11.1 Nutrition Macronutrient metabolism 1839

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ESSENTIALS Food intake is sporadic and, in many cultures, occurs in three daily boluses. At the same time, energy expenditure is continuous and can vary to a large extent independently of the pattern of energy intake, although fixed or predictable demands (e.g. through occupation) means that in most persons food intake and energy expenditure are soon balanced. The body has developed complex systems that direct excess nutrients into storage pools; as they are needed, they also regu- late the mobilization of nutrients from these pools. Analogous to the fuel tank of a car and the throttle that regulates fuel oxida- tion, supply and need are closely linked, except that in the vehicle there is just one fuel and just one engine. In contrast, in humans there are three major nutrients and a variety of tissues and organs, each of which may have its own preferences for fuels that vary with time. Carbohydrate, lipid, and protein (the latter a source of amino acids) are the three types of energy supply that are stored variably and assimilated from food each day. That we can carry on our daily lives without thinking about whether to store or mo- bilize fuels, and which to use, attests to the remarkable efficiency and refinement of these systems of metabolic control. Overview of metabolism The body requires energy for chemical and mechanical work in order to maintain homeostasis; functions including maintenance of ionic gradients, transport, biosynthesis, heat generation, and loco- motion. This energy is derived from three groups of energy-rich substrates: carbohydrates, lipids, and amino acids. Multiple groups are utilized because they all have chemical and thermodynamic ad- vantages and disadvantages, and together they provide energy under widely varying conditions and demands. All three nutrient groups exist in large, energy-rich macromolecular storage forms. The prin- cipal macronutrient stores are listed in Table 11.1.1 and are related to daily fluxes of energy substrates in the body. For energy mobilization these are sequentially broken down into less energy-rich metabolites, the energy liberated being captured by intermediary reduction-oxidation molecules which carry the energy to a common pathway of oxidation linked to the phosphorylation of ADP to ATP. Hence, the energy is used to synthesize ATP, the common energy carrier to which most energy-requiring biological processes are linked. At a whole-body level, this process is termed ‘catabolism’. Conversely, in energy-rich states when energy intake exceeds expenditure, these metabolic pathways can be reversed, whereby ingested nutrients from all three groups are assembled into large storage

macromolecules (‘anabolism’) (Fig. 11.1.1). Lipids (fats) are the most energy-dense metabolic fuels (c.37 kJ/g). The storage form of lipids for energy provision is triacylglycerol (TAG), which comprises three fatty acids esterified to a glycerol backbone. Being highly hydrophobic and reduced, TAGs are very energy dense and a highly efficient energy store. However, TAGs are relatively slow to mobilize, must be oxidized to yield energy and cannot provide energy anaerobically, and the nonesterified fatty acids (NEFAs) from which they are assembled are amphipathic (detergent-like) and hence potentially toxic in high concentra- tions. Furthermore, fatty acids cannot be converted into carbohy- drates or proteins, limiting their metabolic flexibility. Carbohydrates such as glucose are less reduced and more soluble than lipids and hence contain only about half the energy density of fats (c.17 kJ/g), but they are nontoxic, quickly mobilized/utilized, and can provide some energy anaerobically. They are stored as the glucose polymer glycogen. Glycogen, as a polar molecule, is stored with water (about three times its own weight), so its low energy density makes this an inefficient energy store, and only limited amounts are synthesized (Table 11.1.1). Carbohydrates are able to supply intermediary metabolites to maintain pathway integrity (anaplerosis) in contrast to lipids, oxi- dation of which leads to depletion of intermediary metabolites (cataplerosis): hence some carbohydrate is always required for me- tabolism to proceed efficiently (captured in the old aphorism ‘fat burns in the fire of carbohydrate’). Proteins (polymers of amino acids) have similar energy content to carbohydrates (c.17 kJ/g), but each protein has a specific function and they are not used as dedicated energy stores. In catabolic states of carbohydrate deple- tion (e.g. starvation), however, proteins are broken down to their constituent amino acids for conversion into glucose for energy and anaplerosis—hence proteins constitute a virtual carbohydrate store. 11.1 Nutrition: Macronutrient metabolism Keith N. Frayn and Rhys D. Evans

SECTION 11 Nutrition 1840 Whole body metabolic strategy therefore comprises breaking down large macronutrient storage molecules (TAGs, glycogen, protein—by lipolysis, glycogenolysis and proteolysis respectively) into smaller energy-rich substrates (NEFAs, glucose, amino acids) with distinct characteristics and roles. In the next stage of metab- olism these small substrates are converted into a common fuel, acetyl-CoA (by β-oxidation, glycolysis, and amino acid metabolism, respectively). In the final stage of metabolism the acetyl-CoA is fully oxidized by the tricarboxylic acid (TCA) cycle into carbon dioxide within the mitochondria. The step-wise release of energy from these pathways is carried as a hydride (H-) ion by NAD+ and FAD as their reduced forms, NADH and FADH2: these redox carriers are then reoxidized by the electron transport chain, the energy derived being used to phosphorylate ADP to ATP (oxidative phosphorylation). By contrast, in anabolism these pathways are reversed, chemical energy being used to synthesize complex energy-rich storage macromol- ecules from simple precursor substrates. Three key features of metabolism impact metabolic strategy and energy provision: • Most energy stored in the body is in the form of lipid (TAGs); • This lipid cannot be converted to carbohydrate; and • All tissues require some glucose for normal metabolic func- tioning, and some tissues (glycolytic, lacking mitochondria, such as erythrocytes) have an absolute requirement for glucose, or cannot utilize NEFAs (brain). Since very little carbohydrate is stored (c.100 g hepatic glycogen; <1 day if sole fuel), in catabolic states glucose is rapidly depleted and alternative mechanisms are required to provide or replace glu- cose: under these conditions, breakdown of protein to amino acids, and then conversion of these to glucose by gluconeogenesis, becomes an essential pathway. Indeed, the ability of the body to divert protein from its primary (e.g. contractile) function to a secondary function of glucose provision has been the adaptation that has allowed such limited stores of the energy density-inefficient glycogen to be per- mitted. Another mechanism is ketogenesis, whereby

the liver con- verts TAG-derived NEFAs into small, soluble (nonamphipathic) ketone bodies, which can be utilized by many tissues, including brain, hence acting as a ‘glucose-sparing’ substrate. Hence during conditions of energy repletion, energy in ex- cess of current requirements is stored in a tissue-specific manner (lipid as TAGs principally in adipose tissue; carbohydrate as glycogen in most tissues but specifically in liver for glucose re- lease to maintain blood glucose concentration; amino acids ‘vir- tually’ in labile proteins, e.g. muscle). In subsequent periods of limited energy ingestion (postabsorptive, fasted) this substrate resource can be mobilized in a regulated fashion and directed to specific tissues according to their metabolic requirement. These pathways are illustrated schematically in Fig. 11.1.2. Table 11.1.1 The principal macronutrient stores Macronutrient Total amount in body Energy equivalent (MJ) Days’ supply if the only energy source Daily intake (g) Daily intake as percentage of store Carbohydrate 0.6 kg 8.5 <1 300 60 Free glucose 12 g Liver glycogen 100 g Muscle glycogen 500 g Fat (triacylglycerol) 12–18 kg 550 56 100 0.7 Circulating in plasma 5 g Stored in adipocytes 12–18 kg Protein and amino acids 12 kg 200 (20) 100 0.8 Free amino acids 100 g Protein 12 kg Note: These are very much typical rounded figures. Days’ supply is the length of time for which this store would last if it were the only fuel for oxidation at an energy expenditure of 10 MJ/day: the figure for protein is given in parentheses since protein does not fulfil the role of an energy store in this way. Energy-rich substrates: • Carbohydrates • Lipids • Proteins Complex molecules: • Polysaccharides • Lipids • Nucleic acids • Proteins Energy-poor end-products: • CO2 • H2O • NH3 Precursor molecules: • Amino acids • Hexoses • Fatty acids ANABOLISM CATABOLISM chemical energy • ATP • NAD(P)H Fig. 11.1.1 Anabolism and catabolism. Anabolism refers to the synthesis of complex molecules from simpler ones, and in the context of metabolism refers to energy storage within carbohydrate, lipid and protein macromolecules; catabolism refers to the breakdown of molecules to smaller ones which contain less energy; the energy difference is released and used for biological work (including providing the energy for anabolism). Biological energy is carried in the form of ‘high energy’ phosphate groups (e.g. ATP) and as electrons (hydride ions, e.g. NADH).

