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### 2.3.3 Protein and amino acid metabolism

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#### Introduction

The liver is a major organ of amino acid metabolism. It is responsible for the disposal of much of the dietary amino acid load; it is the only organ with a complete urea cycle; it is capable of synthesizing some amino acids; and it produces glucose from muscle-derived amino acids during starvation and in diabetes. Hepatic amino acid metabolism is finely regulated. There is evidence that the liver is largely responsible for maintaining circulating amino acid homeostasis. The liver also plays a critical role in the biosynthesis of key molecules from amino acids, e.g. creatine and glutathione. It also uses amino acids and glutathione in the conjugation of xenobiotics and toxic molecules to ensure their elimination from the body. In addition, there is good evidence that amino acids play a crucial regulatory role in controlling the turnover of hepatic proteins.

#### Amino acid pools and amino acid transport

Rapidly frozen rat liver contains a total of 20  $\mu\text{mol/g}$ , which translates into an intracellular concentration of about 40 mM [1]. This figure ignores compartmentation between and within cells, however. Nevertheless, it gives an accurate picture of the magnitude of the hepatic intracellular amino acid pool. Given that the liver cell, like plasma, experiences an osmotic pressure of about 305 mOsM, it is apparent that free amino acids account for about 13% of all intracellular osmolytes. The amino acids with the highest hepatic concentrations include taurine (7.5 mM), aspartate (6.3 mM), glutamate (2.8 mM), glutamine (10.6 mM), glycine (4.0 mM) and alanine (4.7 mM). By also measuring plasma amino acids, intracellular/extracellular concentration ratios can be calculated (Table 1). Very high ratios (> 10) were identified for taurine, aspartate, glutamate, glutamine, glycine, alanine and histidine. No amino acid displayed a ratio significantly less than 1. These ratios are largely a result of the operation of hepatic amino acid transporters.

Hepatocytes communicate with their environment via their plasma membranes, either by signal transduction or via transport. Enormous progress has been made in the field of amino acid transport as our knowledge has advanced from kinetic to molecular characterization. Different amino acid transporters are responsible for the transport of groups of structurally similar amino acids. Transport of the three classes of amino acids (zwitterionic, cationic and anionic) is effected by a number of different transporters with different (though overlapping) specificities. Energetically, we can divide these transport systems into two classes: sodium-linked transporters (an example of secondary active transport) that employ the  $\text{Na}^+$  electrochemical

**Table 1** Hepatic intracellular amino acid concentrations and intracellular/extracellular concentration ratios.

Amino acid	Hepatic concentration (mM)	Intracellular/extracellular ratio
Taurine	7.53	57
Aspartate	6.27	412
Threonine	0.46	1.8
Serine	0.53	3.0
Asparagine	0.13	3.2
Glutamate	2.83	29
Glutamine	10.63	18
Proline	1.21	6.5
Glycine	3.97	17
Alanine	4.65	11
Citrulline	0.09	1.4
Valine	0.27	1.7
Methionine	0.18	3.3
Isoleucine	0.19	2.4
Leucine	0.30	2.2
Tyrosine	0.09	0.8
Phenylalanine	0.12	2.1
Ornithine	0.31	5.2
Lysine	0.98	2.9
Histidine	1.01	20
Arginine	0.10	0.8

From ref. 1.

gradient to drive inward amino acid translocation against a concentration gradient; and sodium-independent transporters that facilitate amino acid transport in either direction, depending on the concentration gradient (an example of facilitated diffusion).

Among the most important hepatic amino acid transporters are systems A, ASC, L and N for neutral (zwitterion) amino acids, system  $\gamma^+$  for cationic amino acids and  $X_{AG}^-$  and  $X_C^-$  for anionic amino acids [2]. System A is a sodium-dependent transporter with preference for neutral amino acids with small side-chains (e.g. alanine and serine). System ASC is also sodium dependent and exhibits a preference for alanine, serine, cysteine and threonine, although it can also transport bulky amino acids such as leucine, valine and methionine at a lower rate. This transporter exhibits a marked trans-stimulation and is thought to exchange the extracellular and intracellular pools of a number of amino acids. System L favours amino acids with bulky side-chains (branched-chain and aromatic amino acids). It is not sodium dependent, and its active exchange properties suggest that it may mediate efflux from cells rather than influx. System N is a sodium-dependent transporter with rather narrow substrate specificity. It transports glutamine, asparagine and histidine – amino acids that contain nitrogen in their side-chains. The most active cationic transporter is system  $\gamma^+$ , a high-affinity sodium-independent transporter that acts on lysine, histidine and arginine. Two anionic transporters are of note. System  $X_{AG}^-$  transports glutamate and aspartate, and this is accompanied by sodium cotransport and potassium countertransport. System  $X_C^-$  transports cystine into hepatocytes in exchange for glutamate

