Human metabolism: metabolic pathways and clinical aspects

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Abstract

All mammalian energy is derived from food, in three chemical forms: carbohydrate, lipid, and protein, each providing a unique advantage in energy provision. 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 mobilization/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. Many common diseases have a metabolic aetiology, whilst others affect patient metabolism with significant clinical consequences. Understanding metabolism is key to the treatment of many common diseases, notably diabetes, as well as providing a rational basis for managing critically ill patients with sepsis and trauma, and 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). In animals this energy must be ultimately derived from food. The rate of energy production measured under basal conditions – 'basal metabolic rate' (BMR; at normothermia and without voluntary muscle contraction) – 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 the process of degrading substrates to mobilize biologically useable energy is termed catabolism. Imbalance of these pathways leads to cachexia or obesity. Tissues have specialized metabolic functions (e.g. adipose tissue stores energy substrate, muscle oxidizes substrate, lactating mammary gland exports substrate). The liver is a metabolic 'transformer' that regulates substrate supply between tissues, whilst pancreas detects and signals nutritional status.

Metabolic energy is carried in two main forms (both of which carrier molecules are nucleotides): (i) 'high energy' phosphate groups including ATP, GTP and creatine phosphate; and (ii) hydride (H^-) 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 (i.e. the degree to which the nucleotide energy carriers are phosphorylated or reduced (electron-rich)) are major regulators of metabolism.

Energy substrates

Energy is derived from three groups of energy-rich compounds: carbohydrates, lipids (fats) and proteins (amino acids) (Figure 1). Carbohydrates (hydrated carbon: $C(H_2O)_n$) are soluble and hence easy to transport and fast, 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 very insoluble and highly reduced, and hence energy-dense, therefore 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 workload/oxygen-challenged tissues such as myocardium and exercising skeletal muscle. (Measuring the Respiratory Quotient (RQ) – the ratio of CO_2 produced to O_2 consumed – permits assessment of whole body substrate metabolism: RQ=1 indicates carbohydrate oxidation whilst RQ=0.7 indicates lipid oxidation.) Also, lipids in the form of non-esterified ('free') 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). Hence energy (ATP) yield from oxidative

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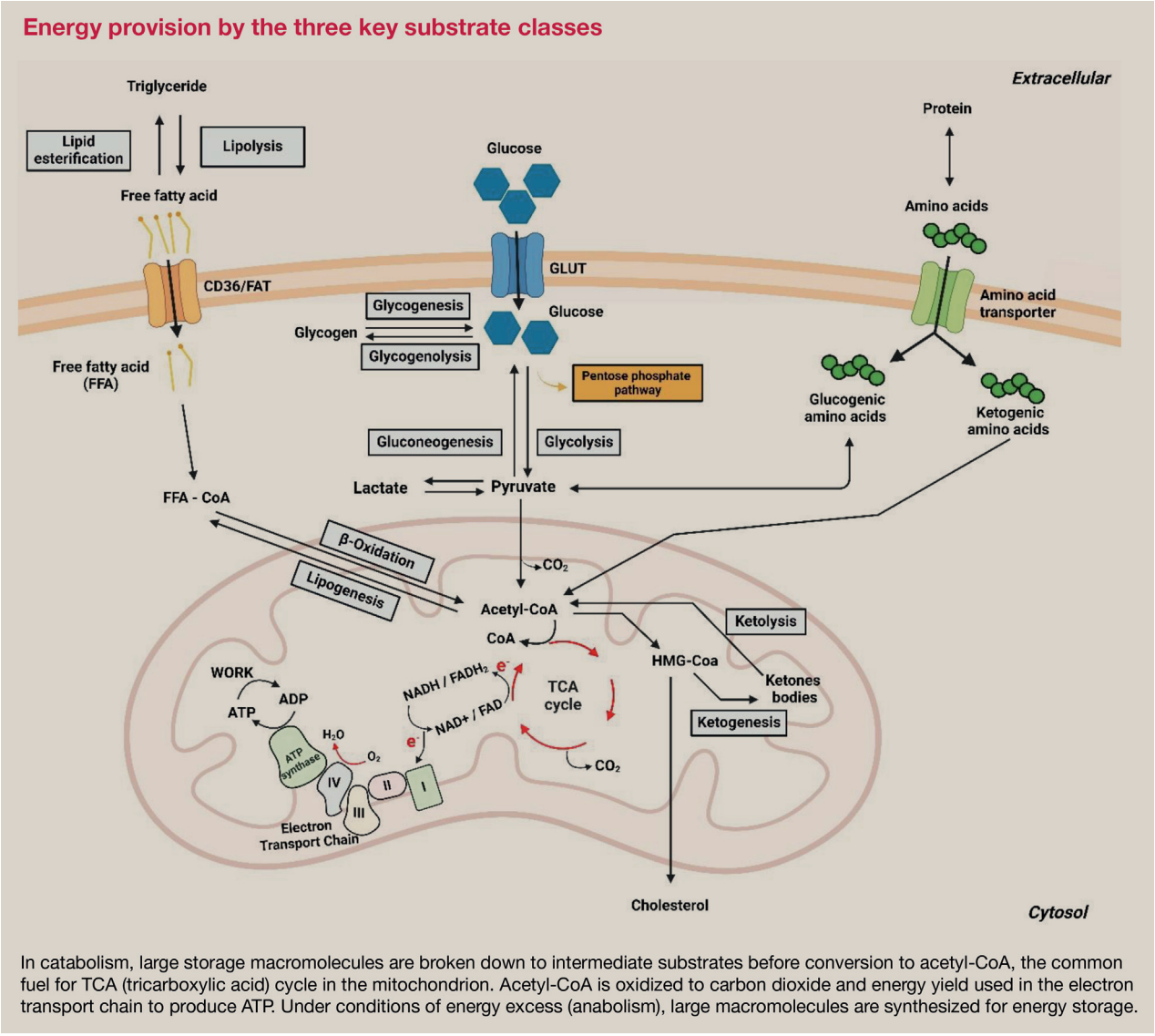