11.1 Nutrition: Macronutrient metabolism 1841 Carbohydrate metabolism in the postabsorptive and postprandial states Pathways of glucose metabolism Glucose is a ubiquitous sugar which may be derived from dietary carbohydrate or synthesized in the body. As noted here already, it is stored in polymeric form as glycogen: this removes the osmotic problems that would arise if it were stored in cells as free sugars. Glucose, as a polar molecule, cannot cross membranes composed of phospholipid bilayers by diffusion, and a family of glucose trans- porter proteins, expressed in a tissue-specific manner, facilitates its movement in and out of cells. Within cells, the first step of glu- cose metabolism is always phosphorylation to glucose 6-phosphate, brought about by a member of a family of enzymes (‘hexokinases’— the form expressed in liver and pancreatic β-cells is generally known as glucokinase) again expressed in a tissue-specific manner. Phosphorylation ensures that the molecule does not diffuse again out of the cell. Glucose (molecular formula $C_6H_{12}O_6$) is broken down by the pathway of glycolysis to pyruvate ($C_3H_4O_3$, showing that H has been lost relative to C and O; i.e. this is a partial oxidation). A small amount of ATP is generated by so-called substrate-level phosphor- ylation, as opposed to the oxidative phosphorylation pathway men- tioned earlier: indeed, this is a cytosolic pathway and can occur even in cells that lack mitochondria. Pyruvate, as described next, may be reduced to lactate ($C_3H_6O_3$, so exactly half a glucose molecule with no redox changes). Pyruvate can also enter mitochondria where it can be a substrate for the enzyme complex known as pyruvate de- hydrogenase (pyruvate dehydrogenase complex, PDC). PDC not only further oxidizes pyruvate, but also removes one carbon, re- sulting in

the formation of acetyl-CoA which, as described earlier, can be fully oxidized in the TCA cycle. The pathway of glucose synthesis, gluconeogenesis, occurs primarily in liver cells and is basically a reversal of glycolysis al- though with some specific steps, circumventing energy-yielding and largely irreversible steps of glycolysis. The major substrate for gluconeogenesis under most conditions is lactate. Amino acids whose carbon skeletons can be converted to lactate or pyruvate (discussed later) can also contribute, as can the glycerol released from lipolysis of TAGs in adipose tissue. Note that glucose may be broken down to lactate in red blood cells, for instance: the lactate may be transferred via the bloodstream to the liver where it is used glucogenic amino acids (≥3C) ketogenic amino acids (≡2C) glucose (6C) pyruvate (3C) GLYCOGEN acetyl-CoA(2C) 2CO2 nonesterified fatty acids (x x 2C) TRIACYLGLYCEROL 5C-sugars NADPH ketone bodies PDH e- O2 ADP ATP H2O PROTEIN CoA TCA cycle NADH NAD+ e- CO2 WORK lactate 1 2 3 4 5 6 7 8 9 10
Fig. 11.1.2 Stages of metabolism. Flux in a downward direction is catabolic (energy-yielding) while upward flux denotes energy storage, hence is anabolic. In the first stage of energy mobilization, large storage macromolecules are broken down to smaller, energy-rich, substrates (NEFAs, glucose, amino acids). In the second stage, these small energy-rich substrates are converted into the common ‘fuel’ for oxidation, acetyl-CoA. While these pathways can be reversed in order to store excess energy (anabolism), in the case of carbohydrates the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH) is essentially irreversible, therefore lipids (which are assemblies of two carbon groups themselves derived from acetyl-CoA) cannot be converted into pyruvate, and hence carbohydrates. In the third stage of energy mobilization, the acetyl-CoA is fully oxidized by the tricarboxylic acid (TCA) cycle; the energy released is carried by electron transporters down the electron transport chain and converted into ATP by oxidative phosphorylation. 1. esterification 2. lipolysis 3. glycogenesis 4. glycogenolysis 5. protein synthesis/ proteolysis 6. lipogenesis 7. β-oxidation 8. gluconeogenesis 9. glycolysis 10. pentose phosphate pathway.

SECTION 11 Nutrition 1842 to make new glucose. This cycle is sometimes called the Cori cycle. It does not result in irreversible loss of glucose from the body. That occurs after the action of PDC, as acetyl-CoA can no longer be re- converted to glucose. Glycogen synthesis starts with glucose 6-phosphate and involves sequential polymerization of glucose units on a protein backbone (glycogenin). Glycogenolysis involves the reverse: sequential re- moval of glucose units. In most tissues this will result in glucose 6-phosphate that can enter the pathway of glycolysis. In the liver specifically (and to some extent in kidney, especially during star- vation) the enzyme glucose 6-phosphatase may convert glucose 6- phosphate to glucose: thus, glucose released from glycogenolysis or produced by gluconeogenesis may be released into the bloodstream to maintain blood glucose concentrations in the postabsorptive or the fasting state. Breakdown of glucose as far as acetyl-CoA can also be part of a synthetic process. Acetyl-CoA produced from glucose is the starting point for the pathways of lipid synthesis: lipogenesis, which usu- ally refers to the synthesis of fatty acids from glucose, and choles- terol synthesis. These pathways, like most biosynthetic pathways, are cytosolic, and the acetyl-CoA must be transferred out of the mitochondria. One further pathway of glucose metabolism will be mentioned briefly: the pentose phosphate pathway. This involves the metab- olism of glucose 6-phosphate through a complex series of reactions that generate pentose sugars, used in nucleic acid synthesis, and also reducing power in the form of NADPH. NADPH is used in many biosynthetic pathways such as lipogenesis. Lactate and ethanol metabolism Glycolysis requires an electron acceptor, and NAD+ acts in this role, becoming reduced to NADH; however, the NAD+ must be replenished for glycolysis to continue. In the

aerobic state this is achieved by oxidizing NADH back to NAD+ in the electron trans- port chain, with useful energy yield. However, in the absence of oxygen the electron transport chain is inhibited and the NADH is reoxidized back to NAD+ by linking this to the reduction of pyru- vate to lactate, as just described, by lactate dehydrogenase (LDH). Hence lactate (instead of pyruvate) accumulates but NADH does not, and NAD+ is replenished; glycolysis proceeds, providing limited ATP production by substrate-level phosphorylation (anaerobic metabolism) (Fig. 11.1.3). This is a true fermentation pathway: 'homolactic fermentation'. Lactate is produced by tissues lacking mitochondria (and hence electron transport chains, e.g. red blood cells) and physiologically by oxidative tissues in the presence of oxygen, where it likely rep- resents a redox buffering mechanism, but excessive lactate produc- tion is associated with the hypoxic (and ischaemic) state. However, the lactate contains considerable energy (more than pyruvate as it is further reduced), and when oxygen availability is restored and oxi- dative metabolism is again possible, it makes an excellent substrate, being converted back to pyruvate (LDH being near-equilibrium) for subsequent oxidation via the TCA cycle (e.g. in myocardium). Furthermore, lactate is a principal substrate for gluconeogenesis as discussed above. This Cori cycle is constitutive (lactate being con- stantly produce by glycolytic tissues such as erythrocytes). However, since the concentration of lactate in the blood is dependent not only glucose pyruvate lactate acetaldehyde ethanol acetate acetyl-CoA ATP ADP NAD+ NADH NAD+ NADH ATP ADP CO2 NADH NAD+ NAD+ CO2 glycolysis 1 2 3 4 Fig. 11.1.3 Fermentation reactions. Glucose is split to pyruvate by glycolysis, with small amounts of ATP formed. In the absence of oxygen or a functioning TCA cycle, NAD+ must be regenerated to allow glycolysis to continue. In humans (black lines) this is achieved by lactate production; the lactate can be used to resynthesize glucose by gluconeogenesis or oxidized when oxygen becomes available. In yeasts (orange lines), NAD+ regeneration is achieved by ethanol formation. Metabolism of ethanol by humans alters the NAD+:NADH ratio with significant effects on metabolism. Pyruvate dehydrogenase (red) common to both. 1. pyruvate dehydrogenase 2. lactate dehydrogenase 3. alcohol dehydrogenase 4. acetaldehyde dehydrogenase.

