liver pyruvate kinase to prevent conversion of phosphoenolpyruvate back to pyruvate: the liver isoform is allosterically inhibited by ATP and by alanine (an important pyruvate/gluconeogenic precursor; see description of the alanine cycle below). It is dependent on activation by fructose 1,6-bisphosphate (and so will follow changes in phosphofructokinase activity) and also inhibited by phosphorylation by PKA in response to glucagon. Finally, the amounts of these key enzymes are adaptive, that is, affected by the nutritional and hormonal state, so the key glycolytic enzymes are increased by a high carbohydrate diet, whereas the key gluconeogenic enzymes are increased by a low carbohydrate diet or starvation. This involves regulation by insulin phosphorylation cascades and also carbohydrate responsive transcription factors. In recent years, there has been increasing emphasis on transcriptional control of PEPCK and glucose 6-phosphatase [8, 9].

Note that the citric acid cycle is a central station or reservoir in metabolism. Pyruvate carboxylase is one way of filling up the cycle (anapleurosis), and PEPCK one way of depleting it. In addition, there is flow in and out through various other reactions such as those involving the amino acids glutamate and aspartate and their citric acid cycle counterparts α -ketoglutarate and oxaloacetate, respectively (see Fig. 2). In skeletal muscle, which lacks pyruvate carboxylase, the citric acid cycle can be replenished by the conversion of aspartate to fumarate in the reactions of the purine nucleotide cycle [10].

The Cori cycle is an inter-organ cycle involving muscle glycolysis and liver gluconeogenesis. In muscle, pyruvate from glycolysis is converted to lactate in the lactate dehydrogenase reaction, together with the conversion of glycolytically generated NADH back to NAD. The lactate then moves through the blood to the liver, where it is converted back to pyruvate in the lactate dehydrogenase reaction, with the production of NADH. Gluconeogenesis in the liver then utilizes both the pyruvate and the NADH (the latter in the reversal of the glyceraldehyde 3-phosphate dehydrogenase reaction). The glucose so formed can then be exported through the blood to the muscle to complete the cycle. The alanine cycle is similar, with pyruvate being converted to alanine by transamination (see "Protein and Amino Acid Metabolism" section).

Glycerol released by fat cells during lipolysis is another gluconeogenic substrate and is especially important during prolonged starvation. The fat cell lacks glycerol kinase and so cannot recycle glycerol back into glycerol lipids. However, the released glycerol can be phosphorylated to glycerol 3-phosphate in liver and then converted to the glycolytic/gluconeogenic intermediate dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. This explains the ability of very obese individuals to survive a prolonged fast of many weeks despite the need for glucose for the brain.

Glycogen Metabolism

Glycogen is a branched polymer of glucose. Most glucose residues are connected by α -1,4-glycosidic bonds, but branches are created by α -1,6-glycosidic bonds roughly every ten residues. The branching allows for a more compact molecule and also greatly multiplies the concentration of the “nonreducing” ends that serve as substrate for addition and removal of glucosyl residues. Synthesis of glycogen by glycogen synthase uses UDP-glucose as an activated donor molecule:



UDP-glucose in turn is made by UDP-glucose pyrophosphorylase (named for the reverse reaction):



The reaction is pulled to the right by cleavage of PP_i by pyrophosphatase. Glucose 1-phosphate comes from glucose 6-phosphate through the phosphoglucomutase equilibrium reaction (Fig. 1). The UDP is phosphorylated back to UTP with ATP in the nucleoside diphosphate kinase reaction. Glycogen synthase only makes α -1,4-glycosidic bonds. Branches are formed by branching enzyme taking a terminal chain of seven residues and transferring it further down, making the α -1,6-glycosidic linkage.

The major stores of glycogen are in liver and muscle. Liver can have a greater concentration of glycogen per gram, but the total muscle glycogen is greater because of the much larger muscle mass. The purpose of liver glycogen is a reserve to be broken down to supply glucose to other tissues, in particular the brain, in time of need; thus liver glycogenolysis is stimulated by glucagon or epinephrine, along with gluconeogenesis. The purpose of muscle glycogen is for local glycolytic fuel for muscular contraction, and its breakdown is stimulated by epinephrine. (Note: there are no glucagon receptors on muscle.)

Most of the breakdown of glycogen is catalyzed by phosphorylase, which breaks α -1,4-glycosidic bonds with phosphate, not water, so that the product is glucose 1-phosphate, together with a shortened glycogen. (There are other phosphorylase enzymes besides glycogen phosphorylase, but this one was discovered first and so is commonly called just phosphorylase.) The glucose 1-phosphate produced in glycogenolysis is converted to glucose 6-phosphate in the phosphoglucomutase reaction and so joins the glycolytic pathway in muscle and glucose production in liver.

Phosphorylase cannot break the α -1,6-glycosidic bonds; in fact it cannot get within four residues of a branch point. So first a transferase enzyme takes three of the last four residues in a branch and transfers them to the end of another chain, and then the last residue is cleaved off as free glucose by α -1,6-glucosidase. Phosphorylase can then proceed further down the chain. The fact that phosphorylase generates a phosphorylated sugar without expenditure of ATP means that three molecules of ATP can be generated in glycolysis per glucose residue from glycogen, rather than the two from free glucose, so in a sense glycogen is a more efficient fuel

for muscular contraction. Of course, there had to be an expenditure of two ATP equivalents per residue to synthesize the glycogen when the muscle was at rest.