In catabolism, large storage macromolecules are broken down to intermediate substrates before conversion to acetyl-CoA, the common fuel for TCA (tricarboxylic acid) cycle in the mitochondrion. Acetyl-CoA is oxidized to carbon dioxide and energy yield used in the electron transport chain to produce ATP.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

Figure 1

metabolism (~32 ATP) is much greater than that from anaerobic metabolism (2 ATP).

The pancreas is the key organ detecting metabolic status. Pancreatic islet β-cells sense high blood glucose and release insulin in response. Pancreatic islet α-cells release glucagon in response to low blood glucose concentration. Carbohydrate and lipid utilization are reciprocally regulated (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: glucokinase); G6P is a central hub in carbohydrate metabolism and can be spilt in glycolysis or assembled 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, anaerobic, 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 available, 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 G6P from glycogen breakdown is dephosphorylated by glucose-6-phosphatase into free glucose, which is released into the bloodstream 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. The liver stores about 100 g of glycogen, enough to supply the body for only about 12–24

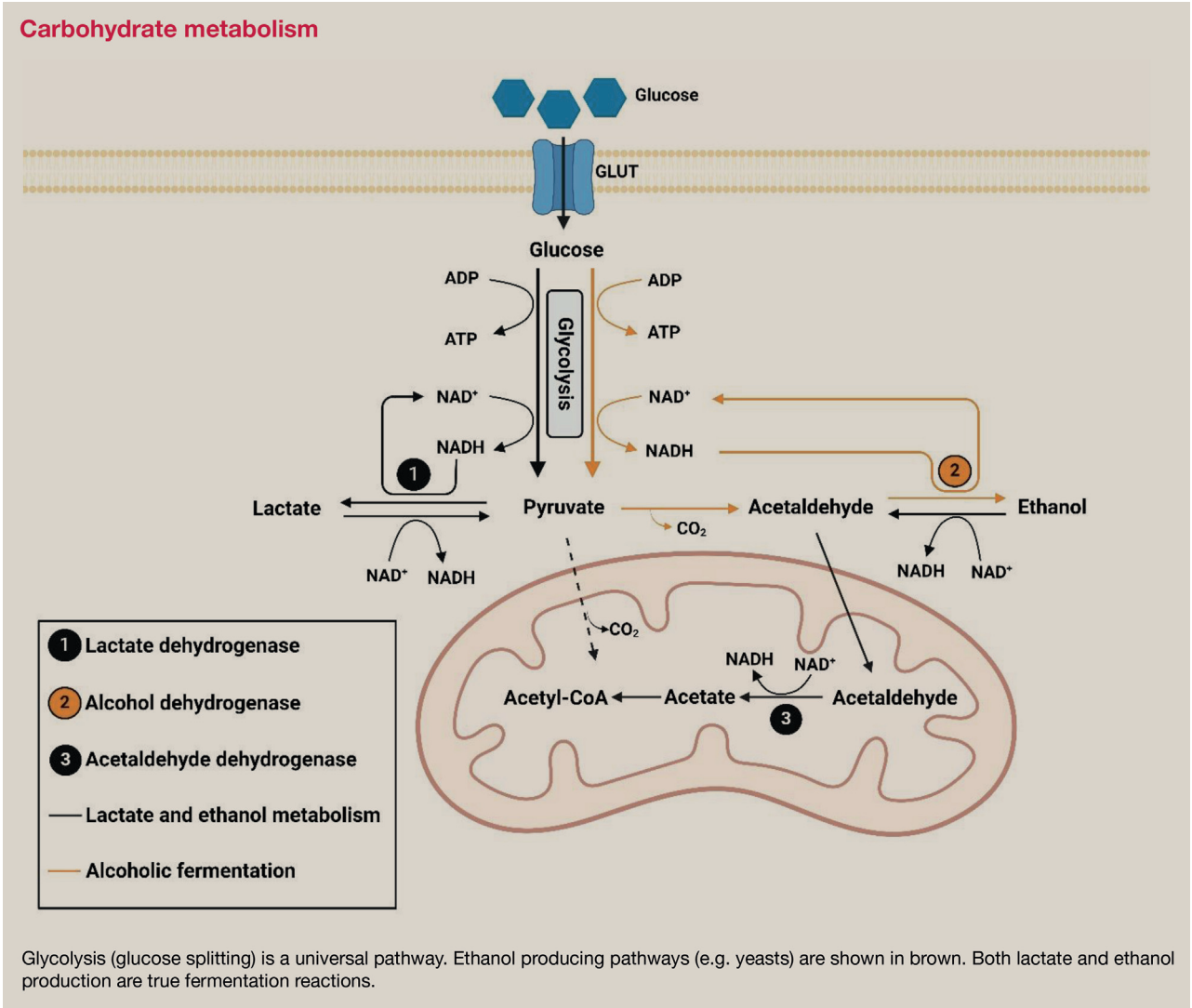
hours, whereas skeletal muscle stores about 350 g of glycogen, sufficient for about 70 minutes of muscle contraction.

Glycolysis

Glucose is cleaved into pyruvate by glycolysis in the cytosol of all cells (Figure 2), and this generates some energy without the need for 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 (the common fuel for oxidation in the mitochondrion; 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 glucose synthesis from non-carbohydrate sources and is typically active in catabolic states (e.g. post-prandial/starvation, exercise), occurring mainly in the liver (consistent with its role in maintaining blood glucose levels) and with some limited activity in the kidney. It allows the body to make new glucose when dietary carbohydrate sources are limited (i.e. catabolic states), 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, and this helps prevent substrate cycling whereby the two opposing pathways are active simultaneously. 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), 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



Glycolysis (glucose splitting) is a universal pathway. Ethanol producing pathways (e.g. yeasts) are shown in brown. Both lactate and ethanol production are true fermentation reactions.