 on peripheral tissue production (e.g. hypoxic/ischaemic muscle) but also on hepatic disposal (uptake and gluconeogenesis), hep- atic blood flow and liver function must both be considered when interpreting hyperlactataemic states. Certain organisms, such as yeasts, have an alternative strategy to regenerate NAD+ for glycolysis—alcoholic fermentation. Here, the pyruvate is decarboxylated to acetaldehyde with carbon dioxide evolved, and acetaldehyde is then reduced to ethanol; the alcohol dehydrogenase enzyme responsible is linked to the oxidation of NADH, regenerating NAD+ and glycolysis continues. The ethanol accumulates and inhibits competing microorganisms. When ethanol is ingested by humans, its metabolism has multiple effects as a result of this link to the NAD+:NADH ratio (redox potential). Following absorption, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase, and acetaldehyde is further oxidized to acetate by aldehyde dehydrogenase; both these enzymes are linked to NAD+ and generate 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 the oxidation of lactate to pyruvate, limiting the availability of lactate as a precursor for gluconeogenesis (and causing a mild metabolic lactic acid- osis). In addition, the NAD-dependent malate shuttle is inhibited, limiting availability of alanine also as a gluconeogenic substrate. The result is decreased gluconeogenesis and potentially hypoglycaemia. Furthermore, the TCA cycle and fatty acid β-oxidation are inhibited, while lipogenesis is increased (increased acetyl-CoA), leading to hepatic lipid (TAG) accumulation and alcoholic fatty liver. Glucose

metabolism in the postabsorptive state (overnight-fasted) In the overnight-fasted (postabsorptive) state, no glucose enters the plasma from the small intestine. Glucose enters and leaves the plasma at about 2 mg/kg body weight per min (200 g/24 h). About one-half of this will be consumed by the brain. Of the remainder, a considerable proportion will be utilized by blood cells and periph- eral tissues by anaerobic glycolysis, thus returning lactate to the liver for reconversion to glucose (Fig. 11.1.4). This is the Cori cycle. Glucose is produced by hepatocytes from glycogen breakdown and from gluconeogenesis. Net glycogen breakdown is stimulated by the relatively low insulin/glucagon ratio after overnight fasting. The major substrates for gluconeogenesis are pyruvate, derived from lactate (released from blood cells and peripheral tissues) and alanine (from proteolysis), and glycerol (from lipolysis). The pathway of gluconeogenesis predominates over that of glycolysis, again because of the relatively low insulin/glucagon ratio. Glucose metabolism after a meal When a meal enters the system (the postprandial state), this pattern of metabolism changes rapidly. About 12 g of free glucose are present in the circulation and extravascular space. Typically, a single meal Brain Insulin Insulin Glycogen Muscle Glycogen + + + + – – Insulin Insulin Insulin Insulin Insulin Insulin Liver Adipose tissue + Glucose Small intestine Glucose + G6P Pyruvate, Lactate Pyruvate, Lactate Lactate CO_2 CO_2 CO_2 GLUT4 GLUT4 GLUT2 GLUT2 GLUT2 GLUT3 G6P Pancreas GLUT2 Glucose Fig. 11.1.4 Overview of carbohydrate metabolism. Pathways in the liver shown as regulated by insulin are probably controlled by the insulin/glucagon ratio (high in the fed state, low in fasting). In muscle, contraction is an important stimulus for glycogen breakdown and glycolysis. Adrenaline also contributes. Not shown is the significant glucose uptake by red blood cells and other glycolytic tissues, returning lactate to the liver (Cori cycle). GLUT2 is the high-Km non-insulin-regulated glucose transporter (i.e. the glucose flux is determined by concentration), GLUT3 is the low-Km brain glucose transporter (the glucose flux is relatively independent of concentration within the normal range), and GLUT4 the insulin-regulated glucose transporter (insulin brings about movement of GLUT4 to the cell surface from intracellular vesicles). G6P is glucose 6-phosphate.

SECTION 11 Nutrition 1844 will provide about 100 g of glucose, entering the circulation over perhaps 60 min. In order to minimize variations in plasma glucose concentration, coordinated mechanisms come into play to suppress the production of endogenous glucose and to increase the rate of re- moval of glucose from the circulation. Much of the incoming glucose may be taken up by hepatocytes as described earlier, but some enters the systemic circulation and stimulates pancreatic insulin secretion (and somewhat suppresses glucagon secretion). Insulin is liberated into the portal vein. Thus, the liver is exposed to high concentrations of glucose (from the small intestine) and insulin. The net effect is to reverse glycogenolysis, so that glycogen synthesis begins. In addition, gluconeogenesis is sup- pressed and glycolysis favoured (Fig. 11.1.4). Hepatocyte glucose output is therefore rapidly suppressed and converted to an uptake of glucose. At the same time, utilization of glucose by insulin-sensitive peripheral tissues such as skeletal muscle and adipose tissue is in- creased. The main mechanism of this short-term change is the re-cruitment of the insulin-regulated glucose transporter GLUT4 to the cell membrane (Fig. 11.1.4). However, the decrease in concen- tration of plasma NEFAs (see following paragraphs) will also remove inhibition of glucose uptake caused by fatty acid oxidation. Within muscle, glycolysis and glycogen synthesis will be stimulated by in- sulin. In adipose tissue, increased glucose uptake provides glycerol 3-phosphate (formed from glycolysis) for esterification of fatty acids (see following paragraphs). Thus, insulin is the key regulator of the rapid changes that occur in glucose metabolism in the postprandial state: it brings about glucose storage as glycogen, and promotes the utilization of glucose at the expense of fatty acids. Lipid metabolism in the postabsorptive and

postprandial states As discussed earlier, lipids are stored primarily as TAGs in adipo- cytes. When energy is required in other tissues, TAGs are hydrolysed by enzymes (lipases) within adipocytes, and NEFAs are transported through the circulation, bound to albumin as they are not in them- selves water-soluble, to those tissues that can take them up and use them (Fig. 11.1.2). This is the pathway of fat mobilization. However, adipocyte TAG stores remain relatively constant in amount over many years (if body weight is stable), showing that there must also be matching pathways for deposition of TAGs in adipocytes (fat storage). In principle adipocyte TAGs could arise from direct uptake of plasma NEFAs, by uptake of plasma TAGs, and by de novo syn- thesis of fatty acids from glucose or amino acids within the adipo- cytes by the pathway of lipogenesis. The first of these pathways, direct uptake of NEFAs, can be demonstrated by tracer methods but does not achieve net fat storage, as the NEFAs will have arisen from adipo- cyte TAG stores. It has been suggested that this pathway may serve to redistribute lipids between adipose depots. Uptake of fatty acids from plasma TAGs (rather than uptake of the TAGs themselves) is, how- ever, an important pathway, and indeed the predominant pathway for fat storage in humans. It will be considered further next. The last of these options, de novo synthesis of glucose from glucose or amino acids, can be demonstrated to occur, but much evidence points to this being a very minor pathway for adipose tissue lipid deposition in humans. (It may be more prominent in rodents, which typically consume a low-fat, high-carbohydrate diet.) There is also the pos- sibility of lipogenesis in the liver with export of TAG-fatty acids to adipose tissue. This pathway undoubtedly contributes to lipid storage but is small quantitatively under most conditions. The major path- ways of fat metabolism are summarized in Fig. 11.1.5. Triacylglycerols in the circulation TAGs are not water-soluble—this is one reason that they are such an efficient form of energy storage. However, this poses problems when they must be transported through the plasma. Evolution has solved this problem by the development of lipoproteins, submicroscopic lipid droplets in which a core of neutral lipid (TAGs or cholesteryl esters) is stabilized by a surface monolayer of amphipathic phospho- lipids. This is an oil-in-water emulsion and is highly analogous to the stable lipid droplets in milk. Plasma lipoproteins are heterogeneous and are usually classified by their density—essentially, the greater the core lipid content per particle, the less dense the particle (lipid being less dense than water), and this enables different fractions to be separated in an ultracentrifuge. The main lipoprotein carriers of TAGs in the circulation are known as the TAG-rich lipoproteins: chylomicrons, secreted from the small intestine and transporting dietary lipid, and very low- density lipoprotein (VLDL) particles secreted from the liver, trans- porting endogenous TAGs (i.e. lipid from hepatocytes). In the postabsorptive state, chylomicron-TAG secretion is virtually zero. Secretion of VLDL is a means of exporting lipid from the liver to peripheral tissues. Insulin -

- Insulin

- Catecholamines NEFA Chylomicrons (via lymphatics) CO2 LPL LPL TAG TAG HSL, ATGL FA Muscle, myocardium renal cortex etc Small intestine Adipose tissue Ketone bodies & CO2 VLDL NEFA Insulin +

- Insulin Liver Fig. 11.1.5 Overview of fat metabolism. Dietary triacylglycerols (TAGs) enter the circulation in the form of chylomicrons. Fatty acids are taken up by tissues through the action of the enzyme lipoprotein lipase (LPL). Adipose tissue is the major TAG store. TAGs are mobilized in times of energy demand by the enzymes adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL; for more detail see Fig. 11.1.6), liberating

nonesterified fatty acids (NEFAs) into the circulation, from where they may be taken up by several tissues and used for synthesis of new TAGs and for oxidation. Major points of hormonal regulation are shown (italic).

