Human metabolism: pathways and clinical aspects

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Abstract

Metabolism describes the series of chemical reactions that are concerned with the provision of energy to biological systems. They may be divided into reactions involved in energy yield (catabolism: demand exceeds supply), and energy storage (anabolism: supply exceeds demand). Regulation of these pathways is critical for homeostasis, and derangements in metabolism are seen in a wide variety of pathological processes. Understanding metabolism is key to the treatment of many diseases, notably diabetes, as well as underpinning clinical nutritional support.

Keywords Carbohydrates; diabetes; lipids; metabolism; proteins

The word metabolism is derived from the Greek 'to change', and describes the series of biochemical reactions that provide the body with the energy it requires to maintain biological functions (e.g. biosynthesis, maintenance of ionic gradients, muscle contraction, heat generation). This energy must ultimately be derived from food. The rate of energy production measured under basal conditions — 'basal metabolic rate' (BMR) — is affected by many factors, including muscle contraction, food ingestion, size, gender, age, temperature, sepsis, cancer and several hormones, including thyroid hormones and catecholamines. The metabolic rate can be estimated by measuring oxygen consumption (VO_2; indirect calorimetry).

The process of converting excess energy-rich substrate precursors in food into complex energy storage molecules is termed anabolism, whereas processes degrading substrates to mobilize biologically useable energy, are termed catabolism. Imbalance of these pathways leads to cachexia or obesity. Tissues have specialized metabolic functions (e.g. adipose tissue stores substrate, muscle oxidizes substrate, lactating mammary gland exports substrate). The liver is a metabolic 'transformer' that regulates substrate supply between tissues, and pancreas detects and signals nutritional status.

Metabolic energy is carried in two main forms: (i) 'high energy' phosphate groups including ATP, GTP and creatine phosphate; and (ii) hydride ion (effectively, electron) carriers such as NADH, $FADH_2$ and NADPH. These molecules are used in chemical reactions throughout the cell that would not occur without external energy input, because they are energetically unfavourable. Besides carrying energy in metabolic pathways, the cellular energy charge and redox potential are major regulators of metabolism.

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Energy substrates

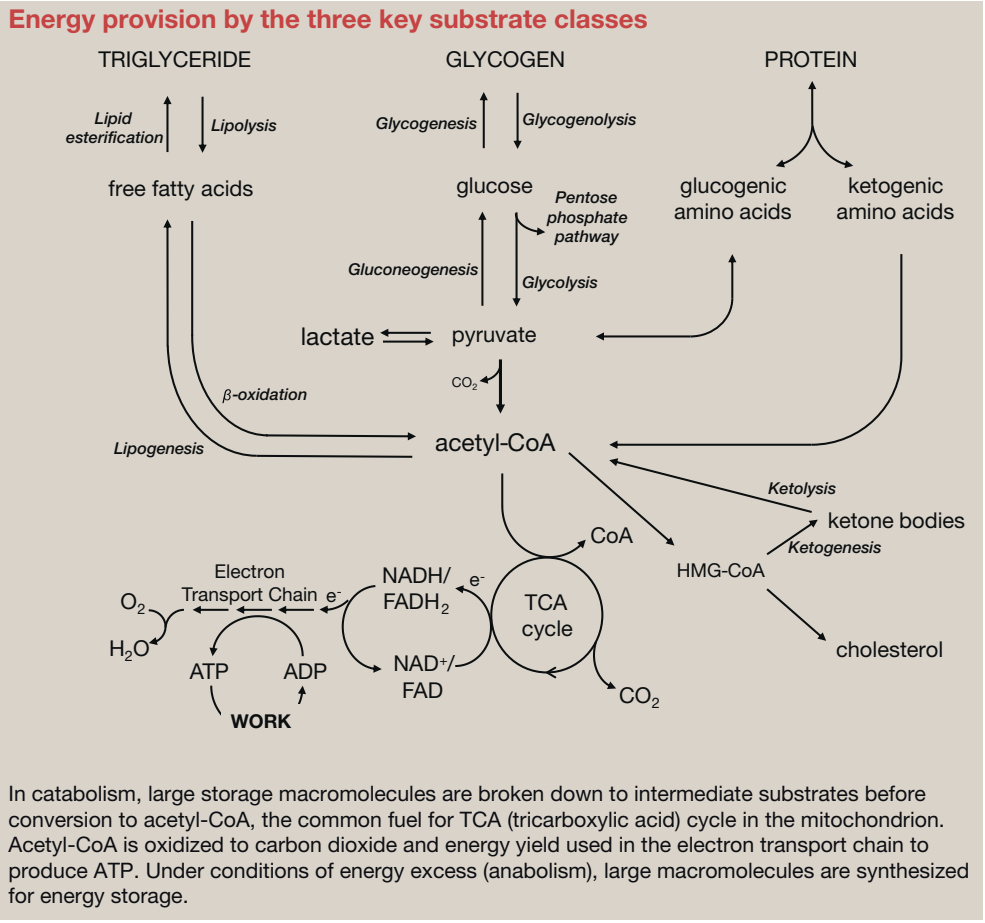
Energy is derived from three groups of energy-rich compounds: carbohydrates, lipid (fats) and proteins (amino acids) (Figure 1). Carbohydrates (hydrated carbon: $C(H_2O)_n$) are soluble, fast and easy to transport, relatively non-toxic, and can yield some energy anaerobically in hypoxia or ischaemia when oxygen availability is limited. However, their water solubility means that in storage form as glycogen they retain significant amounts of water; in addition, carbohydrates are partially oxidized and hence do not contain as much energy as lipids. Therefore only limited amounts are stored. By contrast, lipids are highly reduced and very energy-dense, hence they function as the principal energy store for free-living animals and are major energy providers to most tissues. However, their water-insolubility makes lipids slow to mobilize, and unlike carbohydrates they cannot yield energy anaerobically, so cannot be used by erythrocytes and renal medulla. Furthermore, they cannot cross the blood—brain barrier so cannot be used by the CNS. Because lipids are more reduced, relatively more oxygen is required to extract energy from them (2.8 ATP/O_2) compared to carbohydrates (3.7 ATP/O_2) and this may be critical in high work-load/oxygen-challenged tissues such as myocardium and exercising skeletal muscle. Also, lipids in the form of non-esterified fatty acids are amphipathic (detergent-like) and hence disruptive to membranes and potentially toxic. Amino acids have similar energy yields to carbohydrates, and most can be converted to glucose. Under conditions of carbohydrate depletion (e.g. starvation) certain proteins can be broken down to yield amino acids for conversion into carbohydrates to supply glucose-dependent tissues. Although proteins are not stored specifically to supply energy, they act as a virtual carbohydrate supply in catabolic states of carbohydrate exhaustion (e.g. starvation).