and is thought to provide the limiting substrate (cysteine) for hepatic glutathione synthesis. Much progress has recently been made in the molecular and kinetic characterization of these transporters. Nevertheless, it is apparent that the combination of a multitude of transporters with overlapping specificity and differing energetics, as well as facilitation of both uptake and exchange, makes the physiological interpretation of the roles of these transporters challenging.

These amino acid transporters are regulated with regard to both their expression and the cell types in which they are expressed. For example, system A activity is rapidly stimulated by glucagon via a mechanism that does not require protein synthesis. System A activity is also upregulated by insulin via a mechanism that requires gene transcription. Uptake of the anionic amino acids, glutamate and aspartate, is much more rapid in hepatocytes at the perivenous terminus of the hepatic acinus than in the periportal hepatocytes [3].

### Metabolic disposal of dietary amino acids

Adults ingesting a typical western diet consume ~ 80–100 g of protein per day. The common textbook statement that these amino acids are oxidized in the liver cannot be true because, even if no other fuel were oxidized there, it would oblige the consumption of more oxygen than the liver actually consumes [4]. The solution to this conundrum is threefold. First, there is appreciable oxidation of a number of amino acids in the gastrointestinal tract [5]. Secondly, the branched-chain amino

acids (which comprise about 20% of total dietary amino acids) largely escape splanchnic metabolism and are mostly metabolized in skeletal muscle. Thirdly, even in the fed state, the hepatic disposal of amino acids involves the conversion of their carbon skeletons to glucose and to ketone bodies, which are released, as universal fuels, for other tissues. This enables the liver to accommodate dietary amino acid disposal within the bounds of its oxygen consumption. In an adult in nitrogen balance, the daily dietary amino acid intake must be oxidized in a 24-h period. If the liver were too efficient in the clearance of absorbed amino acids, none would be available for peripheral tissues. Rather, after meals, the liver must consume only excess amino acids. An equal priority, however, is to have sufficient amino acids available for protein synthesis both in peripheral tissues and in the liver itself. In the postprandial period, the liver will metabolize amino acids released by peripheral tissues, principally muscle.

The catabolism of individual amino acids is tightly controlled, particularly the essential amino acids. This control is accomplished in a number of ways. An important role may be ascribed to glucagon, which is secreted at a higher rate upon ingestion of a high-protein meal. Glucagon activates phenylalanine hydroxylase by means of phosphorylation; it stimulates glycine and glutamine catabolism; and, together with glucocorticoids, it induces the synthesis of a number of amino acid-catabolizing enzymes. Glucagon can also regulate the catabolism of a variety of amino acids, as evidenced by the generalized hypoaminoacidaemia in patients suffering from glucagonoma. A variety of mechanisms prevent the depletion of amino acids, in particular the essential amino acids. Many enzymes that initiate amino acid catabolism exhibit rather high  $K_m$ s for their amino acid substrates. However, simple Michaelis–Menten kinetics do not provide sufficient sensitivity to substrate concentrations. A number of additional mechanisms have evolved to conserve these amino acids. The hepatic isoform of *S*-adenosylmethionine synthase, the first enzyme of methionine catabolism, is activated by its product, *S*-adenosylmethionine. This regulatory feature provides the necessary sigmoidal dependence on amino acid concentrations [6]. Another regulatory stratagem is provided by the inducibility of some of the enzymes of essential amino acid catabolism. Enzymes such as tyrosine aminotransferase, ornithine aminotransferase, tryptophan dioxygenase, threonine dehydratase and histidase exhibit large-amplitude (as much as 10-fold) changes in activity. They also have short half-lives, so that they promptly return to very low activities when the stimulus is removed. The importance of these effects is clear – the induction of these enzymes permits increased catabolism of these amino acids when the supply is high, whereas the return to very low activities when the supply is low conserves these essential amino acids [7].