Stimulation of glycogen breakdown by hormones uses a regulatory cascade (Fig. 3). Binding of glucagon to its receptor in the liver or of epinephrine to β -adrenergic receptors in muscle causes activation of adenylyl cyclase, which synthesizes 3',5'-cyclic AMP (cAMP) from ATP. cAMP then activates protein kinase A (PKA), which in turn phosphorylates and activates phosphorylase *b* kinase. Phosphorylase *b* kinase then phosphorylates and activates phosphorylase, converting it from the nonphosphorylated form (named phosphorylase *b*) that requires high AMP for activity to the phosphorylated form (named phosphorylase *a*) that does not require high AMP. Phosphorylase *b* kinase can also be activated allosterically by a rise in intracellular free Ca^{2+} , such as occurs with muscular contraction. In liver cells, physiological concentrations of epinephrine bind to α -adrenergic receptors that are coupled to the release of Ca^{2+} from intracellular stores, thereby activating

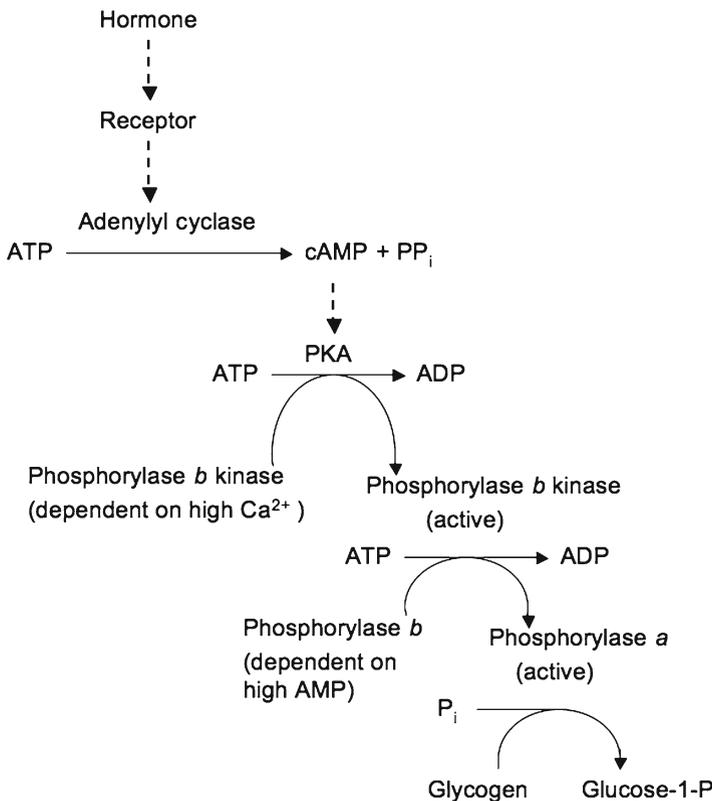


Fig. 3 The cascade. Hormone (epinephrine in muscle or glucagon in liver) binding to its plasma membrane receptor stimulates the formation of cAMP and activation of protein kinase A (PKA), leading to glycogen breakdown as shown. PKA also phosphorylates and inhibits glycogen synthase, liver pyruvate kinase, and liver PFKFB. Phosphorylation of perilipin and hormone sensitive lipase (HSL) by PKA is also important in the stimulation of triglyceride breakdown in adipose tissue

phosphorylase *b* kinase without phosphorylation. I-strain mice, which lack phosphorylase *b* kinase and so cannot make phosphorylase *a*, nevertheless breakdown muscle glycogen during exercise; this presumably is due to allosteric activation of phosphorylase *b* by AMP (or its deamination product IMP).

The cAMP cascade also operates to inhibit glycogen synthesis, since PKA phosphorylates and thereby inhibits glycogen synthase, converting it from the active, nonphosphorylated form (named glycogen synthase *a*) to the phosphorylated form (named glycogen synthase *b*) that requires high glucose 6-phosphate for activity. In contrast to phosphorylase, which has only one phosphorylation site, glycogen synthase has at least ten phosphorylation sites and can be phosphorylated by at least nine different protein kinases whose importance/hierarchy is still being studied. In addition to PKA, others of importance are glycogen synthase kinase 3 and AMPK.

Reversal of the signals in the cascade involves hydrolysis of cAMP to AMP by phosphodiesterase and removal of phosphates on enzymes by phosphoprotein phosphatases. The phosphodiesterase is inhibited by methylxanthines such as caffeine, theophylline, and theobromin, accounting in part for the stimulatory effects of the popular drinks coffee, tea, and cocoa. The phosphatases are also subject to regulation, by phosphorylation and dephosphorylation, by protein inhibitors whose activity is regulated by phosphorylation and dephosphorylation, and by allosteric effects inducing conformational changes in their substrates. For example, binding of AMP to phosphorylase *a* causes the phosphate to be tucked in where the phosphatase cannot reach it. Glucose, on the other hand, causes the phosphate to become accessible.

Insulin stimulates glycogen synthesis in three ways: First, it activates glycogen synthase by causing phosphorylation and inhibition of glycogen synthase kinase 3. Second, it reduces cAMP by activating phosphodiesterase. Third, in muscle, it increases glucose transport by activating Glut4. The latter may be the dominant action and the one impaired in diabetes, though impairment of glycogen synthase is also of importance [11, 12]. Interestingly, in liver, where glucose transport is not limiting, a relatively high proportion of glucosyl residues incorporated into glycogen appears to come via an indirect pathway of gluconeogenesis after peripheral catabolism of a glucose load [13].

Oxidative Phosphorylation

The bulk of ATP generation in most cells occurs via electron transport coupled to mitochondrial ATP synthase on the inner mitochondrial membrane, a process known as oxidative phosphorylation. The electron transport chain takes reducing equivalents from NADH and passes them in a series of steps to molecular oxygen (see Fig. 4). The classical sequence of the carriers of the electron transport chain is NADH to complex I (NADH:Q reductase, which contains bound flavin mononucleotide, FMN, and nonheme iron) to CoQ (ubiquinone) to complex III (QH₂:cytochrome *c* reductase, which contains cytochromes *b* and *c*₁) to cytochrome *c* to complex IV (cytochrome oxidase, which contains cytochromes *a* + *a*₃) to O₂, reducing the