 Direct uptake of TAGs from plasma only occurs in small amounts when whole lipoprotein particles are taken into cells by receptor- mediated pathways. No TAG transporter protein has been identi- fied. Instead, the main route for cellular uptake of plasma TAGs is hydrolysis within the capillaries to release fatty acids that may be taken up by simple diffusion across the phospholipid bilayer, or by carrier-mediated diffusion. As with glucose transport, there are sev- eral fatty acid transport proteins, although rather more disparate than the GLUT family of glucose transporters. Hydrolysis of lipoprotein TAGs in the capillaries of many tissues is mediated by the enzyme lipoprotein lipase, situated in the ca- pillaries and bound to the capillary endothelial cells. This occurs in skeletal muscle and heart, adipose tissue, mammary glands, and other tissues that use fatty acids, although not the liver. (In the liver there is a related enzyme, hepatic lipase.) Lipoprotein lipase acts on the circulating TAG-rich particles to liberate fatty acids which may be taken up by the parenchymal cells (muscle fibres, adipocytes, and so on). Adipose tissue lipoprotein lipase is upregulated by insulin, giving that tissue a special role in clearance of dietary lipid from the chylo- microns in the postprandial state. In the postabsorptive state, muscle lipoprotein lipase is likely to predominate as the site of removal of TAGs from the VLDL particles. Muscle lipoprotein lipase expression is upregulated in response to physical activity, and during fasting. The fatty acids can then be used as an oxidative fuel by the muscle. In this process, VLDL particles lose their TAG core and become rela- tively enriched with cholesterol and phospholipids. After several cycles through such capillary beds, they shrink to become simple particles with a core of cholesteryl ester and an outer phospholipid shell: these are known as low density lipoprotein (LDL) particles, the main carrier of cholesterol in the circulation.
Fat mobilization, nonesterified fatty acids and ‘energy transport’ from adipose to other tissues Lipid is mobilized from adipose tissue stores in the form of NEFAs (Fig. 11.1.2, Fig. 11.1.5). The adipocyte has a central droplet of TAGs, which is hydrolysed by intracellular enzymes (Fig. 11.1.6), releasing glycerol and NEFAs. Fat mobilization is stimulated by cat- echolamines but powerfully suppressed by insulin (see Fig. 11.1.5). Thus, fat mobilization is active in the postabsorptive state when in- sulin levels are low, and there is a call upon the body’s fat stores. It is also activated during exercise, mainly by catecholamine stimulation. Atrial natriuretic peptide (ANP), released from the heart during ex- ercise, also stimulates lipolysis. The turnover of NEFAs in the plasma is rapid. They are the major oxidative fuel in muscle after overnight fast. In the liver, fatty acids are both a fuel for oxidation and a sub- strate for synthesis of TAGs that will be exported as VLDL.
A typical concentration of nonesterified fatty acids in the plasma after over- night fast is 500 μmol/litre, one-tenth that of glucose, but because of their rapid turnover, their larger molecular mass and more reduced state fatty acids account for about twice the energy turnover of glu- cose in the circulation. Disposition of dietary fat Dietary fat is almost entirely (typically 95% or more) in the form of TAGs. A typical meal might contain 30 to 40 g of lipid. The typ- ical plasma TAG concentration in a healthy subject is 1 mmol/litre, confined to the vascular space; this means that about 3 g of TAGs is present in the circulation. Therefore, as in the case of glucose, the amount in a meal could overwhelm the system unless coordinated mechanisms come into play to ensure its rapid dispersion. Dietary TAG is digested in the stomach and small intestine and packaged by the enterocytes of the duodenum and proximal je- junum into chylomicrons, which enter the circulation via the lymph- atics (Fig. 11.1.5). Therefore, unlike other nutrients absorbed from TAG Perilipin FA

FA FA DAG ATGL CGI-58 MAG Glycerol MGL HSL Fig. 11.1.6 Fat mobilization in adipocytes. The pathway of adipocyte lipolysis is mediated by a series of three enzymes which progressively remove NEFAs from stored TAGs. Adipose triglyceride [triacylglycerol] lipase (ATGL) is active against TAGs, whereas hormone-sensitive lipase (HSL) is active mainly against diacylglycerols (DAGs), with some activity against TAGs also. Monoacylglycerol (MAG) lipase (MGL) acts to release free glycerol together with one NEFA. Free glycerol is exported from the adipocyte and will eventually be taken up by the liver. The pathway of adipocyte lipolysis is highly regulated. HSL is activated on a very short-term basis by reversible phosphorylation by protein kinases A and G, brought about by changes in the intracellular concentrations of cyclic AMP and cyclic GMP. The regulation of ATGL is less direct. ATGL has an essential coactivator protein called CGI-58 (comparative gene identification member 58). There is additional regulation at the surface of the lipid droplets where these lipases act. The lipid droplet is coated with specific proteins, a major one of which in the adipocyte is perilipin 1. Perilipin 1 is itself subject to phosphorylation, under the same conditions as HSL, and this appears to cause a conformational change that allows lipases to access the lipid droplet. CGI-58 is bound to perilipin in the unstimulated state, but released, and hence is free to activate ATGL, upon perilipin phosphorylation. The figure shows a lipid droplet surrounded by perilipin. Upon phosphorylation of perilipin, and also of HSL, a conformational change in perilipin (i) allows the lipases access to the lipid droplet and (ii) allows CGI-58 to dissociate and thus to activate ATGL. N.B. The lipases act much closer to the surface of the lipid droplet than is shown here. Fatty acids (FA on diagram) may be released from the cell for transport to other tissues, although a proportion is always re-activated (thio-esterified to CoA) and re-esterified to form TAGs. Largely reproduced from Gurr MI, Harwood JL, Frayn KN, Murphy DJ, Michell RH. Lipids: Biochemistry, Biotechnology and Health (Lipid Biochemistry: an Introduction, 6th edn), 2016 (Wiley, Oxford).

SECTION 11 Nutrition 1846 the small intestine, they bypass the liver on first passage. The chylo- microns also carry other lipid constituents of food, including choles- terol and fat-soluble vitamins. In the circulation their fate is similar to that of VLDL particles, although the tissue-specific regulation of lipoprotein lipase ensures that adipose tissue (where lipoprotein lipase is upregulated by insulin) is a major site of clearance of their TAGs. The pathway of TAG synthesis from fatty acids in adipocytes, as in the liver, is stimulated by insulin. Therefore, there is a short and energy-efficient pathway for storage of dietary fatty acids in adipose tissue (Fig. 11.1.5). The half-life of chylomicron-TAGs in the circu- lation is about 5 min. After hydrolysis of most of the TAGs, the rem- nant particles are removed by receptors in the liver and other tissues. Thus dietary cholesterol, which remains in the remnant particles along with fat-soluble vitamins, is transported mainly to the liver. Provided that a meal contains carbohydrate or protein, stimu- lation of insulin secretion will rapidly suppress the mobilization of adipose tissue TAG stores, and concentrations of NEFAs in the plasma fall after a meal. Therefore, utilization of fatty acids by tis- sues such as skeletal muscle and liver will be decreased simply by lack of availability. This reduces competition for oxidation in muscle, further increasing glucose utilization. In liver, the lack of NEFAs is likely to decrease the secretion of VLDL-TAGs. Within the liver, insulin powerfully stimulates esterification of fatty acids (for TAG synthesis) at the expense of oxidation of fatty acids (see following paragraphs). Interrelationships between carbohydrate and lipid metabolism Links between carbohydrates and lipids Carbohydrates and lipids are our main energy fuels. Oxidation of each of them is regulated by how much we ingest, our physical activity level and many other factors. However, it should not be surprising that the metabolic fates of these two important fuels are also intertwined