Metabolism of the three major substrate groups converges at a common intermediate, acetyl-CoA, in mitochondria (Figure 1). Acetyl-CoA can enter the tricarboxylic acid (TCA; Krebs) cycle and be completely oxidized to 3 NADH, 1 $FADH_2$, 1 GTP and 2 CO_2. The hydride carriers convey electrons to the electron transport chain in the presence of oxygen, and result in the generation of large amounts of ATP via oxidative phosphorylation (and H_2O).

Pancreas is the key organ detecting metabolic status. Pancreatic β-cells sense high blood glucose and release insulin in response. Pancreatic α-cells release glucagon in response to low blood glucose concentration. Carbohydrate and lipid utilization are reciprocally related (Randle cycle), a mechanism partly orchestrated by insulin.

Carbohydrate metabolism

Carbohydrate metabolism centres around the hexose sugar glucose ($C_6H_{12}O_6$). Following uptake into the cell by glucose transporters (GLUT), glucose is rapidly phosphorylated to glucose-6-phosphate (G6P) by the enzyme hexokinase (liver and pancreas:



In catabolism, large storage macromolecules are broken down to intermediate substrates before conversion to acetyl-CoA, the common fuel for TCA (tricarboxylic acid) cycle in the mitochondrion. Acetyl-CoA is oxidized to carbon dioxide and energy yield used in the electron transport chain to produce ATP. Under conditions of energy excess (anabolism), large macromolecules are synthesized for energy storage.

Figure 1

glucokinase); G6P is a central hub in carbohydrate metabolism and can be used for glycolysis and glycogen synthesis (glycogenesis), but it may also be derived from glycogen breakdown (glycogenolysis) and from non-carbohydrate precursors (gluconeogenesis), depending on tissue and prevailing metabolic state.

Glycogen

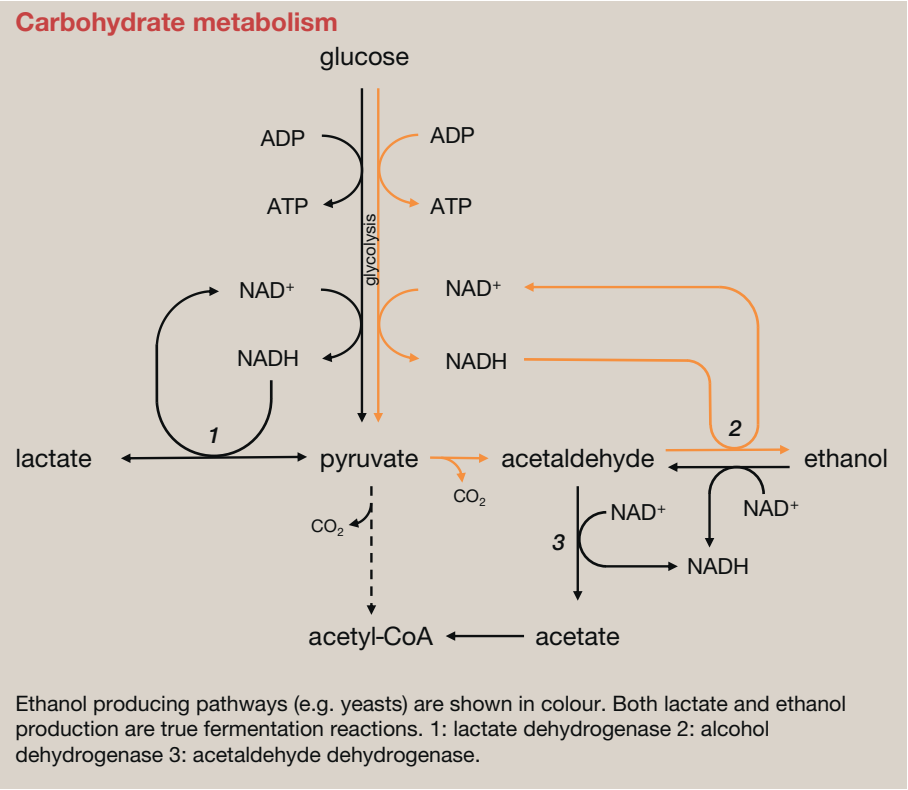
Carbohydrate is stored in limited amounts as cytoplasmic glycogen granules in most tissues, as an energy resource available within the tissue (and hence independent of blood supply) for rapid utilization when required. Glycogen is a polymer of glucose that glycogen synthase assembles into a linear chain, but every 8–10 glucose residues a branch point is introduced. This produces a highly branched tree-like structure with many free ('non-reducing') ends, which enables rapid glucose release during glycogenolysis by the enzyme glycogen phosphorylase. In tissues which store glycogen for their own utilization (e.g. muscle), the G6P generated by glycogenolysis undergoes glycolysis for energy production; however, in liver the G6P is dephosphorylated by glucose-6-phosphatase into free glucose, which is released into the blood stream to maintain blood glucose levels. Genetic lack of glucose-6-phosphatase gives rise to von Gierke's disease, the most common of the glycogen storage diseases. Liver stores about 100 g of glycogen, enough to supply the body for only about 12–24 hours, whereas skeletal muscle stores about 350 g of glycogen, sufficient for about 70 minutes of muscle contraction.