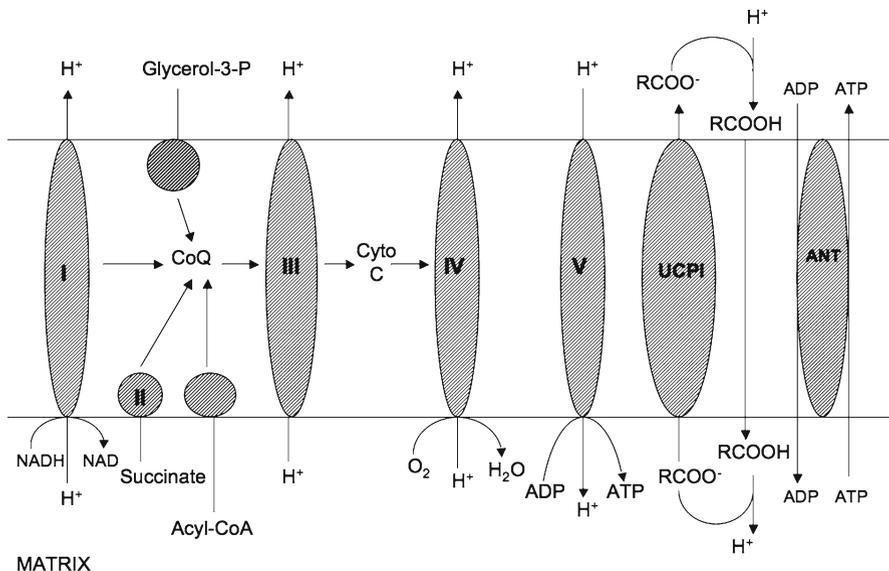


Fig. 4 The electron transport chain and oxidative phosphorylation. Complexes I, III, and IV are proton pumps. Complex II is succinate dehydrogenase; other flavin proteins transfer reducing equivalents from glycerol 3-phosphate and acyl-CoA to CoQ. The proton gradient is then used to drive ATP synthesis by complex V. Uncoupling protein I (UCPI) in brown fat uncouples by transporting fatty acid anions out, such that the proton gradient can be consumed while bypassing complex V, thereby generating heat. The adenine nucleotide translocase (ANT) carries ATP out in exchange for ADP

oxygen to H_2O . Complexes I, III, and IV are proton pumps that move protons out of the matrix and establish a proton gradient and membrane potential across the inner mitochondrial membrane. According to the chemiosmotic hypothesis of Peter Mitchell, this provides the driving force for protons moving back through a channel in the F_0 component of the ATP synthase (complex V) to drive a molecular motor, the F_1 component, that synthesizes ATP from ADP and P_i . Complex II of the electron transport chain is succinate dehydrogenase of the citric acid cycle, a flavin enzyme that passes reducing equivalents to CoQ. Two other flavin enzymes, mitochondrial glycerol 3-phosphate dehydrogenase of the glycerol phosphate shuttle and acyl-CoA dehydrogenase of fatty acid β -oxidation, also feed into the electron transport chain at CoQ. Classically it was thought that complexes I, III, and IV each generated the energy for the synthesis of 1 ATP, such that the yield would be 3 ATP for NADH and 2 ATP for succinate and other substrates feeding in at CoQ. However, it is now considered that the yield is 2.5 ATP for NADH and 1.5 for succinate. Part of the reason for the decreased yield is that the ATP synthesized by complex V is in the matrix, and that when ATP^+ is transported out to the cytoplasm by the adenine nucleotide translocase in exchange for ADP^{3-} , the charge difference consumes part of the proton gradient or membrane potential.

Because the site for NADH on complex I faces the matrix of the mitochondria and there is no carrier for NADH to cross the inner mitochondrial membrane, metabolic shuttles are used to carry the reducing equivalents from NADH produced

in the cytoplasm by glycolysis. In the glycerol phosphate shuttle, the glycolytic intermediate dihydroxyacetone phosphate is used as the initial acceptor, being reduced by NADH to glycerol 3-phosphate by the cytosolic, NAD-linked glycerol-3-phosphate dehydrogenase. Then the glycerol 3-phosphate is oxidized back to dihydroxyacetone phosphate by the mitochondrial, FAD-linked glycerol-3-phosphate dehydrogenase, with the reducing equivalents being subsequently transferred from FADH₂ to CoQ. The site for glycerol 3-phosphate on the latter, inner-membrane-bound dehydrogenase faces out, so glycerol 3-phosphate does not need to be transported into the matrix. In the malate–aspartate shuttle, the initial acceptor is cytosolic oxaloacetate, which is reduced by NADH to malate by malate dehydrogenase present in the cytoplasm. Malate crosses the inner mitochondrial membrane via a dicarboxylic acid transporter and is oxidized back to oxaloacetate by malate dehydrogenase in the matrix, with the conversion of NAD to NADH. There is no carrier for oxaloacetate to go back out, so instead oxaloacetate is converted to aspartate by glutamate-oxaloacetate transaminase, using glutamate and producing α -ketoglutarate. The α -ketoglutarate exits (in exchange for malate), as does the aspartate, and the transamination is reversed in the cytoplasm, regenerating oxaloacetate and glutamate. Glutamate reenters the mitochondria (in exchange for aspartate).

Electron transport, and therefore oxygen consumption or respiration, is normally coupled to ATP synthesis or phosphorylation. If there is limited ADP available for complex V because of low ATP usage by the cell, then there will be limited consumption of the proton gradient, and the high gradient will inhibit the proton-pumping electron transport chain. Uncouplers, such as 2,4-dinitrophenol and FCCP, are weak acids that are lipid soluble in both the protonated and unprotonated states. Thus, they can catalyze transport of protons across the inner mitochondrial membrane, collapsing the proton gradient and the membrane potential. This will stimulate respiration but without ATP synthesis. Ionophores, such as valinomycin which carries K⁺ across membranes, can collapse the membrane potential and therefore have a partial uncoupling action. Classical inhibitors of electron transport are the site 1 inhibitors rotenone and amytal (inhibit complex I), the site 2 inhibitor antimycin A (inhibits complex III), and the site 3 inhibitors cyanide, azide, carbon monoxide, and hydrogen sulfide (analogs of O₂ that block complex IV). Oligomycin is the classical inhibitor of phosphorylation (complex V); it blocks the F₀ channel, preventing the flow of protons that powers the ATP synthase. Atractyloside and bongkrekic acid inhibit the adenine nucleotide translocase. Inhibition of phosphorylation or the translocase will indirectly inhibit respiration of coupled oxidative phosphorylation.