Covalent modification of key enzymes is also employed as a regulatory stratagem. The branched-chain  $\alpha$ -keto acid dehydrogenase (BCKDH), the controlling enzyme complex of branched-chain amino acid catabolism, is inhibited by phosphorylation; removal of the phosphate by a protein phosphatase

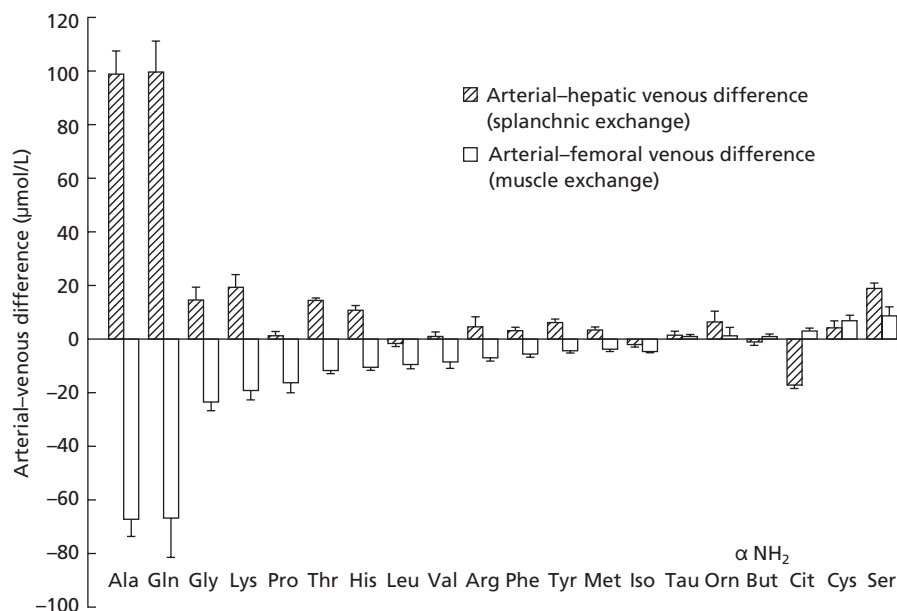
reverses the inhibition. The BCKDH phosphatase is activated by  $\alpha$ -ketoisocaproate. This mechanism produces a system that is particularly sensitive to  $\alpha$ -keto acid concentration as it serves both as a substrate and as an agent that facilitates the conversion of the BCKDH to the active form. Phenylalanine hydroxylase, the flux-generating step in phenylalanine catabolism, affords another example of regulation by substrate concentration and by covalent modification. The enzyme can exist in both dimeric and tetrameric forms. Glucagon activates the enzyme by means of protein kinase A phosphorylation. In addition to its role as a substrate, phenylalanine promotes its conversion to the more active, tetrameric form. Both types of activation appear to act synergistically. Again, as in the control of BCKDH, this mechanism produces a regulatory system that is particularly sensitive to substrate (phenylalanine) concentration, much more so than could be accomplished via Michaelis–Menten kinetics alone.

### Hepatic zonation of amino acid metabolism

Metabolic heterogeneity of hepatocytes along the hepatic acinus is also an important feature of hepatic amino acid metabolism. This phenomenon is well established for both glutamine and ornithine metabolism. The liver contains both glutaminase and glutamine synthetase. However, they are not expressed in the same hepatocytes. Glutaminase is found in the periportal region, for about the first third of the hepatic acinus, and can efficiently provide ammonia to carbamoylphosphate synthetase for urea synthesis. Glutamine synthetase is found in the perivenous hepatocytes where it is restricted to a layer of no more than one or two cells, which surround the terminal hepatic venule. Glutamine synthetase plays an important role in determining the low ammonia concentration of blood that leaves the liver, as it removes ammonia which has escaped the urea cycle [8]. Ornithine aminotransferase is also restricted to the same perivenous hepatocytes as glutamine synthetase; indeed, the entire pathway of arginine catabolism occurs in these hepatocytes [9]. Thus, the localization of the arginine catabolic pathway in hepatocytes that do not contain the urea cycle ensures that urea synthesis is not compromised by catabolic depletion of the cycle intermediates.

### Hepatic amino acid metabolism during starvation

Gluconeogenesis from amino acids plays a crucial role during starvation in providing glucose for obligatory glucose-utilizing tissues, particularly the brain. These amino acids are primarily derived from skeletal muscle although, in the very short term, amino acids derived from hepatic proteolysis are also employed. The pattern of amino acids released by muscle is not a reflection of the amino acid composition of muscle protein. Rather, there is a considerable intramuscular amino acid metabolism that reconfigures the pattern of released amino acids. More than 50%



**Fig. 1** Net splanchnic and peripheral exchange of amino acids in normal, postabsorptive man. Reproduced, with permission, from ref. 10.