Glycolysis

Glucose is cleaved into pyruvate by glycolysis in the cytosol of all cells (Figure 2), and generates energy without the use of oxygen. One molecule of glucose yields two molecules of pyruvate, 2 NADH and 2 ATP, the latter via substrate level phosphorylation. Pyruvate can be imported into the mitochondria and decarboxylated to acetyl CoA (see Figure 1), remain in the cytosol and be reduced to lactate, or be transaminated to the amino acid alanine. Its fate is determined by the tissue, oxygen availability and circulating hormones. Hence, in muscle, glycolysis splits glucose in order to provide energy (pyruvate completely oxidized to CO_2 via acetyl-CoA), but in liver, excess glucose is broken down by glycolysis to pyruvate, then acetyl-CoA, and used for lipid synthesis (see Figure 1). Glycolysis is tightly regulated by hormonal and metabolic signals and is linked to the energy status of the cell via allosteric effects of AMP, ATP and citrate.

Gluconeogenesis

Gluconeogenesis is synthesis of glucose from non-carbohydrate sources and is typically active in catabolic states, e.g. postprandial/starvation, exercise, occurring mainly in the liver (consistent with its role in maintaining blood glucose levels) and



Ethanol producing pathways (e.g. yeasts) are shown in colour. Both lactate and ethanol production are true fermentation reactions. 1: lactate dehydrogenase 2: alcohol dehydrogenase 3: acetaldehyde dehydrogenase.

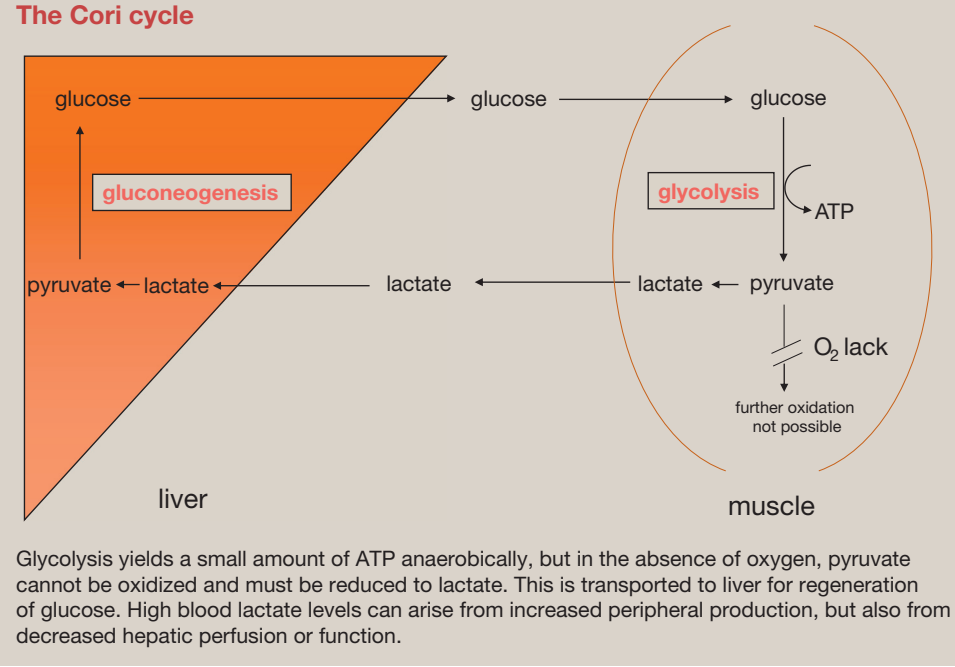
Figure 2

with some limited activity in kidney. It allows the body to make new glucose when dietary carbohydrate sources are limited, and is regulated by hormones such as glucagon, and by the supply of substrates. The pathway is not simply a reversal of glycolysis, as several reactions of glycolysis are irreversible, but many of the steps are shared by both pathways. The substrate for gluconeogenesis is pyruvate, derived from transamination of the amino acid alanine (derived from proteolysis of body protein) or from re-oxidation of lactate produced from anaerobic metabolism. Glycerol, derived from lipolysis of triglycerides (triacylglycerols, TAG), can also be used for glucose synthesis (and hence although fatty acids cannot be converted into glucose, breakdown of storage lipids does yield a small amount of carbohydrate). Regulation of blood glucose concentration being a major function of liver, liver failure is characterized by falling blood glucose levels.