Brown Fat and Uncoupling Proteins

Dinitrophenol is a poison. However, there is physiological uncoupling of mitochondria that does reduce ATP production and instead produces heat. The clearest example is in brown fat, so-called because the cells are indeed colored from the high amount of mitochondria with their colored cytochromes and the multiple small lipid droplets, in contrast to the large lipid droplet of the mature standard or white

fat cell. Rodents use brown fat for heat production when placed in a cold environment. Adult humans do have small amounts of brown fat, perhaps to warm certain critical areas, but in a cold environment largely use muscular nonproductive thermogenesis, that is, shivering. Nevertheless, it has recently been suggested that brown fat may consume significant energy in an adult human under some conditions [14, 15]. There is a thought that obesity could be treated by increasing energy expenditure by increasing brown fat (or even converting white fat to brown fat), though whether elevated body temperature would be a problem remains to be seen. Uncoupling in brown fat occurs because of the presence of uncoupling protein 1 (UCP1), which transports fatty acid anions across the inner mitochondrial membrane (see Fig. 4). Protonated, that is, uncharged fatty acids can easily dissolve in and flip across the lipid bilayer membrane, thus carrying in protons down the gradient, but normally the anion cannot flip back to repeat the process. UCP1 allows the fatty acid anion to go back and pick up another proton and thus catalyze consumption of the proton gradient. Thus, the uncoupling action of UCP1 is dependent on fatty acids. Physiologically its activity is initiated by neuronal adrenergic (β 3) stimulation of lipolysis in the brown fat cell. Analogous proteins (by sequence homology), UCP2 and UCP3, exist in other tissues (notably UCP2 in muscle), but their mechanism of action has not been established.

Triglyceride and Fatty Acid Metabolism

Triglyceride (or triacylglycerol) consists of a glycerol with three esterified fatty acids. Fatty acid simply means long chain carboxylic acid, normally 14–20 carbons. The fatty acids at the end positions tend to be saturated (palmitate, C16; stearate, C18), whereas the fatty acid at the two position tends to be unsaturated (oleate, 18:1) or polyunsaturated (linoleate, 18:2; linolenate, 18:3; arachidonate, 20:4). Myristate (saturated C14) is not normally important in triglyceride, but is important in post-translational modification of some proteins for targeting them to membranes. Triglyceride stores in fat tissue are the major energy reserve of the body, though other tissues may have triglyceride for internal usage. Excessive triglyceride in nonadipose tissues (notably fatty liver) can cause insulin resistance and loss of metabolic function.

Lipolysis

Triglyceride breakdown in fat cells was originally thought to begin with hormone sensitive lipase (HSL), activated by phosphorylation by PKA in response to epinephrine or glucagon. Additionally, PKA phosphorylation of the lipid droplet protein perilipin allows movement of HSL to the lipid droplet. However, knockout of HSL did not greatly reduce such stimulated lipolysis. It was then discovered that

the major triglyceride lipase in fat cells was another protein, named adipose triglyceride lipase (ATGL). (This, too, is a misnomer, because ATGL is likely the dominant triglyceride lipase in other tissues as well.) The current view is that phosphorylation of perilipin also releases CGI-58, a protein activator of ATGL. The ATGL then hydrolyzes triacylglycerol to diacylglycerol. The HSL, which actually has a preference for diacylglycerol, then hydrolyzes diacylglycerol to monoacylglycerol, and a monoglyceride lipase finally hydrolyzes monoacylglycerol to free glycerol (see Fig. 5). Fat cells lack glycerokinase, so the release of glycerol is commonly used as a measure of lipolysis. The glycerol can be used for gluconeogenesis in liver, which is why very obese people can survive many weeks of starvation. The released fatty acids may be re-esterified into triglyceride, carried to other organs bound to albumin in the circulation or oxidized in the mitochondria. Oxidation is a relatively minor fate of fatty acids in fat cells, whereas heart and muscle are major consumers of fatty acids for fuel.

Insulin inhibition of lipolysis in part involves stimulation of phosphodiesterase 3B, the primary enzyme for degradation of cAMP in fat cells. More recently, a second important mechanism has been proposed, whereby insulin stimulates

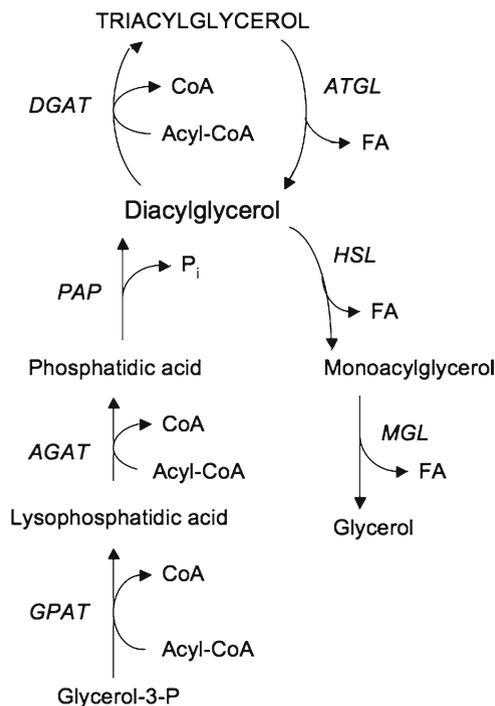


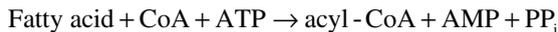
Fig. 5 Triglyceride synthesis and hydrolysis. *GPAT*, glycerophosphate acyl transferase; *AGAT*, acylglycerophosphate acyltransferase; *PAP*, phosphatidic acid phosphohydrolase (lipin); *DGAT*, diacylglycerol acyltransferase; *ATGL*, adipose triglyceride lipase; *HSL*, hormone-sensitive lipase; *MGL*, monoglyceride lipase; *FA*, free fatty acid

glucose uptake (Glut4) and metabolism to lactate, and the lactate then acts in an autocrine manner on the orphan receptor GPR81, which in turn acts via G_i G-protein to inhibit adenylyl cyclase, in contrast to the G_s -mediated action of epinephrine and glucagon to activate adenylyl cyclase [16]. The inhibitory action on lipolysis of some other compounds, such as adenosine, β -hydroxybutyrate, and nicotinic acid, is also due to their binding to receptors linked to G_i . Note that insulin also stimulates triglyceride synthesis, in part by the stimulation of glucose uptake and thus the synthesis of the precursor molecule glycerol 3-phosphate.