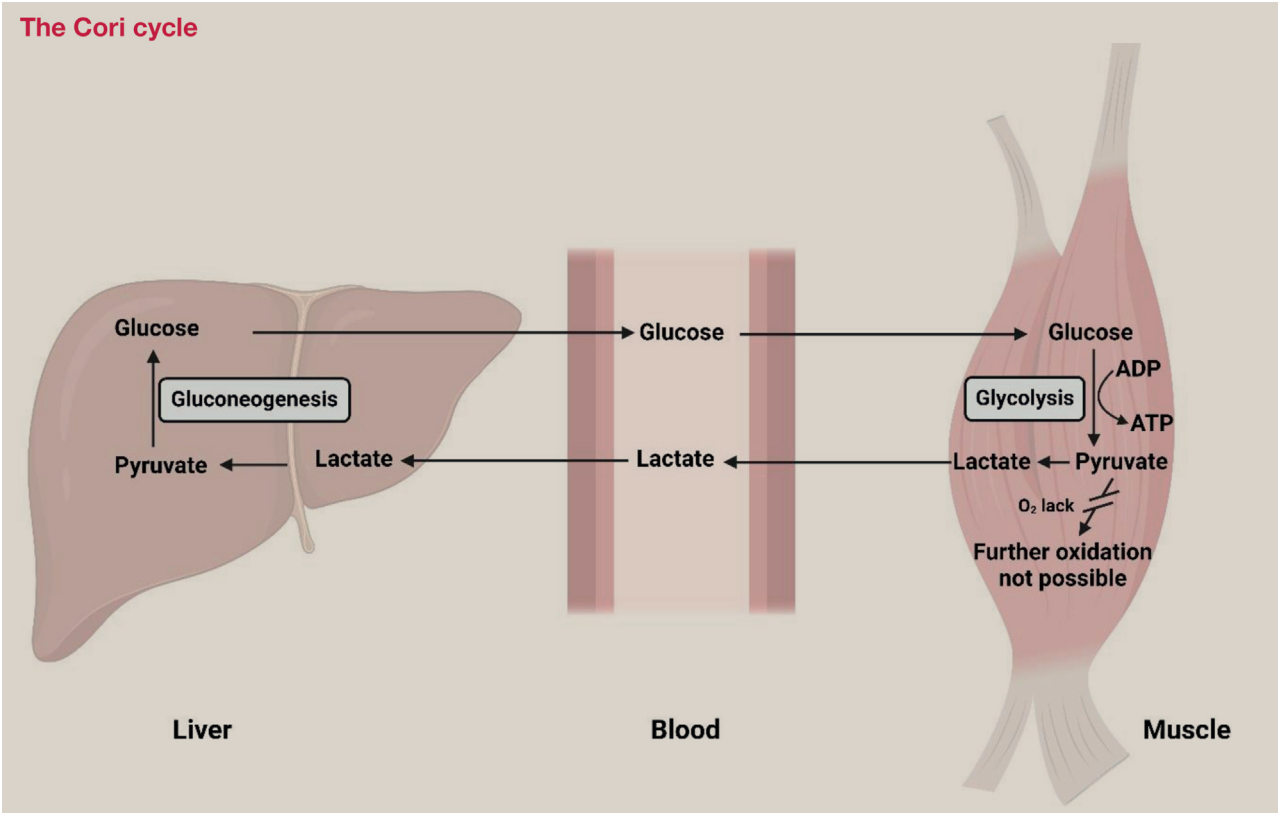
Figure 2

function of the liver, hepatic 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 further 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 (2 ATP) 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 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') and interleukins (e.g. IL-1β); oncometabolites) are likely involved in the (inefficient) 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 CO_2, the characteristic gaseous product of brewing), which is then reduced to ethanol by alcohol dehydrogenase, linked to the 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



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

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 (and hence hunger sensation). 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 hence 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 (GSH) activity within 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 for carbohydrates, 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 (FFA) are more correctly termed non-esterified fatty acids (NEFA)). Esterification of three fatty acids to glycerol yields the very hydrophobic triacylglycerol (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 depending on BMI), 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 (e.g. muscle, heart) for ATP production. Other tissues store TAG in lipid droplets as an intracellular energy resource for their own utilization. Recent evidence suggests that excessive cellular 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 triacylglycerols, or migrate into 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 of adipose tissue-derived TAG-FA 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 alternatively 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

In anabolic (fed) states 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 the two opposing pathways. Three FAs are then esterified to glycerol to form TAG. TAG synthesized in the liver must be exported to adipose tissue for storage.

Water-insoluble 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 Fredrickson 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 (LDL) 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 mature 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 atheroma.