metabolically. As noted earlier, in mammals lipids cannot be converted to glucose in a net sense. Glucose can, however, be converted to lipid. Acetyl-CoA produced by PDC leaves the mitochondrion (it is transported across the mitochondrial membrane as citrate), and is then a substrate for the pathway of de novo lipogenesis, which begins with the enzyme acetyl-CoA carboxylase, forming malonyl-CoA. Principal among the way these fuels interact is carbohydrate- induced insulin secretion. Insulin, as outlined earlier (Fig. 11.1.5), acutely suppresses the release of NEFAs from adipose tissue. Therefore, when carbohydrate is readily available, lipid stores are conserved. In the longer term, ingestion of a high-carbohydrate diet will induce enzymes of lipid synthesis and downregulate enzymes of fatty acid oxidation, through insulin- and carbohydrate-response elements in the promoter regions of the relevant genes (see para- graph on ‘Regulation of macronutrient flux’). Glucose–fatty acid cycle Beyond this, there are specific cellular mechanisms that regulate the relative oxidation of carbohydrate and lipid. These probably operate in several tissues, although they have been most studied in skeletal and heart muscle and in liver. In 1963, Philip Randle and colleagues described the glucose–fatty acid cycle, which encompasses one aspect of this mutual relationship between carbohydrate and lipid oxidation. It is summarized in Fig. 11.1.7. The concept was based upon observations that availability of fatty acids reduced the oxi- dation of glucose in skeletal and cardiac muscle. This basic obser- vation has been confirmed many times both in vitro and in vivo. The glucose–fatty acid cycle describes both the normal interplay between fat and carbohydrate oxidation, and also pathological situ- ations involving excess availability of lipids and insulin resistance (e.g. type 2 diabetes and obesity). Glucose and the regulation of fatty acid oxidation An additional mechanism was first described in 1977 by Denis McGarry and Daniel Foster. They were following up a long-standing observation that the generation of ketone bodies by the liver was suppressed by insulin. They showed that malonyl-CoA, the first committed intermediate in the pathway of de novo lipogenesis (pro- duced by acetyl-CoA carboxylase; see earlier), strongly inhibits fatty acid oxidation. This inhibition is mediated via the enzyme carnitine palmitoyltransferase-1 in the mitochondrial membrane. Carnitine palmitoyltransferase-1 is responsible for the transport of fatty acids from the cytoplasm to the mitochondrion for β-oxidation. Acetyl- CoA carboxylase is activated by insulin (both by increased gene transcription and by reversible dephosphorylation). Hence, in a carbohydrate-replete state, malonyl-CoA will be formed and fatty acid oxidation inhibited (Fig. 11.1.7). This is now recognized as a widespread regulatory mechanism. There are two isoforms of acetyl-CoA carboxylase. Acetyl-CoA carboxylase 1, expressed in lipogenic tissues such as liver and adi- pose tissue, is involved in de novo fatty acid synthesis. Acetyl-CoA carboxylase 2 is expressed more in tissues oxidizing fatty acids such as heart and skeletal muscle and is thought to produce malonyl-CoA for regulatory, rather than synthetic, purposes. Muscle carnitine palmitoyltransferase-1 is more sensitive to inhibition by malonyl- CoA than is the liver enzyme. The ability of glucose to inhibit the oxidation of fatty acids in muscle has been clearly demonstrated in vivo, and has been termed the ‘reverse glucose–fatty acid cycle’. Protein and amino acid metabolism and their regulation The body pools of protein and amino acids, and their turnover, are summarized in Fig. 11.1.8. Insulin exerts a net anabolic effect on body protein, mainly in skeletal muscle, whereas thyroid hormones and cortisol are generally catabolic. Anabolism is also stimulated by anabolic steroids, by physical training, and during growth by the insulin-like growth factors (IGF-1; IGF-2). Dietary protein, digested in the small intestine and absorbed as free amino acids and short peptides, enters the portal vein. Enterocytes of the small intestine remove some amino acids, espe- cially glutamine, for use as an oxidative fuel. The remaining products of digestion next enter the liver, where further preferential extrac- tion occurs. Amino acid oxidation is, under most circumstances, the major oxidative pathway in the

liver—about 60% of incoming amino acids may be directed into immediate oxidation. The rate of

11.1 Nutrition: Macronutrient metabolism 1847 hepatic protein synthesis is also high, and since much of the pro- tein is secreted (e.g. albumin), this represents a net loss of amino acids from the liver (perhaps a further 20% of the incoming amino acids). The remaining mixture of amino acids, around 20% of those absorbed, enters the systemic circulation. This mixture is enriched in the branched-chain amino acids (BCAA) leucine, isoleucine, and valine, which have a special role in muscle. BCAA make up approxi- mately one-third of all amino acids in the body; while the other amino acids are metabolized principally in the liver, these essen- tial amino acids are metabolized in peripheral (nonhepatic) tissue, especially skeletal muscle. Amino acids must be synthesized, obtained from the diet or de- rived from proteolysis (although no dedicated protein exists whose sole function is to supply amino acids for energy). 'Nonessential' amino acids can be synthesized from intermediary metabolites (or from other amino acids); 'essential' amino acids cannot be synthesized by humans and therefore must be obtained from the diet. 'Conditionally essential' amino acids can be synthesized in only limited amounts, and this must be supplemented by the diet in states of rapid protein synthesis (e.g. growth). Free amino acids (dietary, synthetic, proteolytic) constitute a soluble amino acid pool; this is quantitatively small, but dynamic. From this pool, amino acids are used for biosynthetic functions as well as degradation for energy production, their carbon skeletons entering the common metabolic pool of intermediary metabolites shared with carbohydrate and lipid metabolism. While amino acids are used to synthesize proteins, proteins are broken down to amino acids, this constituting the protein turnover rate (Fig. 11.1.8), which varies between individual proteins. For a protein to be useful as a source of amino acids for energy production, Fig. 11.1.7 The glucose–fatty acid cycle. When glucose and insulin concentrations are high, release of nonesterified fatty acids (NEFAs) from adipose tissue is suppressed, and glucose utilization predominates in insulin-sensitive tissues such as skeletal muscle—its uptake is stimulated by GLUT4. In addition, glucose metabolism inhibits NEFA oxidation: stimulation of acetyl-CoA carboxylase (ACC; 1 in image) produces malonyl-CoA which inhibits uptake of fatty acyl-CoA (FA-CoA) into the mitochondrion by inhibiting carnitine palmitoyl-transferase-1 (CPT-1; 2 in image), hence β-oxidation of fatty acids is inhibited. In the fasting state (glucose and insulin concentrations are low), insulin-mediated inhibition of adipose tissue lipolysis is decreased, and NEFA utilization predominates, reinforced by inhibitory effects of fatty acids and their products of β-oxidation on glucose uptake and oxidation. This may have pathological significance, in that states in which NEFA concentrations tend to be high (e.g. type 2 diabetes mellitus) will be associated with resistance of glucose utilization to insulin. Protein 100 g/day 10 kg protein ? 100 g amino acids Nucleotides, hormones etc CO2 plus urea, NH3, equivalent to protein 100 g/day 300 g/day 300 g/day Fig. 11.1.8 The body pools of protein and free amino acids and their turnover. Numbers are approximate.

SECTION 11 Nutrition 1848 its turnover rate must be relatively high, and there must be a rela- tively large amount of it in the body. Dietary amino acids surplus to synthetic requirements (for proteins, nucleotides, hormones, neurotransmitters, creatine, porphyrins, sphingolipids) are utilized directly for energy pro- duction. Although many amino acids exist in vivo (20 'standard' amino acids are coded for in proteins, but there are others that are not used for peptide bond formation), and some have indi- vidual amino acid metabolic pathways, most follow a common biochemical route to yield their energy. Amino acids comprise a central (α-) carbon bonded to a carboxyl, amino, hydrogen and side chain ('R group') group—hence they contain C, H, O atoms, like