Fatty Acid Oxidation

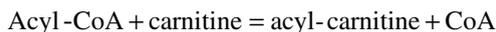
There are three steps in fatty acid oxidation, to be described in detail below. First, the fatty acid is activated to the CoA ester by acyl-CoA synthetase. Second, the acyl group is transported into the mitochondrial matrix attached to carnitine. Third, the regenerated acyl-CoA undergoes multiple cycles of β -oxidation, leading to cleavage into two-carbon fragments in the form of acetyl-CoA, which can then be fully oxidized in the citric acid cycle.

Activation. Nearly all enzymatic reactions of fatty acids utilize the CoA ester form (with the exception of prostaglandin and leukotriene synthesis from arachidonate). Fatty acids are activated to the CoA ester in the acyl-CoA synthetase reaction:



The thio ester bond of acyl-CoA has the same energy as an ATP phosphate anhydride bond. Therefore, the acyl-CoA synthetase reaction is pulled to the right by hydrolysis of the pyrophosphate (PP_i) by pyrophosphatase.

Translocation. Acyl-CoA synthetase for fatty acids is located in the cytoplasm (or on membranes facing the cytoplasm), and there is no carrier for acyl-CoA itself to cross the inner mitochondrial membrane for β -oxidation in the matrix. Therefore, the acyl group is transferred to carnitine in a reaction catalyzed by carnitine acyl transferase I (or CPT-I, for carnitine palmitoyl transferase I, after its principal substrate, since the abbreviation CAT was already in use for the reporter enzyme chloramphenicol acetyltransferase):



A translocase then carries the acyl-carnitine across the inner mitochondrial membrane. In the matrix the acyl group is transferred back to CoA by the isoform CPT-II, regenerating acyl-CoA and carnitine. The carnitine is then transported back out by the translocase.

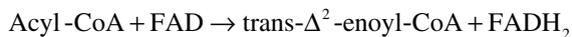
The purpose of this complex transport system is to provide regulation of fatty acid oxidation, since the free fatty acids could diffuse across the membrane but cannot be activated in the matrix. CPT-I is inhibited by malonyl-CoA, which is

made by the highly regulated enzyme acetyl-CoA carboxylase. Acetyl-CoA carboxylase is activated by citrate, the precursor for cytosolic acetyl-CoA, and inhibited by phosphorylation by AMPK. A drop in cellular energy state and hence rise in AMP activates AMPK, which phosphorylates a number of targets to increase energy production and decrease energy usage [17]. One important target is acetyl-CoA carboxylase, decreasing malonyl-CoA, and thus deinhibiting CPT-1 and promoting fatty acid oxidation. Also, malonyl-CoA decarboxylase, which degrades malonyl-CoA, is phosphorylated but activated by AMPK.

Malonyl-CoA is also the substrate for fatty acid synthesis (see below), so a rise in malonyl-CoA, say by high glucose which would promote the formation of citrate and reduce AMP and AMPK activity, would provide a shift from fatty acid oxidation to fatty acid synthesis. In tissues that do not do much fatty acid synthesis, the inhibition of fatty acid oxidation may shift the use of acyl-CoA from oxidation to complex lipid formation. This may also serve a signaling function, in that diacylglycerol is an activator of protein kinase C; furthermore, acyl-CoA itself is an allosteric regulator of a number of enzymes as well as the substrate for protein acylation. Such a role for malonyl-CoA, cytosolic acyl-CoA, and diacylglycerol has been proposed to be part of the stimulation of insulin secretion in pancreatic β -cells by glucose metabolism and its amplification by fatty acid metabolism [18].

Interestingly, medium and short chain acyl-CoA synthetases, including acetyl-CoA synthetase, are located in the mitochondrial matrix, and therefore for them transport can bypass the carnitine translocase system, and their β -oxidation is unregulated.

β -Oxidation. β -Oxidation is so named because it involves oxidation of the β carbon, that is, the second carbon from the carboxylic acid group of the fatty acid. One round of β -oxidation involves the following sequence of steps. First, the carbon-carbon single bond between the α and β carbons is oxidized to a double bond by acyl-CoA dehydrogenase:



Second, water is added across the double bond by enoyl-CoA hydratase to form an alcohol:



Third, the alcohol is oxidized to a carbonyl by L-3-hydroxyacyl-CoA dehydrogenase:



Finally, the β carbonyl is attacked by CoA in the β -ketothiolase reaction:



The process is repeated with the shortened acyl-CoA, until it is completely cleaved to acetyl-CoA fragments. Thus, palmitoyl-CoA (C16) would undergo seven cycles of β -oxidation to generate 8 acetyl-CoA, plus 7 FADH₂ and 7 NADH to enter the electron transport pathway.

Most fatty acids have an even number of carbons, because the synthetic pathway involves the addition of carbons two at a time. The occasional odd chain fatty acid (say from bacterial sources) is degraded by β -oxidation down to propionyl-CoA, which is then carboxylated to methylmalonyl-CoA and converted to the citric acid cycle intermediate succinyl-CoA.