Lactate and ethanol metabolism

In tissues with mitochondria and in the presence of oxygen, NADH from glycolysis is reoxidized to NAD^+ by the electron transport chain in the inner mitochondrial membrane, with generation of ATP. In the absence of mitochondria (e.g. erythrocytes) or in ischaemic/hypoxic states, NAD^+ must be regenerated from NADH in the cytosol, by linking pyruvate reduction to lactate by lactate dehydrogenase (LDH) to allow glycolysis to continue (see Figure 2). Lactate accumulates and glycolysis proceeds, providing limited ATP production by anaerobic metabolism: 'homolactic fermentation'. Tissues lacking oxygen (e.g. exercising muscle, ischaemic myocardium) or mitochondria (erythrocytes) export lactate to the liver where it is re-oxidized to pyruvate, which then undergoes gluconeogenesis, regenerating glucose for re-export to muscle or erythrocytes: the Cori cycle (Figure 3). In the clinical context, elevated blood lactate is a common finding in critically ill patients. Hyperlactataemia/lactic acidosis (blood [lactate] >2.5 mM) may be due to increased peripheral (extrahepatic) production (e.g. tissue ischaemia/hypoxia) but may also be due to decreased central (hepatic) gluconeogenesis or liver blood flow, and is an early sign of mesenteric/hepatic ischaemia. This cycle also operates in malignancy. Cancer cells are highly glycolytic, producing lactate from glucose even in the presence of adequate oxygen; this aerobic glycolysis is termed the Warburg effect. The lactate is recycled by the 'host' liver in an energetically inefficient cycle. Tumours also typically utilize much glutamine; a likely explanation for these processes is that this provides the tumour with biosynthetic substrates (including products of the pentose phosphate pathway) to support its rapid growth. The cause of the weight loss and wasting (cachexia) seen in cancer is uncertain but is probably not solely due to the energetic burden the tumour places on the host — a variety of signals, both host and tumour derived (e.g. cytokines such as TNFα ('cachectin', oncometabolites) are likely involved in the metabolic reprogramming seen in this condition.

Certain organisms (e.g. yeasts) have an alternative strategy to regenerate NAD^+ for glycolysis — alcoholic fermentation. Here, pyruvate is decarboxylated to acetaldehyde (and carbon dioxide, the characteristic gaseous product of brewing), which is then reduced to ethanol by alcohol dehydrogenase, linked to the



Glycolysis yields a small amount of ATP anaerobically, but in the absence of oxygen, pyruvate cannot be oxidized and must be reduced to lactate. This is transported to liver for regeneration of glucose. High blood lactate levels can arise from increased peripheral production, but also from decreased hepatic perfusion or function.

Figure 3

oxidation of NADH, regenerating NAD^+ (see Figure 2). Ethanol accumulates, and inhibits competing microorganisms. When ethanol is ingested by humans, its metabolism has multiple effects on the NAD^+:NADH ratio (redox potential). In the liver, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase, and acetaldehyde is further oxidized to acetate by aldehyde dehydrogenase, both enzymes generating NADH (and potentially reactive oxygen species). The acetate is converted into acetyl-CoA, providing an abundant energy source. However, the high levels of NADH inhibit oxidation of lactate to pyruvate, limiting the availability of the gluconeogenic precursor and causing a mild metabolic lactic acidosis. The result is decreased gluconeogenesis and hypoglycaemia. Furthermore, the TCA cycle and fatty acid β-oxidation are inhibited, while lipogenesis is increased (increased acetyl-CoA), leading to hepatic lipid accumulation and alcoholic fatty liver.

Pentose phosphate pathway

G6P is also used in the cytosolic pentose phosphate pathway (PPP). The pathway generates NADPH, and the pentose (5-carbon) sugar ribulose-5-phosphate, which is used for synthesis of nucleotides and aromatic amino acids. NADPH provides energy for certain anabolic reactions, such as lipogenesis, and also maintains the antioxidant glutathione in its reduced (active) form (GSH). The initiating step of PPP reduces $NADP^+$ to NADPH and is catalysed by glucose-6-phosphate dehydrogenase (G6PDH). Deficiency of this enzyme is common in equatorial regions; it is an X-linked condition and many variants of G6PDH deficiency occur. NADPH deficiency secondary to low G6PDH activity results in low levels of reduced glutathione and increased oxidative damage to erythrocytes, denatured haemoglobin appearing as blister cells and Heinz bodies, presenting as haemolytic anaemia. Haemolytic crises may be induced by various drugs and chemicals, including methylene blue, and ingested fava beans (favism). G6PDH mutations have probably been tolerated in evolution because lack of NADPH-derived anti-oxidant activity in erythrocytes inhibits the malaria parasite.

Regulation of carbohydrate metabolism

Insulin is the major anabolic signal for carbohydrate metabolism. Pancreatic β-cells secrete insulin in response to increased blood glucose, which stimulates glucose uptake from the blood, and anabolic processes such as hepatic glycogenesis and glycolysis, resulting in lowered blood glucose. Insulin also inhibits catabolic pathways such as glycogenolysis and gluconeogenesis. Sulphonylurea drugs act on ATP-sensitive potassium channels in the pancreatic β-cells to modify the mechanism linking glucose sensing and insulin secretion by that tissue, causing increased insulin output. Sympathetic activation and several hormones, including catecholamines, cortisol and growth hormone, stimulate hepatic glycogenolysis and gluconeogenesis, with glucose release into the blood, but glucagon is the major catabolic signal, raising blood glucose by stimulating hepatic glucose production and inhibiting the reciprocal anabolic pathways. Hepatic gluconeogenesis is inhibited by the biguanide metformin, decreasing hepatic glucose production in diabetes.

Lipid metabolism

Fatty acids are used for energy production in oxidative tissues. Since they are amphipathic and potentially toxic, their plasma concentration does not rise to more than ~0.5 mM physiologically and they are transported bound to albumin ('free' fatty acids are more correctly termed non-esterified fatty acids [NEFA]). Esterification of three fatty acids to glycerol yields the very hydrophobic TAG — a highly efficient energy storage molecule. TAG transport in the aqueous plasma requires it to be carried in the hydrophobic core of TAG-rich lipoproteins (TGRLP).