carbohydrates and lipids, but also a distinguishing N-atom. The common feature of amino acid metabolism is removal of the amino-N group (deamination), which is excreted as urea or ammonia (or incorporated into certain biomolecules), followed by utilization of the remaining carbon skeleton (α-keto acid; 2-oxoacid) (Fig. 11.1.9). The fate of the 2-oxoacid carbon skeleton depends on where it enters the common metabolic pool of inter- mediary metabolism. Nitrogen disposal Deamination of amino acids is achieved by two types of reaction which function in a complementary manner (Fig. 11.1.10). The first is transamination, in which the amino group from one amino acid is transferred to another 2-oxoacid (carbon skeleton), forming its corresponding amino acid (i.e. amino acid-1 + 2-oxoacid-2 ↔ 2- oxoacid-1 + amino acid-2). The enzymes responsible for transamin- ation reactions are aminotransferases (transaminases), all of which contain pyridoxal phosphate, a derivative of vitamin B6, in their ac- tive centres. Aminotransferases are widespread in most tissues and are near-equilibrium, hence readily reversible. Each is specific for a limited number of amino acids, but most utilize 2-oxoglutarate (α- ketoglutarate) as the amino (N) acceptor, producing the carbon back- bone of the donor amino acid together with glutamate (amino acid + 2-oxoglutarate → 2-oxoacid + glutamate). Hence 2-oxoglutarate and glutamate are central to amino acid catabolism as these reactions ‘funnel’ the various amino acids into glutamate (and note that 2- oxoglutarate is common to the TCA cycle, and its utilization by this pathway depletes TCA intermediates—cataplerosis). Alanine aminotransferase (ALT) transfers the amino group of ala- nine to 2-oxoglutarate, forming pyruvate and glutamate. Aspartate aminotransferase (AST) transfers the amino group of aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate—however, this enzyme usually works in the reverse direction, its function being to convert glutamate (derived from amino acid funnelling, de- scribed earlier) into aspartate, which is required to donate a second urea N-atom to the urea cycle. Since ALT and AST are both intra- cellular enzymes and widespread, necrosis of many tissues causes them to increase in plasma; they are commonly used to diagnose hepatocellular damage. The second type of reaction responsible for amino acid de- amination is oxidative deamination. Since most amino acids have been deaminated (funnelled) into glutamate by transamination, glutamate is the only amino acid that undergoes direct, oxida- tive, deamination, by glutamate dehydrogenase, regenerating 2-oxoglutarate and producing ammonia (NH_3). Unusually for a highly regulated enzyme, glutamate dehydrogenase is revers- ible, and can use NAD^+ or $NADP^+$ as electron acceptors. In the ‘forward’ direction of deamination (catabolic, amino acid break- down), it uses NAD^+, but in the ‘reverse’ direction of amination of 2-oxoglutarate to glutamate (anabolic, amino acid synthesis) it uses NADPH, reflecting the different roles of these cofactors as redox energy carriers in different metabolic states. Hence, aminotransferases (transamination) and glutamate dehydrogenase (oxidative deamination) work together to produce ammonia for detoxification to urea in the urea cycle, and carbon skeletons for further intermediary metabolism (Fig. 11.1.10). The urea cycle occurs in the liver (the pathway is partially pre- sent in kidney, and also in the brain, but this is not a significant site of blood urea production.) Urea ($CO(NH_2)_2$) contains two ni- trogen atoms: one derives from ammonia (produced by oxidative deamination of glutamate, described earlier), the other derives from AST (also from glutamate; Fig. 11.1.10); the body excretes nitrogen with minimal carbon (and energy) loss. Because urea is very water- soluble, much nitrogen can be excreted for relatively little water loss, an important adaptation in terrestrial animals. Urea lacks toxicity at physiological concentrations; it is (neuro)toxic only in extremely high concentrations, for example those seen in untreated renal failure, but considerably less so than ammonia. Besides urea formation, there is another route of ammonia excre- tion. In peripheral tissues (e.g. muscle) ammonia may be formed by the oxidative deamination of glutamate (by glutamate dehydro-

genase). This reaction, in combination with the aminotransferases, can be seen to capture amino nitrogen from several amino acids. However, blood ammonia concentrations are very low (it is highly toxic) and instead it is exported by being fixed in the amido (side chain) group of glutamine by the enzyme glutamine amino acid carbon skeleton (2-oxoacid) TCA intermediates (≥ 3 C; glucogenic) acetyl-CoA acetoacetyl-CoA (≡2C; ketogenic) glucose CO_2+ H_2O + ATP ketone bodies fatty acids deamination NH_3 urea urea cycle 3 1 2 Fig. 11.1.9 Amino acid metabolism. In order to yield energy, amino acids must have their α-amino group removed (deamination). This is achieved by transamination and oxidative deamination (see Fig. 11.1.10). The resulting ammonia is converted to urea (urea cycle) in order to decrease its toxicity. The remaining carbon skeleton (2-oxoacid; ‘α-keto acid’) enters the common metabolic pool of intermediary metabolites for immediate oxidation, or conversion into glucose (glucogenic amino acids; blue) or lipids (ketogenic amino acids; green) for subsequent oxidation.

 synthetase: hence glutamine is carrying two nitrogen atoms. In liver, the enzyme glutaminase removes the amido nitrogen of glutamine as ammonia for rapid incorporation into urea. In the kidney, glutaminase also removes the amido group of glutamine to form ammonia (and glutamine; glutamate dehydrogenase then deaminates this to form another ammonia), but here the resulting ammonia is excreted directly into the urine as a urinary buffer. There is also a supply of ammonia from the small intestine. Metabolism of carbon skeleton Following amino acid deamination, the remaining 2-oxoacid enters the common metabolic pool. All amino acid carbon skeletons ultim- ately yield just seven products of intermediary metabolism: pyru- vate, 2-oxoglutarate, succinyl-CoA, fumarate, oxaloacetate, acetyl-CoA, and acetoacetyl-CoA. The first five of these represent at least three carbons, hence amino acids producing these metab- olites can be used for glucose synthesis (‘glucogenic’). The acetyl-CoA and acetoacetyl-CoA, however, yield two carbon groups or equivalent, and amino acids which produce them cannot be used for gluconeogenesis (Fig. 11.1.9)—they can be directly oxidized in the TCA cycle, undergo lipogenesis or be used to synthesize ketone bodies (‘ketogenic’). The glucogenic amino acids therefore confer on proteins the property of acting as a carbohydrate reserve in states such as starvation. Intertissue amino acid flux Considerable flux of amino acids occurs between tissues as part of intermediary metabolism. Liver is the site of both ureagenesis (amino-N metabolism) and gluconeogenesis (carbon skeleton me- tabolism), and diet-derived amino acids enter the liver through the portal circulation for immediate processing. However, many amino acids are derived from endogenous proteolysis in many peripheral (nonhepatic) tissues. Amino acid transport from peripheral tissues to the liver for catabolism involves transport of both the N-group (for deamination and excretion) and the carbon skeleton (for oxida- tion/glucose synthesis) (Fig. 11.1.11). Hence, amino acids released from proteolysis in peripheral tissues must transfer their amino nitrogen to the liver. This re- sults in considerable interaction between the pathways of amino acid, carbohydrate, and fat metabolism. Measurements of arterio- venous differences across muscle and adipose tissue show that the release of the amino acids alanine and glutamine predomin- ates. Since glutamine carries two nitrogens it is, under most cir- cumstances, the predominant nitrogen carrier. Arteriovenous difference measurements across the splanchnic bed (by cath- eterization of the hepatic vein) show an almost identical pattern for uptake: removal of alanine and glutamine far exceeds that of other amino acids. Therefore, amino acids in tissues including muscle must transfer their amino nitrogen to alanine (by trans- amination with pyruvate) and glutamine (formed by glutamine synthetase from glutamate, itself arising by transamination with 2-oxoglutarate). It is important that the 2-oxoacid acceptors, pyruvate and 2-oxoglutarate, are

common metabolic intermedi- ates and thus readily available. All three BCAA are transaminated by a single branched-chain aminotransferase, and the resulting branched-chain 2-oxoacids undergo oxidative decarboxylation (branched-chain α-ketoacid de- hydrogenase (BCKD) complex). Branched-chain amino acids ab- sorbed from the diet are not removed from the portal circulation by the liver (therefore avoid the hepatic first pass effect of other amino acids) and appear in high concentration in the blood from the splanchnic bed; furthermore, branched-chain amino acids may amino acid 2-oxoglutarate glutamate NH3 urea CO2 transamination oxidative deamination urea cycle carbon skeleton (2-oxoacid) intermediary metabolism aspartate transamination glutamate 2-oxoglutarate OAA aminotransferase glutamate dehydrogenase aspartate aminotransferase
Fig. 11.1.10 Amino acid deamination. Multiple amino acids are ‘funnelled’ into one amino acid, glutamate, which acts as the universal donor of the α-amino group. Transamination reactions, catalysed by aminotransferase enzymes (transaminases), are responsible for the funnelling of most amino acids into glutamate; the remaining 2-oxoacid (carbon skeleton) can then be used for energy generation. These transamination reactions occur both in tissues of amino acid supply (e.g. muscle) and in the site of urea synthesis (liver); alanine is used for amino acid transport and therefore an important aminotransferase is alanine aminotransferase (ALT), yielding the 2-oxoacid pyruvate. Aspartate aminotransferase (AST) also utilizes glutamate for deamination. Glutamate provides both nitrogen atoms for the urea cycle (urea: CO(NH2)2) and is the only amino acid to undergo direct (oxidative) deamination by glutamate dehydrogenase. OAA, oxaloacetate.