Ketone Bodies

Excess fatty acid catabolism in the liver, generating more acetyl-CoA than can be oxidized in the citric acid cycle, may lead to the formation of the ketone bodies acetoacetate and β -hydroxybutyrate. These can be carried in the blood to other organs and converted back to acetyl-CoA. The acidity that can be caused by diabetic ketoacidosis is a serious concern. On the other hand, ketone body production during prolonged starvation is an advantage, in that they can be used as fuel by the brain, in contrast to fatty acids, thus reducing the demand for gluconeogenesis.

Triglyceride Synthesis

Triglyceride synthesis begins with glycerol 3-phosphate, made by reduction of the glycolytic intermediate dihydroxyacetone phosphate with NADH by glycerol 3-phosphate dehydrogenase (see Figs. 1 and 5). Two acyl chains are added from acyl-CoA in the glycerol-phosphate acyltransferase (GPAT) reaction, followed by the acylglycerol-phosphate acyltransferase (AGAT) reaction. Then the phosphate is cleaved off in the phosphatidic acid phosphohydrolase reaction, to form diacylglycerol. Finally, the third acyl chain is added in the diacylglycerol acyltransferase (DGAT) reaction, forming triglyceride. Insulin promotion of triglyceride synthesis in the fat cell occurs at several steps: translocation of Glut4 to the plasma membrane to increase input into glycolysis and hence glycerol 3-phosphate synthesis; phosphorylation and activation of PFKFB3 to raise fructose 2,6-bisphosphate and thereby activate PFK; increased amount of GPAT.

However, the simple idea that triglyceride synthesis is largely controlled by glycolytic generation of glycerol 3-phosphate, especially via insulin regulation of Glut4, has been complicated by recent work showing that much of the triglyceride glycerol portion comes from glyceroneogenesis, that is, the reactions of gluconeogenesis from pyruvate up to dihydroxyacetone phosphate [19]. (Fat cells, like most cell types other than liver or kidney cortex, lack glucose 6-phosphatase and so cannot synthesize free glucose.) It should be recognized that triglyceride synthesis/lipolysis is a dynamic process, with much of the released fatty acids being re-esterified. This cycling must occur even during times of relative insulin lack. Perhaps this is the optimal way to provide fuel for the rest of the body as needed, without flooding the body with free

fatty acids that could lead to deleterious triglyceride accumulations in nonadipose tissue. Free fatty acids are just siphoned off by albumin in the blood as needed. The recycling, although energy consuming, is not that expensive. Much of the energy needs of the fat cell may be provided by glucose, and only a small amount of fatty acid is oxidized, compared to that exported or re-esterified. Yet the oxidation of a single fatty acid would provide the energy for the re-esterification of 50 fatty acid molecules, or over 30 even if the cost of glyceroneogenesis is included.

Fatty Acid Synthesis

Fatty acids are synthesized from acetyl-CoA. However, the synthesis occurs in the cytosol, whereas the acetyl-CoA from carbohydrate metabolism is generated in the mitochondrion in the PDH reaction. Because there is no carrier for acetyl-CoA to cross the mitochondrial membrane, it is first converted to citrate, which has a carrier. Citrate can exit the mitochondrion and be cleaved back to acetyl-CoA and oxaloacetate by ATP-citrate lyase. Much of the acetyl-CoA to be used is then converted to malonyl-CoA by acetyl-CoA carboxylase. As mentioned above, acetyl-CoA carboxylase is activated by citrate, the precursor for cytosolic acetyl-CoA, and inhibited by phosphorylation by AMPK.

The fatty acid synthase actually starts with an acetyl group transferred to an acyl carrier protein. Then successive malonyl groups are reacted, also bound to an acyl carrier protein, adding two carbons at a time, with the reaction driven by the decarboxylation. A cycle on the synthase includes reduction of the carbonyl of the adduct to a β -hydroxyl, followed by dehydration, and then reduction of the resulting double bond, in steps analogous to reversing the process seen in fatty acid β -oxidation. However, the reducing steps use NADPH, generated in the pentose phosphate pathway. Alternatively, NADPH can be generated in a malate-pyruvate cycle, whereby the cytosolic oxaloacetate from the citrate lyase reaction is converted to malate, the malate is converted to pyruvate in the malic enzyme reaction (with NADP conversion to NADPH), and the pyruvate is converted to oxaloacetate back in the mitochondria by pyruvate carboxylase. Insulin stimulates the synthesis of fatty acid synthase and acetyl-CoA carboxylase.

In humans most fatty acids for triglyceride synthesis are obtained from the diet or recycled from lipolysis. However, some *de novo* fatty acid synthesis may still occur. The rate is more substantial in rodent models; hence, triglyceride synthesis from labeled glucose may include incorporation of label into the fatty acid components as well as the glycerol component, but a distinction can be made by saponification of the sample.

Protein and Amino Acid Metabolism

Amino acids differ from fatty acids and sugars as fuels in that their storage forms, proteins, all have other functions as enzymes or transporters, contractile or structural elements. Muscle protein constitutes the major reserve. Although there is

constant turnover of proteins, gross proteolysis only occurs during prolonged starvation or wasting diseases. Pathways for protein synthesis and degradation and the synthesis and degradation of many individual amino acids will not be considered in detail here, and the reader is referred to a standard biochemistry textbook (e.g., Stryer [20]). Focus instead will be on connections of amino acid metabolism to that of glucose and lipid. Insulin, as a general storage signal, promotes protein synthesis as well as glycogen and triglyceride synthesis.

Amino acids can be divided into two classes: “ketogenic,” that is, those that are metabolized to form ketones or acetyl-CoA and therefore can be oxidized in the citric acid cycle or in theory used for fatty acid biosynthesis, but cannot be converted to glucose; and “glucogenic,” that is, those that can be used for gluconeogenesis. Ketogenic amino acids include, for example, the branched chain amino acids (leucine, isoleucine, and valine). Important glucogenic amino acids include alanine, aspartate, glutamate, and glutamine.