Lipid mobilization

Lipid mobilization occurs during catabolic states such as fasting, starvation and exercise. Adipose tissue stores the greatest amount of TAG (~15 kg), which undergoes lipolysis by the action of lipase enzymes (including hormone sensitive lipase, HSL) releasing three fatty acids (FA) and one glycerol into the circulation for use elsewhere in the body: glycerol is utilized by the liver for gluconeogenesis, the fatty acids are utilized by oxidative tissues for ATP production. Other tissues store TAG in lipid droplets as an intracellular energy resource for their own utilization. Recent evidence suggests that excessive tissue lipid accumulation, for example, in insulin-resistant states such as type 2 diabetes, leads to tissue dysfunction (and further insulin resistance: 'lipotoxicity').

NEFA uptake from the plasma involves both diffusion across the cell membrane as well as facilitated uptake via CD36/FAT (FA translocase) and FA binding and transport proteins Following uptake, FAs may be re-esterified in the cytosol to intracellular triglycerides or migrate to the mitochondria for oxidation. However, long-chain FAs cannot cross the highly selective inner mitochondrial membrane when bound to their carrier, CoA, therefore, the FA is transported across on the carnitine shuttle, initiated by carnitine acyl transferase-1 (CAT-1). CAT-1 is inhibited by malonyl-CoA, the first committed intermediate of lipogenesis, a reciprocal mechanism preventing simultaneous FA synthesis and breakdown, and a major regulatory mechanism of FA oxidation.

β-Oxidation

Fatty acids within the mitochondria now undergo β-oxidation. The β-carbon of the FA chain is attacked and a 2-carbon segment of the FA chain is released as acetyl-CoA. This oxidative cycle is repeated until the entire FA chain has been broken down to multiple acetyl-CoA, NADH and $FADH_2$. The acetyl-CoA undergoes further oxidation in the TCA cycle; all the NADH and $FADH_2$ generated are then oxidized by the electron transport chain, yielding large amounts of ATP (see Figure 1). Many forms of mitochondrial disease based on enzyme mutations are now recognized, often presenting with muscle weakness but demonstrating a wide variety of phenotypes. Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency impairs the β-oxidation of fatty acids within the mitochondria, limiting their use as a fuel in oxidative tissues and increasing the dependence on glucose metabolism for ATP production.

Ketone bodies

In the liver, acetyl-CoA derived from β-oxidation can also be used for ketone body synthesis (ketogenesis). The ketone bodies, acetoacetate and β-hydroxybutyrate, are water soluble transportable forms of acetyl-CoA, which can be used by the brain and other oxidative tissues as glucose-sparing fuels in catabolic conditions such as starvation. Ketogenesis occurs exclusively in the liver; however, liver lacks the pathway for ketone body utilization (ketolysis), preventing futile substrate cycling. Acetoacetate undergoes spontaneous decarboxylation to acetone, which probably has no physiological function in humans but is volatile and excreted in the breath, with a characteristic sweet-smelling odour present in diabetic ketoacidosis. An intermediate of ketogenesis is hydroxymethylglutaryl-CoA (HMG-CoA); HMG-CoA can also be converted to mevalonate by HMG-CoA reductase, and eventually to cholesterol (see Figure 1). HMG-CoA reductase is the rate-limiting step of cholesterol synthesis, and is the enzyme inhibited by the statin class of drugs.

Lipid synthesis and lipoprotein metabolism

Excess acetyl-CoA derived from surplus carbohydrates and amino acids is assembled into fatty acids for energy storage (lipogenesis) in the cytosol of liver and adipose tissue (see Figure 1). The initiating step involves generation of malonyl-CoA from acetyl-CoA by acetyl-CoA carboxylase (ACC), and is highly regulated. The malonyl group is the donor for fatty acid synthetase (FAS), a multicatalytic polypeptide which elongates the growing fatty acid chain by 2 carbons in a repeated cycle using NADPH for energy. While β-oxidation occurs in mitochondria, lipogenesis occurs in the cytosol, an example of intracellular compartmentation limiting futile substrate cycling of two opposing pathways. Three FAs are then esterified to glycerol phosphate to form TAG. TAG synthesized in liver must next be exported to adipose tissue for storage.

TAG must be transported in the plasma within specialized carrier particles — lipoproteins. The TAG-rich lipoproteins comprise a phospholipid monolayer shell, embedded proteins (apolipoproteins, which direct the fate of the particle), a hydrophobic core of TAG, together with cholesterol (esterified), and fat-soluble vitamins. TGRLPs comprise chylomicrons (CM), synthesized by the intestine from exogenous dietary fat, and very-low-density lipoproteins (VLDL), synthesized by the liver from endogenous lipids. CM and VLDL deliver TAG to FA-utilizing tissues that express the enzyme lipoprotein lipase (LPL), which is tethered to the luminal surface of the endothelium. In type I hyperlipoproteinaemia (chylomicronaemia) syndrome, an autosomal recessive mutation of LPL, plasma TGRLP-TAG cannot be cleared and very high (>50 mM) plasma TAG levels result. (More rarely, a mutation in the LPL-activating apoprotein apo-CII causes a similar clinical picture.)

Following LPL action the lipoprotein shrinks, resulting in a smaller, denser, TAG-depleted particle named a 'remnant particle'. The chylomicron remnant is recycled in the liver; the VLDL remnant particle is termed low-density lipoprotein (LDL), and continues in the circulation to deliver its remaining core lipid — cholesterol ester — to peripheral tissue through a lipoprotein receptor-mediated uptake mechanism, resulting in the entire LDL particle being endocytosed into the target cell for cholesterol release. This mechanism is termed 'forward cholesterol transport'. Excess cholesterol is transported back from peripheral tissues to the liver for excretion by the 'reverse cholesterol transport' pathway: nascent high-density lipoprotein (HDL) particles in the plasma assimilate cholesterol from extrahepatic tissues, then the cholesterol-enriched HDL particle is removed by the liver and the cholesterol excreted in the bile. Defects in lipoprotein receptor expression, for example lack of functional LDL receptor in familial hypercholesterolaemia (Fredrickson type II hyperlipidaemia), are characterized by an inability to remove LDL cholesterol from the circulation, resulting in very high plasma LDL-cholesterol levels (>10 mM) and accelerated atherosclerosis. By contrast, high levels of HDL are associated with decreased risk of atherosclerosis.