SECTION 11 Nutrition 1850 also have a role as nutrient signals. Hence, branched-chain-amino acids act as a major source of nitrogen in skeletal muscle to maintain pools of glutamine, glutamate, and alanine (branched-chain amino acid transferase activity is significantly higher than BCKD activity). Much of the alanine released from skeletal muscle comes from transamination of pyruvate formed in glycolysis. Within the liver, the amino group can be transferred further (e.g. to oxaloacetate, forming aspartate, which is one of the immediate donors of nitrogen to the urea cycle). The pyruvate thus formed may be a substrate for gluconeogenesis, producing glucose that can be recycled to periph- eral tissues. This metabolic cycle has been called the glucose-alanine cycle. It closely parallels the Cori cycle (see Fig. 11.1.4). An important aspect of the large depot of muscle protein is that it represents a potential source of synthesis of new glucose during fasting. In that situation, while the brain continues to require glu- cose for oxidation, and as glycogen reserves are depleted, new glu- cose can only be formed from glycerol, released in adipose tissue lipolysis, and from amino acids. Regulation of macronutrient flux The need for the coordinated control of nutrient storage, mobiliza- tion, and flux between tissues and along the many metabolic path- ways, is met by a complex series of control mechanisms. These may be viewed on several levels. The simplest involves the effects of sub- strate concentration, and is dependent upon the kinetic properties of enzymes and transport proteins. The next level involves more specific interaction of nutrients, or pathway intermediates, with enzymes, usually through allosteric effects (binding of the effector alters the conformation of the en- zyme and hence its catalytic properties). There are many examples in the metabolism of carbohydrate, fat, and protein. The enzyme 6-phosphofructo-1-kinase in the glycolysis pathway is a good ex- ample. This enzyme is subject to allosteric regulation by many com- pounds that relate to the energy status of the cell. For instance, it is activated by AMP (indicating energy shortage) and inhibited by ATP. Such mechanisms undoubtedly provide important fine tuning of flux along various pathways, entirely in accord with the modern view that control of flux does not reside in certain rate-limiting steps but is distributed among many steps along a pathway. Related to this, the enzyme AMP-activated protein kinase (AMPK) re- sponds to

the cellular energy status and regulates several metabolic pathways accordingly (see ‘Further reading’). These mechanisms operate essentially within tissues. However, the coordination of nutrient metabolism requires considerable interaction between tissues and organs. This coordination is largely brought about by the hormonal and nervous systems. Certain hor- mones play a particularly important role in regulation of macronu- trient flux (Table 11.1.2). The role of the nervous system in metabolic glucose glucose Liver Intestine Kidney Muscle glucose alanine alanine alanine pyruvate AA AA BCAA BCAA urea urea urea NH3 NH3 NH3 glutamine glutamine glutamate glutamate glutamine pyruvate NH3 2-oxoglutarate 2-oxoglutarate 2-oxoglutarate 2-oxoacid PROTEIN NH3 PROTEIN CO2 + H2O Fig. 11.1.11 Intertissue amino acid flux. Amino acids are provided from the diet and by amino acid turnover from labile protein pools. In catabolic states where amino acids are required for energy (glucose) provision, muscle proteolysis and transamination yield alanine and glutamine for export (muscle is the major source of amino acids during starvation and is shown here for clarity). Alanine is transported to the liver, where it is deaminated, and its carbon skeleton (pyruvate) used for gluconeogenesis; the glucose produced is used by brain, or recycled to the muscle to facilitate further nitrogen transport (glucose-alanine cycle), while the nitrogen is converted to urea for renal excretion. Glutamine exported from peripheral protein sources is transported to several tissues, including kidney, where it is deaminated to yield free ammonia, an important urinary buffer. AA, amino acids; BCAA, branched-chain amino acids.

11.1 Nutrition: Macronutrient metabolism 1851 Table 11.1.2 Major hormonal effects on intermediary metabolism Hormone Origin Target tissue Major metabolic effects Comments Insulin Pancreatic islets (β-cells) Liver Stimulation of glycogen synthesis/suppression of glycogen breakdown Regulates glucose storage in liver Stimulation of glycolysis/suppression of gluconeogenesis Regulates hepatic glucose output Suppression of fatty acid oxidation/ketogenesis Via malonyl-CoA Stimulation of triacylglycerol synthesis Stimulation of cholesterol synthesis Skeletal muscle Stimulation of glucose uptake Via recruitment of GLUT4 (see Fig. 11.1.4) Stimulation of glycogen synthesis Net protein anabolic effect Suppression of protein breakdown may be more important than stimulation of protein synthesis Adipose tissue Activation of triacylglycerol removal from plasma Via lipoprotein lipase Suppression of fat mobilization Via intracellular lipases (see Fig. 11.1.6) Glucagon Pancreatic islets (α-cells) Liver Stimulation of glycogen breakdown/suppression of glycogen synthesis In effect the regulation is via the insulin/glucagon ratio Stimulation of gluconeogenesis/suppression of glycolysis Stimulation of fatty acid oxidation/ketogenesis Somatostatin δ-Cells in pancreatic islets and in gastrointestinal tract; some neuroendocrine neurons in brain Cells secreting other peptide hormones; acid-producing parietal cells of stomach Indirect, via inhibition of secretion of insulin, glucagon, growth hormone and other peptide hormones; and via inhibition of gastric acid secretion Somatostatin or analogues are used to treat growth hormone excess ‘Incretins’ K and L cells in gastrointestinal tract α- and β-Cells of pancreatic islets Indirect, via increasing the effect of ingested carbohydrate on insulin secretion (hence the name ‘incretin’); and reducing glucagon secretion The name ‘incretins’ covers at least two peptide hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (also known as: glucose-dependent insulinotropic polypeptide or GIP). Analogues, or small molecules that block the proteolytic breakdown of incretins, now used in treatment of type 2 diabetes Adrenaline Adrenal medulla Adipose tissue Stimulation of fat mobilization Via intracellular lipases (see Fig. 11.1.6) Skeletal muscle Stimulation of glycogen breakdown Acts in concert with muscle contraction Tri-iodothyronine Thyroid All oxidative tissues Increase in basal metabolism Cortisol

Adrenal cortex Liver Stimulation of gluconeogenesis Skeletal muscle Generally catabolic effect on protein Adipose tissue Promotes site-specific fat deposition (central depots) and fat mobilization (peripheral depots) Growth hormone Anterior pituitary Liver Stimulation of gluconeogenesis Direct effect: other effects are mediated indirectly via insulin-like growth factors Adipose tissue Stimulation of fat mobilization This is an acute effect: chronically, growth hormone promotes mobilization from central fat depots Insulin-like growth factors (IGF) I and II Liver (IGF-I) and other tissues (both) Several Generally insulin-like acute effects on metabolism Physiological role is probably longer-term effects on growth Leptin Adipose tissue Hypothalamus Suppression of appetite; possibly stimulation of energy expenditure Latter effect prominent in rodents, may not occur in humans; low leptin levels (signalling starvation) more important than high levels signalling excess. Recombinant leptin has been used to treat the rare genetic condition of leptin deficiency Reproductive system Signals sufficient fat stores for reproduction to be possible As with effects on hypothalamus, low leptin may be a signal of starvation