Regulation of lipid metabolism

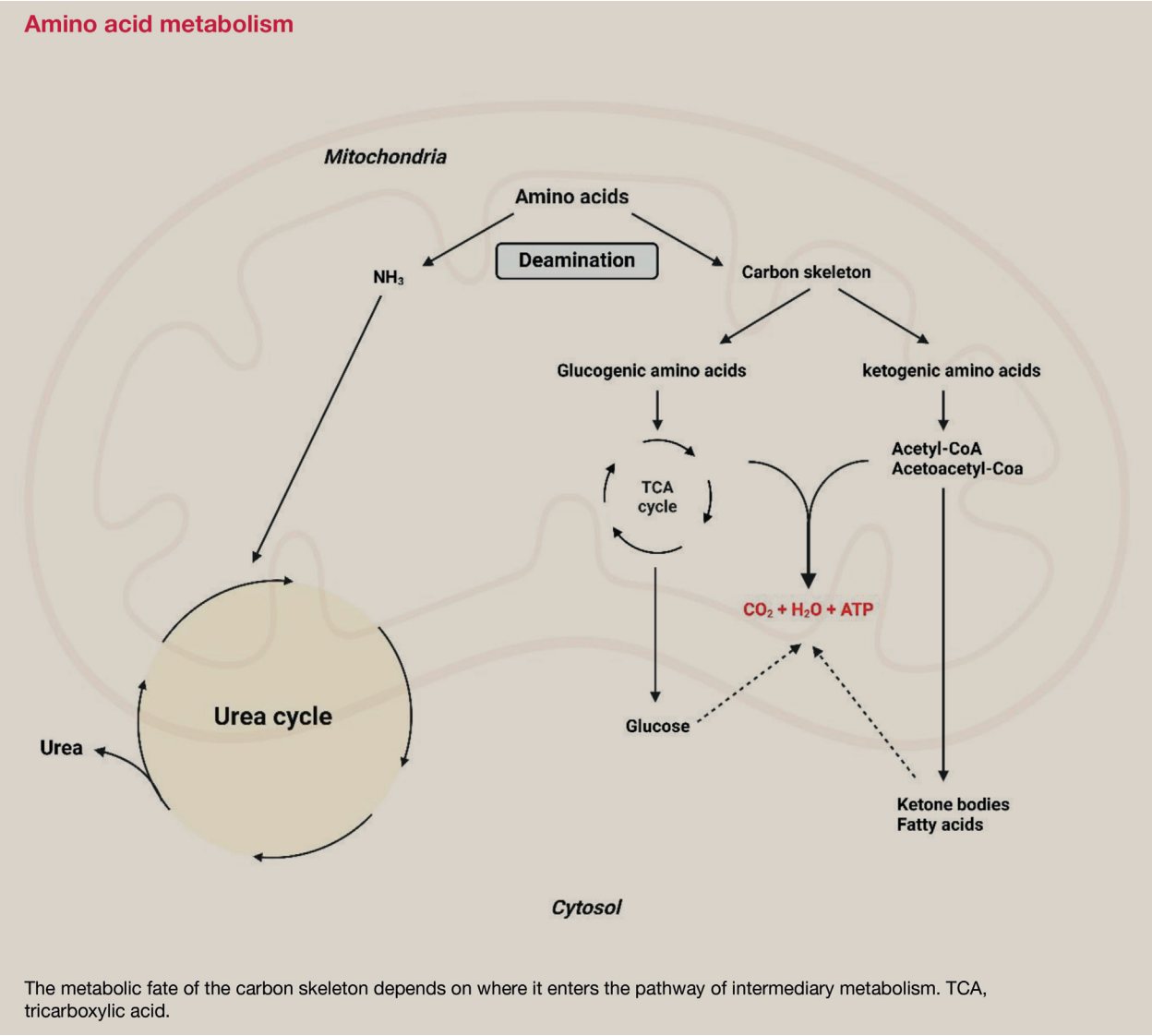
The anabolic state is signalled by insulin: hepatic lipogenesis, TAG and cholesterol synthesis are stimulated, while ketogenesis is inhibited. In adipose tissue, insulin stimulates LPL, enhancing plasma TAG uptake from TGRLP (CM and VLDL), and suppresses TAG lipolysis, thereby lowering plasma NEFA concentrations. 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 energy 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. ('semi-' or 'conditionally-' essential amino acids can be synthesized in limited amounts but during e.g. growth spurts must be supplemented 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 either be 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 oxoacid), 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



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

Figure 4

them to plasma, but they are commonly used to diagnose hepatocellular damage ('liver enzymes'). The second type of deamination reaction is oxidative deamination: following transamination in the 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 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 most common 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 amino acid deamination, the remaining carbon skeleton enters the common metabolic pool (see Figure 1). All amino acid skeletons 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 (and hence amino acids producing them) 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