Regulation of lipid metabolism

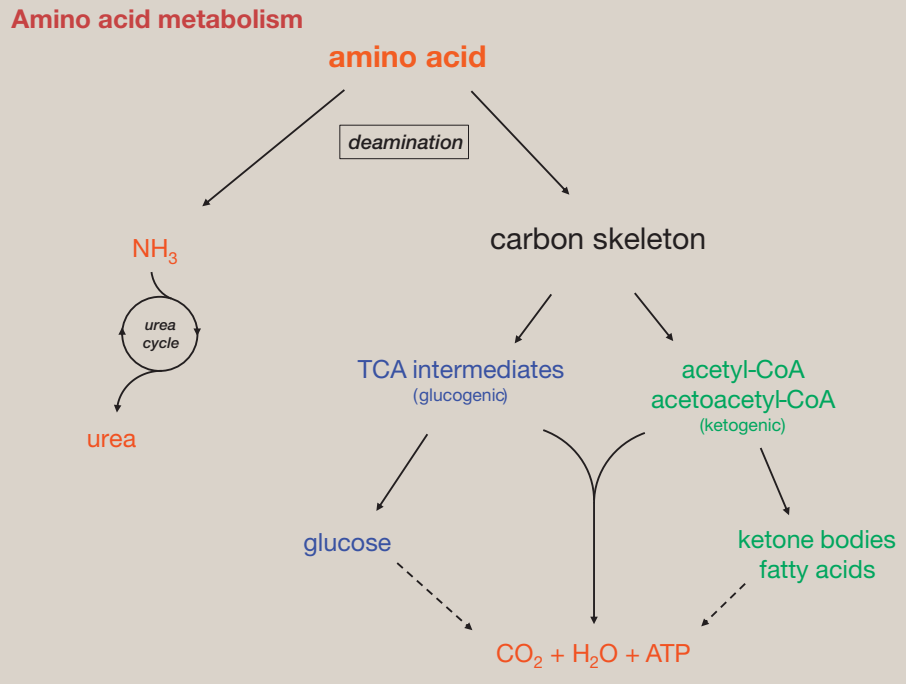
The anabolic state is signalled by insulin: hepatic lipogenesis, TAG and cholesterol synthesis are stimulated, while ketogenesis is inhibited. In adipose tissue, insulin stimulates LPL, enhancing plasma TAG uptake from TGRLPs, and suppresses TAG lipolysis, thereby lowering plasma NEFA concentrations. Conversely, several catabolic 'counter-regulatory' hormones and signals stimulate lipid mobilization and breakdown – catecholamines, increased sympathetic activity, and adrenocorticotrophic hormone (ACTH) stimulate adipose lipolysis, increasing plasma NEFA levels.

Amino acid metabolism

Typical dietary protein intake is ~100 g/day, while the ~10 kg of body protein is turned over at ~300 g/day (~3%). Dietary proteins are absorbed as amino acids and small peptides into the portal circulation, with enterocytes and hepatocytes oxidizing some amino acids as their main substrate. Amino acids are also derived from proteolysis of endogenous proteins, and some ('non-essential') can be synthesized from intermediary metabolites or from other amino acids. In contrast, 'essential' amino acids cannot be synthesized by humans and must be obtained from the diet. Unlike carbohydrates and lipids, amino acids contain nitrogen in the form of an amino group and this must be removed (deamination) before the remaining carbon skeleton (2-oxoacid) can undergo further metabolism (Figure 4). Deamination of amino acids produces ammonia (NH_3), which is highly toxic and must be either excreted directly into the urine by the kidney, or converted into relatively non-toxic urea by the urea (ornithine) cycle in the liver.