SECTION 11 Nutrition 1852 regulation is often difficult to assess. Although the effects of adren- aline are properly regarded as hormonal, liberation of noradrenaline from sympathetic nerve endings in tissues may bring about iden- tical effects and can be difficult to distinguish. The somatic nervous system (motor neurons innervating skeletal muscle) has clear effects (e.g. stimulation of breakdown of muscle glycogen linked to muscle contraction). The autonomic nervous system probably plays mul- tiple roles, but some are indirect (e.g. regulation of blood flow and cardiac output), thus affecting delivery of substrate to tissues, and regulation of the secretion of pancreatic hormones. The effects of hormones are mediated in many ways, but these may be divided into acute effects (usually acting within seconds or minutes), often brought about through reversible phosphorylation or dephosphorylation of enzymes, and longer-term effects (hours or days), brought about by regulation of gene expression. The former are usually exerted through binding to cell surface receptors linked to a variety of second-messenger systems, the latter through nuclear receptors (e.g. for glucocorticoids and thyroid hormones; for more details see Chapter 13.1). However, the distinction is not absolute (e.g. insulin brings about both acute and longer-term effects through binding to the same cell surface receptor). Until recently it was considered that there was a complete distinction between hormones and substrates (or metabolites). One obvious distinction is in typical concentrations in plasma. Table 11.1.3 Some G-protein-coupled receptors (GPCRs) that respond to nutrients and related metabolites GPCR number Other names Gene name Ligand Tissue expression (major tissues) Physiological role and comments GPR40 FFA1, FFAR1 (free fatty acid receptor 1) FFAR1 Long-chain fatty acids (C12–C16) Pancreatic β-cells Potentiates glucose-stimulated insulin secretion GPR41 FFA3, FFAR3 FFAR3 Short-chain fatty acids Adipose tissue, gastrointestinal (GI) tract (enteroendocrine cells) Stimulation of leptin production; stimulation of gut hormone secretion GPR43 FFA2, FFAR2 FFAR2 Short-chain fatty acids Adipose tissue, GI tract (enteroendocrine cells) Adipogenesis, reduction of lipolysis; stimulation of gut hormone secretion GPR70 Taste receptor 1, T1R1 TAS1R1 L-amino acids Taste cells, GI tract, pancreatic islets GPR71 Taste receptor 2, T1R2 TAS1R2 Sugars, artificial sweeteners Taste cells, GI tract, pancreatic islets TAS1R1 and TASR2 act as heterodimers with TASR3 to act as receptors for umami and sweet tastes, respectively Taste receptor 3, T1R3 TAS1R3 See comments on T1R1, T1R2 Taste cells, GI tract, pancreatic islets GPR81 HCAR1 (hydroxycarboxylic acid receptor 1) Lactate Adipocytes System by which lactate reduces adipocyte lipolysis; may act as a paracrine amplifier of insulin action on lipolysis (since insulin increases adipocyte lactate production) GPR109A HM74A, NIACR1 HCAR2 (hydroxycarboxylic acid receptor 2) Probably 3- hydroxybutyrate

(ketone body) Adipocytes Identified initially as the receptor for nicotinic acid (a component of the B- vitamin niacin), used in large doses to treat high triacylglycerol concentrations. Only known metabolic function is to suppress adipocyte lipolysis. Since 3-hydroxybutyrate is a product of hepatic fatty acid oxidation, this could provide a feedback loop GPR109B HCAR3 3-hydroxyoctanoic acid (intermediate of fatty acid oxidation) Adipocytes Reducing lipolysis when fat oxidation is already high (e.g. in pathological situations) GPR119 Oleoylethanolamide receptor GPR119 Oleoylethanolamide and other lipids containing oleic acid, e.g. 2-oleoyl glycerol Pancreatic β-cells, GI tract Oleoylethanolamide has appetite-suppressing activity (although not entirely via GPR119). It is related to the endogenous cannabinoids. 2- monoacylglycerol stimulation in the GI tract may enhance GLP-1 secretion (together with GPR40) GPR120 FFAR4 n-3 Fatty acids Macrophages, GI tract, adipose tissue, brain (hypothalamus) Has been suggested to modulate anti- inflammatory effects of n-3 fatty acids. Human genetic variation associated with obesity and insulin resistance GPR131 GPBAR1 (G protein-coupled bile acid receptor 1), TGR5 GPBAR1 Bile acids Liver, adipose tissue, GI tract, gall bladder Regulates gall bladder filling with bile, gut motility, and secretion of GI tract hormones

11.1 Nutrition: Macronutrient metabolism 1853 For instance, insulin is major regulator of glucose metabolism (see earlier) and yet insulin concentrations in plasma are typic- ally 10–100 pmol/l, whereas glucose concentrations might be 5–10 mmol/litre (a difference of about eight orders of magnitude). However, in recent years it has been appreciated that many nutri- ents and their metabolites also regulate metabolic pathways rap- idly by signalling through receptors that were once thought to be receptors for hormones and neurotransmitters. In particular, this applies to signalling through the G protein-coupled receptors (GPCRs). A variety of compounds that we recognize as nutrients or related metabolites is now known to signal through specific GPCRs. This adds a further level of control of metabolic pathways according to nutrient and metabolite availability. These actions are usually rapid (many mediated via alteration of cellular cyclic AMP concentrations) and so are complementary to the longer- term effects of nutrients and metabolites exerted on gene expres- sion, as discussed next. Some examples of nutrients and their metabolites and the GPCRs through which they may act are given in Table 11.1.3. A further level of coordination is through the effects of nutri- ents themselves, or important cellular components such as chol- esterol, upon gene expression (summarized in Table 11.1.4). This can be seen as a longer-term mechanism to ensure that metab- olism is appropriate to the diet being ingested and the lifestyle followed. A variety of nutrient response elements are known in the promoter regions of genes for enzymes concerned with substrate metabolism. Particular examples are the carbohydrate-response element (which upregulates expression of genes for glucose metabolism such as pyruvate kinase in the glycolysis pathway, and lipogenic genes), the sterol response element (by which insulin activates lipogenic gene expression, as in Table 11.1.4, and cellular sterols downregulate ex- pression of the low density lipoprotein receptor and the enzymes of cholesterol biosynthesis) and response elements for fatty acid de- rivatives. Fatty acids affect gene expression through a family of tran- scription factors known as the peroxisome proliferator-activated receptors, summarized in Table 11.1.4. The expression of many genes is also regulated by insulin. Table 11.1.4 Mechanisms by which nutrients regulate expression of genes involved in macronutrient metabolism Stimulus Transcription factor Examples of proteins whose expression is regulated at the mRNA transcription level Comments Glucose Carbohydrate-response element binding protein Pyruvate kinase (liver isoform) (+) Acetyl-CoA carboxylase 1 (+) Fatty acid synthase (+) SREBP-1c (see next) (+) Insulin (in the pancreatic β-cell) (+) Insulin Various, binding to

a variety of insulin response elements (see ‘Further reading’) GLUT 1, 2, 3, 4 (glucose transporters) (+) Hexokinase, glucokinase (+) Glyceraldehyde-3-phosphate dehydrogenase (+) Glucose-6-phosphatase (–) Acetyl-CoA carboxylase 1 (+) Fatty acid synthase (+) SREBP-1c (+) Glycolysis and lipogenesis are activated, gluconeogenesis suppressed; see ‘Further reading’ for more information Cholesterol (and insulin) Sterol regulatory element binding proteins (SREBP) SREBP-1c: acetyl-CoA carboxylase 1 (+) Fatty acid synthase (+) Stearoyl CoA desaturase (+) SREBP2: LDL receptor (+) HMG CoA synthase (+) HMG CoA reductase (+) The two major isoforms, SREBP-1c and SREBP2 respectively, regulate lipogenesis (in response to glucose and insulin) and cellular cholesterol homeostasis (in response to cellular sterol levels; low sterol levels allow mature SREBP2 to migrate to the nucleus) Fatty acids Peroxisome proliferator- activated receptors (PPARs): PPARα PPARδa PPARγ (Liver): Apolipoproteins; enzymes of peroxisomal fatty acid oxidation Enzymes of mitochondrial oxidation Adipocyte differentiation factors Adipose tissue FABP (also known as aP2) Lipoprotein lipase Adiponectin PPARs act as transcription factors as heterodimers with the retinoid-X receptor; the endogenous ligand is unclear:
it might be a fatty acid (weak affinity) or a fatty acid derivate (e.g. a prostaglandin) (higher affinity) Target for the fibrate lipid-lowering drugs Effects have been documented in adipose tissue, skeletal muscle, and heart; clinical use of agonists uncertain Target for the thiazolidinedione insulin-sensitizing agents Amino acids Mammalian (or mechanistic) target of rapamycin (mTOR); Activating transcription factor 4 (ATF4) IGFBP-1 (–) Asparagine synthase (–) Amino acid transporters (–) (especially neutral amino acid transport system A; cationic amino acid transporter CAT-1) Induction of IGFBP-1 (binds IGF-1) when amino acid supply is restricted limits growth; molecular mechanisms are described in ‘Further reading’ Note: (+), indicates gene induction; (–), gene suppression. a Also known as PPARβ, NUC 1, FAAR (fatty-acid activated receptor). FABP, fatty acid binding protein; GLUT, glucose transporter; HMG, 3-hydroxy-3-methylglutaryl; IGF, insulin-like growth factor; IGFBP, IGF binding protein.

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