The first step in the metabolism of most amino acids is removal of the amino group by transamination or deamination. In transamination reactions, the amino group is transferred to a keto acid, generating a second keto acid (corresponding to the first amino acid) and a second amino acid (corresponding to the first keto acid). One amino-acid/keto-acid pair is generally glutamate/ α -ketoglutarate. Thus, the glutamate-pyruvate transaminase (also called alanine amino transferase) reaction is:



Other transaminases include glutamate-oxaloacetate transaminase (aspartate amino transferase) and the branched chain amino transferase. An important connection between α -amino groups and free ammonia, for both synthesis and degradation of amino acids, is provided by the reversible glutamate dehydrogenase reaction:



The major amino acid put out by muscle is alanine, not because muscle protein has inordinate amounts of alanine, but rather because alanine is produced by transamination of glycolytically generated pyruvate in the glutamate-pyruvate transaminase reaction; the glutamate in turn comes from transamination of other amino acids, such as the branched chain amino acids, or from the glutamate dehydrogenase reaction. The alanine can then go via the blood to the liver, where it is converted back to pyruvate by transamination and the carbon chain then used for gluconeogenesis, with the glucose then cycling back to the muscle. This interorgan cycle of muscle glycolysis and liver gluconeogenesis is known as the alanine cycle and is analogous to the Cori cycle. This is also the reason that liver pyruvate kinase is inhibited by alanine as a signal of substrate for gluconeogenesis. The alanine cycle is also important for bringing the excess amino nitrogen from muscle catabolism of amino acids to the liver for the synthesis of urea (NH_2CONH_2), the mammalian excretion product. In the liver, the amino group of alanine is first transferred to glutamate and then to aspartate (glutamate-oxaloacetate transaminase) and to free ammonia (glutamate dehydrogenase) to provide the substrates for the urea cycle.

Glutamine is the major amino acid put out by other peripheral tissues to carry excess amino nitrogen to the liver for the synthesis of urea. Glutamine is synthesized from glutamate and free ammonia by glutamine synthetase, and it is converted back to glutamate and free ammonia by glutaminase. Because free ammonia is toxic to the brain, the efficient operation of the urea cycle is very important. The complete urea cycle only occurs in liver, and therefore liver disease can lead to a serious rise in blood ammonia levels. Kidney cortex can also use glutamine, putting out ammonium ion in the urine in response to metabolic acidosis; the carbon chain is then used in these cells for gluconeogenesis. Glutamine is a favored substrate for many cells in culture, including fat cells, in addition to glucose, and is often added to tissue culture media. Whether glutamine can contribute substantially to glycerogenesis in fat cells remains to be determined.

AMP-Activated Protein Kinase: An Integrated Modulator of Cellular Metabolism

Until recently, cellular metabolism in the intact organism has been viewed as primarily under the control of insulin and counter insulin hormones (including glucagon, epinephrine, norepinephrine, and the glucocorticoids). On the other hand, it has long been appreciated that some form of regulation by energy state must also occur. Early evidence for this included the inhibition of the key glycolytic enzyme phosphofructokinase by ATP and its activation by AMP and the activation of glycogen phosphorylase by AMP. In the last 15 years, it has become apparent that changes in cellular metabolism to a considerable extent are regulated by the enzyme AMPK. As originally described [21], AMPK is a fuel sensing enzyme present in all eukaryotic cells that senses and responds to a decrease in a cell's energy state as reflected by an increase in the AMP/ATP ratio. For instance, its activation in skeletal muscle and other tissues during exercise both increases the activity of multiple processes that generate ATP (fatty acid oxidation, glucose transport in skeletal and cardiac muscle, and glycolysis in heart) and inhibits others that require ATP but can be downregulated temporarily without jeopardizing the cell (e.g., protein, triglyceride, and cholesterol synthesis) [22]. Conversely, a decrease in AMPK activity appears to have opposite effects [23].

Recently, it has become apparent that AMPK plays an even more fundamental role in metabolic regulation. Thus, upregulation of its activity by a wide variety of hormones (e.g., adiponectin, catecholamines), drugs (metformin and thiazolidinediones, α -lipoic acid, statins), and lack of fuels (e.g., glucose deprivation) as well as its downregulation by other hormones and paracrine factors (e.g., glucocorticoids and endocannabinoids) and by a fuel excess (e.g., hyperglycemia) has been demonstrated. In addition, upstream molecules that phosphorylate and activate AMPK such as LKB1, a tumor suppressor, and Ca^{2+} -dependent CaMKKs have been identified. Thus, it has become increasingly evident that AMPK is a mediator of metabolic events within the cell in response to a wide variety of stimuli including

at least some that act in the apparent absence of a change in energy state. Perhaps most intriguing of all is that decreased AMPK activity has been associated with a metabolic syndrome phenotype (obesity, diabetes, insulin resistance, predisposition to atherosclerosis) in many experimental animals, whereas pharmacological agents and other therapies (e.g., exercise, diet) that activate AMPK have shown benefit in their treatment both in humans and experimental animals [17, 24]. An understanding of how AMPK exerts its many effects and whether its apparent clinical efficacy is related to its actions on intermediary metabolism are exciting questions that will be the object of intense interest in the foreseeable future.

LKB-1, an upstream kinase for AMPK, in turn can be activated by deacetylation by SIRT1, an NAD-dependent deacetylase. SIRT1 is sensitive to the redox state of the NAD/NADH couple. AMPK increases the production of NAD. Therefore, AMPK and SIRT1 may form an integrated system that is sensitive to both the adenine nucleotide energy state and the redox state as well as other factors [24, 25].

As mentioned above, AMPK is important in regulating fatty acid oxidation by phosphorylation and inhibition of acetyl-CoA carboxylase, and activation of malonyl-CoA decarboxylase, thus decreasing malonyl-CoA levels and deactivating CPT1. AMPK also phosphorylates and inhibits GPAT, a key enzyme in triglyceride synthesis, and decreases the levels of fatty acid synthase, GPAT and DGAT. Furthermore, SIRT1 causes the activation of mitochondrial and lipid oxidation genes. Therefore, activation of the AMPK-SIRT1 system should promote lipid consumption. This may be part of the beneficial effect of exercise.