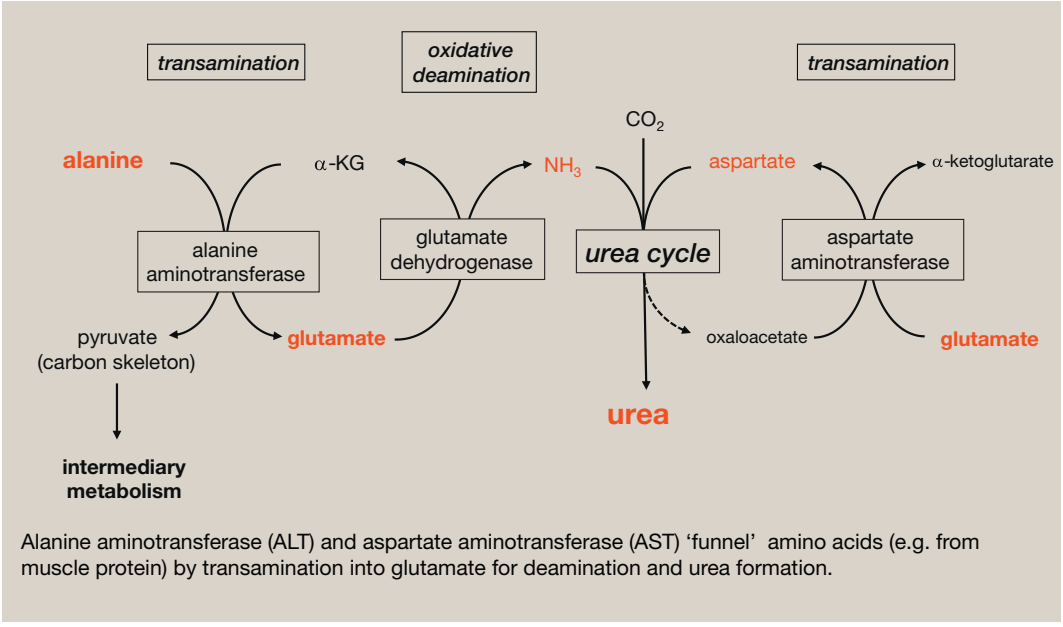
Nitrogen disposal

Deamination of amino acids is achieved by two types of reaction acting together. In transamination, the amino group from one amino acid is transferred to another carbon skeleton (an oxo-acid), forming its corresponding amino acid (i.e. amino acid-1 + oxoacid-2 ↔ oxoacid-1 + amino acid-2). For most amino acids undergoing transamination the amino acceptor is α-ketoglutarate (a TCA cycle intermediate), producing the carbon skeleton of the donor amino acid and glutamate. Hence α-ketoglutarate 'funnels' the various amino acids into glutamate via transamination (Figure 5). Alanine aminotransferase (transaminase; ALT) transfers the amino group of alanine to α-ketoglutarate, forming pyruvate and glutamate. Alanine is a key transport amino acid in the blood, safely conveying nitrogen from peripheral tissues such as muscle to the liver, hence this enzyme is important for inter-tissue amino acid flux. Aspartate aminotransferase (transaminase; AST) transfers the amino group of aspartate to α-ketoglutarate, forming oxaloacetate and glutamate but this enzyme usually works in the reverse direction, converting glutamate (from funnelled amino acids, above) into aspartate, which is required to donate a second N-atom to the urea cycle. Since ALT and AST are both intracellular enzymes and widespread, necrosis of many tissues releases them to plasma, but they are commonly used to diagnose hepatocellular damage. The second type of deamination reaction is oxidative deamination: following transamination in liver, the glutamate undergoes direct oxidative deamination by glutamate dehydrogenase, regenerating α-ketoglutarate and producing NH_3. The ammonia is then detoxified to urea in the urea cycle, and carbon skeletons undergo intermediary metabolism (see below; Figure 5).



The metabolic fate of the carbon skeleton depends on where it enters the pathway of intermediary metabolism. TCA, tricarboxylic acid.

Figure 4



Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) 'funnel' amino acids (e.g. from muscle protein) by transamination into glutamate for deamination and urea formation.

Figure 5

The urea cycle occurs in the liver. Urea ($CO.(NH_2)_2$) contains two nitrogen atoms: one derives from NH_3 via oxidative deamination of glutamate, the other from aspartate via transamination by AST. Each of the six enzymes of the urea cycle may be functionally mutated, impairing ammonia disposal. Ornithine transcarbamylase (OTC) is a mitochondrial urea cycle enzyme which synthesizes citrulline from ornithine and carbamoyl phosphate (the latter formed from ammonia and bicarbonate). OTC deficiency is the commonest disorder of the urea cycle and is characterized by high ammonia concentrations, causing ataxia, lethargy and death, reflecting the extreme neurotoxicity of NH_3. The mechanism of this severe neurotoxicity is not fully understood, but excess free ammonia in the CNS may cause reversal of glutamate dehydrogenase, with glutamate formation – this depletes α-ketoglutarate, a key TCA cycle intermediate and hence depletes ATP. Since glutamate is an excitatory neurotransmitter, this may also account for the observed effects on neural function.

Metabolism of carbon skeleton

Following deamination, the remaining carbon skeleton enters the common metabolic pool (see Figure 1). All amino acids ultimately yield just seven products of intermediary metabolism: pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, acetyl-CoA and acetoacetyl-CoA. The first five of these represent ≥3 carbons, hence amino acids producing these metabolites can be used for glucose synthesis (by gluconeogenesis: 'glucogenic'): it is this property that confers on proteins the ability to act as a carbohydrate reserve. The acetyl-CoA and acetoacetyl-CoA, however, yield two (or two-equivalent) carbons, and amino acids which produce them cannot be used for gluconeogenesis – they can be directly oxidized in the TCA cycle, undergo lipogenesis or be used to synthesize ketone bodies ('ketogenic').

Inborn errors of metabolism of several amino acids exist. In alkaptonuria, the gene for the enzyme homogentisate 1,2-dioxygenase is functionally mutated. This enzyme is required for the metabolism of the carbon skeletons of phenylalanine and tyrosine to acetoacetyl-CoA. Lack of enzyme activity results in accumulation of the intermediate homogentisic acid; its metabolite alkapton gives the urine a black appearance when exposed to air. The inborn error of metabolism phenylketonuria is also due to a mutation in this same pathway, but the striking difference in disease severity lies in the different metabolites that accumulate when the pathway is blocked further upstream.

Inter-tissue amino acids flux

Liver is the site of both ureagenesis (amino-N metabolism) and gluconeogenesis (carbon skeleton metabolism), and removes most dietary amino acids from the portal circulation in the anabolic state, together with amino acids derived from proteolysis in extrahepatic tissues in catabolism. The muscle exports a large amount of its amino acids from proteolysis as alanine, derived from the transamination of multiple amino acids donating their amino group to glycolytically derived pyruvate. The alanine is transported to the liver, where it is transaminated to reform pyruvate, which undergoes gluconeogenesis to glucose (Figure 5). The glucose is re-exported back to muscle (the glucose–alanine cycle). Amino acids are also exported from muscle as glutamine, which contains two amino groups and is a major transporter of amino groups. The kidney utilizes glutamine, removing the side chain amino group with glutaminase, reforming glutamate and free ammonia. Ammonia is excreted directly in the urine as a urinary buffer (achieving buffering capacity in a waste product without losing substrate).