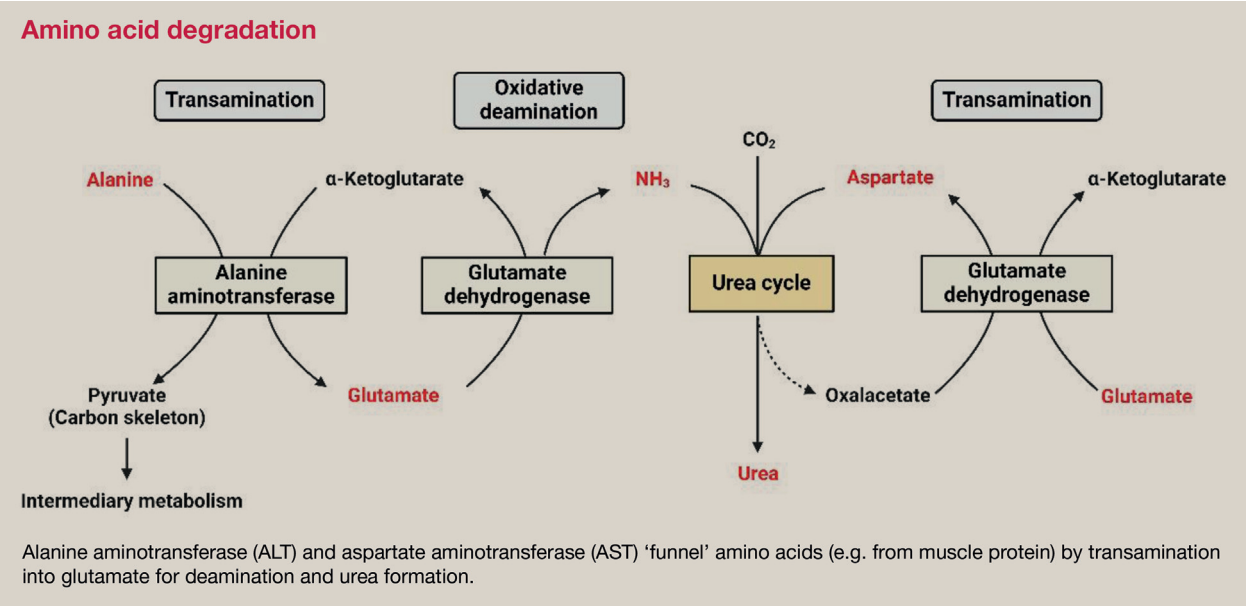


Figure 5

lipogenesis or be used to synthesize ketone bodies ('ketogenic') but cannot synthesize carbohydrate.

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 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

The 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. In starvation, muscle exports a large amount of its amino acids from proteolysis of contractile proteins 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 energetic 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 nutritional state by glucose, while the rest of the body then detects nutritional state by proxy through insulin. Insulin is the sole anabolic hormone, hence responsible for disposition of carbohydrates, lipids and amino acids; its actions are opposed by multiple catabolic signals (neural and humoral). Consequently, insulin deficiency has severe and pleiotropic metabolic consequences; the body defaults to a catabolic (substrate-mobilizing) state if it does not detect insulin (either through lack of the hormone, or resistance to its signalling

mechanism). Inhibition of catabolic 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, results in catabolism and net substrate mobilization. Hence starvation and type 1 (insulin-deficient) diabetes have a similar metabolic profile. In starvation, the pancreas detects falling blood glucose levels, and 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 interprets low insulin as indicating hypoglycaemia, and responds by increasing glucose production and export into the blood by glycogenolysis and gluconeogenesis. The gluconeogenic substrate is alanine, derived from increased proteolysis and muscle wasting. Hence both 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 uptake and utilization via glycolysis and glycogenesis are decreased, in an attempt to spare glucose (for the brain, which is not insulin-sensitive). 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 (FFA) to the liver leads to ketogenesis and ketonaemia, ketone bodies being an important glucose-sparing fuel for the brain, which cannot utilize FAs directly; hence ketonaemia is a characteristic feature of the catabolic (low insulin) state. In type 1 diabetes mellitus, pancreatic β-cells are destroyed, so there is no longer any effective glucose (nutrient) sensing mechanism, nor means to signal the anabolic state. The lack of insulin is interpreted by the body as signalling starvation and the catabolic state results. However, the patient is not starving (indeed is still eating), so dietary glucose continues to enter 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 (FFA) is utilized by muscle, further decreasing glucose utilization (by the Randle cycle), 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 acidosis is compensated by increased ventilation in order to lower PCO_2 — Kussmaul breathing — and the volatile 3-carbon acetone produced from acetoacetate may be noticed by its characteristic ('pear drops') smell on the breath.