Food intake in excess of energy usage leads to the storage of triglyceride in adipose tissue and eventual obesity. Although in humans most fatty acids come from the diet rather than de novo synthesis, preferential oxidation of carbohydrate rather than fat would leave fatty acids available for triglyceride synthesis. Interestingly, some studies indicate that obesity prone individuals have an increased respiratory quotient ($RQ = \text{CO}_2 \text{ expelled divided by O}_2 \text{ consumed}$), implying greater usage of carbohydrate than fat compared with lean individuals [26–28]. Whether obese or obese-prone individuals have dysregulation of the AMPK-SIRT1 system, leading to inappropriate sparing of fatty acids from oxidation, is a tempting hypothesis that requires further study.

The subjects of whole-body regulation and dysregulation of food intake and the involvement of satiety factors and hormones, such as leptin and adiponectin, will be discussed in other chapters.

Transgenic Models: Some Answers and More Questions

An increasingly important approach in metabolic research is the use of transgenic mice or cells, where a gene of interest is knocked out, reduced in expression, or overexpressed, sometimes in a tissue specific manner. Mention has already been made of how the knockout of HSL indicated that the primary triglyceride lipase was in fact a different enzyme, since identified as ATGL. Some other long-held theories have recently received clarification or revision from such experiments.

Knockout of the insulin receptor in muscle (MIRKO) had little effect on blood glucose or insulin levels, and the mice remained normally glucose tolerant [29]. Muscle insulin resistance could be seen in a hyperinsulinemic-euglycemic clamp, and there were effects on protein metabolism, indicated by decreased muscle mass. This calls into question the primary importance of insulin-stimulated glucose disposal in muscle for normal glucose homeostasis and the contribution of muscle insulin resistance to the development of diabetes. On the other hand, perhaps this represents a difference between mice and humans. Interestingly, adipose insulin-stimulated glucose uptake was substantially increased in the MIRKO mice, as was adipose tissue mass, but the enhancement was not seen in isolated adipocytes, suggesting a stimulatory factor released from MIRKO muscle. Knockout of the insulin receptor in liver (LIRKO) led to hyperglycemia and hyperinsulinemia, indicating the importance of the normal suppression by insulin of hepatic glucose output. Levels of the gluconeogenic enzymes PEPCK and G6Pase were elevated; they are normally suppressed by insulin. Hyperinsulinemia leads to insulin resistance in other tissues, which may have exacerbated the situation. Nevertheless, circulating levels of fatty acids and triglycerides were decreased, due to suppression of lipolysis in fat cells by the high insulin levels. On the other hand, knockout of the insulin receptor in fat (FIRKO) perhaps surprisingly led to improved glucose homeostasis and increased insulin sensitivity in the whole animal. It has been suggested that this may be due to alteration in the levels of adipokines (fat secreted signaling molecules), in particular increased adiponectin and leptin. Knockout of the insulin receptor in pancreatic β -cells (β -IRKO) led to the development of abnormal glucose tolerance, smaller islets, and reduced insulin content, indicating that insulin signaling is important in this cell type, too; this presumably is related to the role of insulin as a growth factor rather than as a metabolic regulator.

A major effect of insulin is to cause translocation of Glut4 and hence stimulation of glucose transport in muscle and fat. Knockout of Glut4 in muscle (and heart) (MG4KO) led to hyperglycemia, glucose intolerance, and insulin resistance [30]. The severity of these effects, in contrast to the relatively benign effects of MIRKO, perhaps argues for other/backup mechanisms of activating Glut4 besides insulin. The hyperglycemia in MG4KO also leads to insulin resistance of liver and adipose tissue as well. Knockout of Glut4 in adipose tissue (AG4KO) also led to glucose intolerance and insulin resistance in the whole animal, presumably by altered adipokine communication to other tissues. Adipose mass and adipocyte size were normal, in contrast to the 50% decrease in adipose mass and bimodal distribution of cell size reported for the FIRKO mouse; this may relate to the difference in effects on whole body metabolism and presumably adipokine production in the two knockout models.

One of the key downstream kinases in the insulin signaling cascade is Akt (protein kinase B). Overexpression of Akt1 in skeletal muscle increased the muscle mass through growth of type IIb fibers, which are glycolytic (MyoMouse). This resulted in decreased fat mass accumulation on a high fat/high sucrose diet. The mice did not eat less or exercise more, but burned more fat. This was due to enhanced fatty acid oxidation by the liver, not by the muscle, suggesting a role for muscle-derived myokines [31].

Unexpected connections between glycolytic enzymes and fat metabolism have been published recently. Haller et al. [32] used a photodynamic selection technique to generate a population of Chinese hamster ovary cells deficient in glycerolipid biosynthesis, where the lesion involved a reduction in phosphatidic acid phosphatase activity and downstream glycerolipids and increased α -glycerophosphate; however, the DNA mutation turned out to be a point mutation in the gene for phosphoglucose isomerase. Getty et al. [6] reported that mice deficient in phosphofructokinase-M had greatly decreased fat stores, even though fat contains the other two isoforms of phosphofructokinase, suggesting the possible importance of intrinsic metabolic oscillations for triglyceride synthesis.

Gross obesity is readily apparent, and therefore spontaneous mutations in rodent colonies led to the establishment of such lines even before the development of targeted genetic techniques. The ob/ob (obese) mouse lacks leptin, a satiety hormone produced by fat cells that acts on the hypothalamus. It therefore has hyperphagia, develops obesity, and consequent insulin resistance and diabetes. Interestingly, the ob/ob mouse can outgrow the diabetes, through massive hyperplasia of the insulin-producing β -cells in pancreatic islets. The db/db (diabetic) mouse, which lacks the leptin receptor, has a somewhat more severe phenotype. Zucker diabetic and fa/fa rats also have mutations in the leptin receptor. These rodent models have been frequently used in obesity/diabetes research.

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