The three branched-chain amino acids (BCAA) (leucine, isoleucine and valine) make up approximately one-third of all amino acids in the body. Dietary BCAAs are not removed from the portal circulation by the liver, appearing in high concentration in the splanchnic blood, where they may also have a role as nutrient signals. They are metabolized in extrahepatic tissue,

especially muscle, where they are major sources of nitrogen to maintain pools of glutamine, glutamate and alanine. All three are transaminated by a single branched-chain aminotransferase, and the resulting branched-chain 2-oxoacids (α-ketoacids) undergo oxidative decarboxylation by a branched chain α-ketoacid dehydrogenase. Absence of this enzyme is responsible for maple syrup urine disease, whereby BCAAs are transaminated to their corresponding branched chain α-ketoacids, but absence of the dehydrogenase means these intermediates accumulate, appearing in the urine and giving it its characteristic maple syrup odour.

Regulation of amino acid metabolism

Insulin is the main anabolic signal for protein metabolism, stimulating protein synthesis and inhibiting proteolysis. Net protein synthesis is also stimulated by muscle training, growth factors, growth hormone and anabolic steroids. Protein breakdown is stimulated by cortisol and thyroid hormones. Amino acids act as nutrient signals in pancreatic β-cells, modulating insulin secretion.

Diabetes

Blood glucose and certain amino acids — arginine, leucine — are sensed by pancreatic β-cells: high levels indicate nutrient repletion, and this anabolic state is signalled to the rest of the body by insulin release. Hence, the pancreas detects glucose while the rest of the body then detects insulin. Insulin is the sole anabolic hormone, responsible for disposition of carbohydrates, lipids and amino acids; its actions are opposed by multiple catabolic signals. Consequently, insulin deficiency has severe and pleiotropic metabolic consequences. Inhibition of catabolic (substrate mobilizing) pathways by insulin is as critical as stimulating anabolic (substrate storage) pathways. In low-insulin states, less inhibition of catabolism, together with lack of anabolic stimulation, causes default to catabolism and net substrate mobilization. In starvation, falling blood glucose levels are detected by the pancreas, which responds with decreased insulin secretion. Peripheral tissues interpret falling insulin as indicating whole-body nutrient depletion and respond by becoming catabolic, decreasing substrate uptake and mobilizing energy reserves. The liver responds to low insulin as indicating hypoglycaemia, and increases glucose production by glycogenolysis and gluconeogenesis. The gluconeogenic substrate is alanine, derived from increased proteolysis and muscle wasting. Hence carbohydrate (glycogen) and amino acid (protein) reserves are consumed to increase substrate provision. Because insulin remains low the catabolic state continues and substrate mobilization prevails. In addition to increased hepatic glucose production, peripheral glucose utilization via glucose uptake, glycolysis and glycogenesis is decreased, in an attempt to spare glucose. Adipose tissue responds to decreased insulin by increasing lipolysis, leading to fatty acid and glycerol release, providing substrate for oxidation (FA) as well as limited gluconeogenesis (glycerol). Increased delivery of NEFA to liver leads to ketogenesis and ketonaemia, ketone bodies being an important glucose-sparing fuel for brain, which cannot utilize FAs directly. In type 1 diabetes mellitus, pancreatic β-cells are destroyed, so there is no longer any effective glucose sensing mechanism, nor means to signal the anabolic state. The lack of insulin is interpreted by the body as signalling starvation and the above catabolic state results. However, the patient is not starving (indeed is still eating) so dietary glucose also enters the circulation, resulting in extreme hyperglycaemia. The excess plasma glucose appears in urine (glycosuria) as the renal tubular reabsorption maximum is exceeded, and this carries water with it osmotically, resulting in polyuria (osmotic diuresis), dehydration, thirst and polydipsia. However, lipid metabolism is also involved as adipose tissue responds to low insulin by increasing TAG lipolysis, with excessive NEFA release into the circulation. The NEFA is utilized by muscle, further decreasing glucose utilization, and by liver for ketogenesis. The ketone bodies also inhibit glucose utilization, spill over into the urine (ketonuria), and being acidic cause a metabolic (keto-)acidosis. This is compensated by increased ventilation — Kussmaul breathing — and the volatile 3-carbon acetone produced from acetoacetate may be noticed by its characteristic smell on the breath.

Sepsis, trauma

Besides diabetes and tumour growth, the catabolic state can also be triggered by an excessive inflammatory reaction, characterized by the appearance of pro-inflammatory cytokines, such as TNFα, IL-1β and IL-6 in the blood stream, where their normally local paracrine effects are superseded by more general endocrine effects on metabolism. In sepsis this is a reflection of a generalized and excessive activation of the immune system secondary to pathogen invasion and host immune cell activation, resulting in an unregulated increase in mobilization and availability of all (carbohydrate, lipid, amino acid) substrate. In trauma, a comparable picture occurs. Cuthbertson described the phases of response to trauma as the 'ebb phase' (now usually termed shock: hours), the 'flow phase' (now generally referred to as the catabolic flow phase: days) and 'recovery' (anabolic flow phase: weeks), and we understand this as being the result of generalized neurohumoral activation, stimulating catabolic pathways and inhibiting substrate storage. Sepsis, trauma and burns are therefore associated with a generalized, potentially unregulated inflammation — the systemic inflammatory response syndrome (SIRS). Failure of the body to adequately counter the primary pathological insult and to regulate its response to it leads to multiorgan failure and/or critical illness myopathy/polyneuropathy, with substrate depletion and wastage. ◆

FURTHER READING

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