Unlike type 1 (insulin-deficient) diabetes, type 2 diabetes is caused by a failure of insulin signalling — 'insulin resistance' — by peripheral tissues. Since insulin resistance leads to hyperglycaemia (by the mechanisms mentioned above), the condition may termed 'glucose intolerance'. The condition is not as aggressive and overt as acute insulin deficiency, but the long-term consequences of chronically altered (catabolic) metabolism are grave, with characteristic atherosclerosis and micro-and macrovasculopathy. The aetiology of insulin resistance remains uncertain, but theories have highlighted alterations in the insulin receptor, and its complex downstream second messenger signalling cascade. In contrast to type 1 diabetes, which is associated with severe weight loss/cachexia due to uncontrolled substrate mobilization, type 2 diabetes is, confusingly, often (though not invariably) associated with obesity. This may suggest an anabolic state, in which net TAG synthesis and storage in adipose tissue occurs, but the situation is not this simple. It is likely that different adipose tissue depots have differing metabolic phenotypes, with visceral adipose tissue being metabolically active, and releasing some factor(s) into the circulation which induces insulin resistance in other tissues, including gluteo-femoral adipose depots, muscle and pancreas itself. Factors hypothesized to be involved include fatty acids and cytokines. If fatty acids (or some unidentified lipid product of them) are indeed the cause of insulin resistance, this would explain the efficacy of dieting and exercise on insulin resistance, since both interventions will be expected to decrease FA levels and improve insulin sensitivity.

Cancer

Malignant tumour growth is associated with profound changes in metabolism, typically catabolic, leading to loss of muscle and adipose mass — cancer cachexia. The cancer itself is regarded as a 'parasite', placing metabolic requirements on the patient 'host', which are two-fold — energy provision and biosynthetic substrate provision, and are regardless of energetic efficiency. Again the mechanism is not fully understood but likely involves some metabolic signal (an oncometabolite or a pro-inflammatory cytokine) exported by the tumour, together with autocrine effectors (e.g. myostatin) leading to host substrate release, increased thermogenesis (from brown adipose tissue, adipocytes of which have mitochondria with uncoupled oxidative phosphorylation, leading to heat production instead of ADP phosphorylation). This reprogramming of the patient's metabolism is accompanied by a characteristic metabolic profile of the tumour cell, in which host glucose and glutamine are utilized, the former mainly for energy via anaerobic glycolysis, with the latter being utilized (by an essentially re-programmed TCA cycle) for production of biosynthetic intermediates including nucleotides and other amino acids.

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 bloodstream, 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) substrates. In trauma, a comparable picture occurs. Additionally, activation of the sympathetic nervous system (accompanied by release of adrenal catecholamines) also triggers catabolism. 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). Accompanying insulin resistance prevents cellular substrate uptake even with intravenous nutrition. 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.

Atheroma

The pathogenesis of atheroma/atherosclerosis involves accumulation of both cholesterol and triacylglycerols in the vascular endothelium. (Although cholesterol is not strictly a 'metabolic molecule' as it is not involved in energy provision, it is included in this context because: (1) it is synthesized endogenously from energetic precursors: glucose and fatty acids, via acetyl-CoA; (2) it is co-transported with energetic lipids (TAG) in lipoprotein particles.) Excessive plasma cholesterol (in the form of LDL) and TAG (in the form of VLDL and CM) are both associated with lipid accumulation in endothelium, and this effect is seen in association with metabolic diseases such as diabetes and hyperlipidaemias. Other conditions associated with increased risk of atheroma include obesity, and this may be related to intravascular inflammation. Measures to decrease LDL-cholesterol and plasma TAG (by statins or drugs such as fibrates to limit intestinal absorption of lipids) decrease the severity of the process; however, no drug has been found to date which increases HDL and hence augments reverse cholesterol transport to decrease plasma cholesterol levels. ◆

FURTHER READING

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Practice points

- Metabolism refers to the series of reactions involved with energy provision in the body, and is required for muscle contraction, maintenance of ionic gradients, and heat production
- Energy is derived from food, in the form of three components: carbohydrates, fats (lipids) and proteins. These three energy groups have differing advantages and disadvantages, both energetically and biologically, hence all are used to ensure maximum metabolic efficiency
- The body tends to default into the catabolic (substrate-mobilizing) state, unless the hormone insulin is present to signal the anabolic (energy-storing) state
- Several important diseases have a metabolic basis: these include diabetes, atherogenesis, obesity, and inborn errors of metabolism
- Several important pathologies have a clinically important effect on metabolism: these include cancer, sepsis, trauma, and some endocrine diseases such as hyperthyroidism