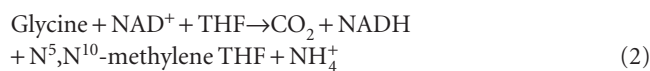
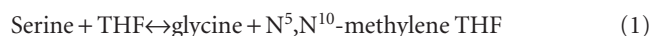
of the released amino acids consist of alanine and glutamine. The carbon skeleton of alanine primarily arises from pyruvate of glycolytic origin. Its amino group arises, via transamination, from other amino acids, principally the branched-chain amino acids. This alanine is converted to glucose by the liver. This 'glucose-alanine cycle' plays a major role in ferrying these amino groups to the liver for detoxification, without the risk of hyperammonaemia. However, it does not really produce 'new' glucose; rather, it recycles glucose carbon, and some investigators have referred to it as 'glucopalaeogenesis' to emphasize the point that it produces 'old' glucose. The glutamine released by muscle has a variety of fates, including renal metabolism for acid-base regulation and use as a fuel by the small intestine. Alanine is an important product of intestinal glutamine metabolism and also becomes available to the liver for gluconeogenesis. Alanine is, therefore, generally acknowledged as the principal glucogenic amino acid. Yet, the role played by other amino acids should not be overlooked. Such amino acids as glycine, lysine, proline, threonine, histidine, arginine, phenylalanine and tyrosine are released by muscle and converted to glucose by the liver (Fig. 1). Although individually of less importance than alanine and glutamine, together they make a substantial contribution to hepatic gluconeogenesis [10].

### Specialized functions of amino acids

Amino acids display considerable metabolic plasticity and, therefore, play a variety of roles. These include roles in one-carbon metabolism, biosyntheses, glutathione function and cell signalling. A number of these roles are highlighted below, rather than providing an exhaustive account.

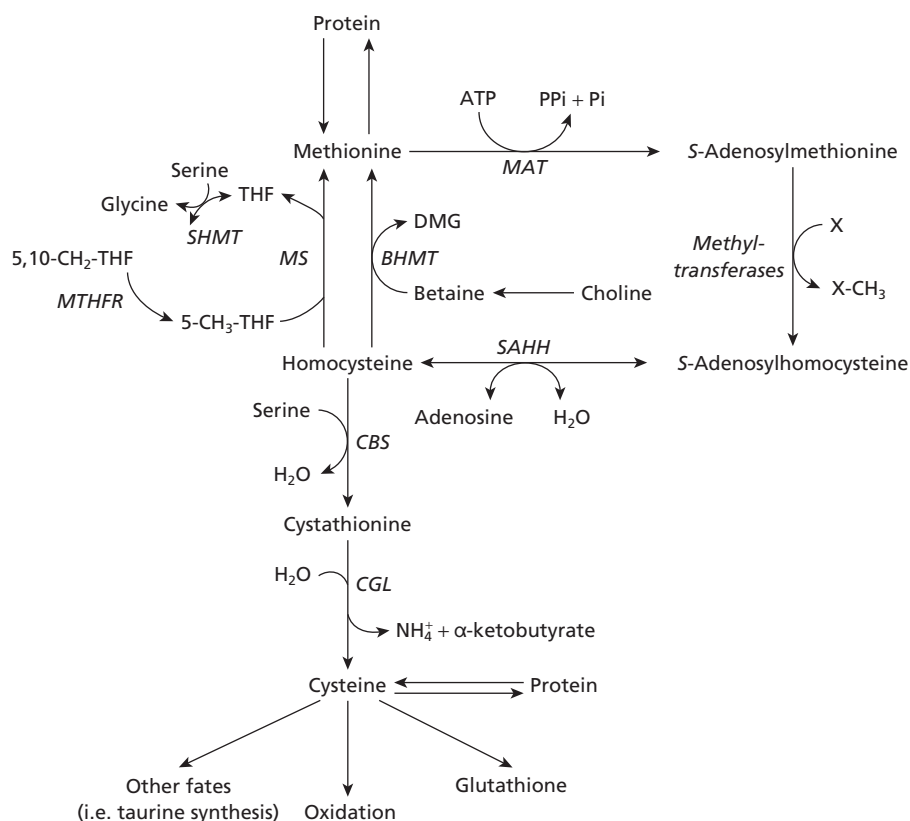
### Amino acids, methylation reactions and one-carbon metabolism

The folic acid, one-carbon pool provides cells with considerable flexibility in providing one-carbon groups of different oxidation states for biosynthetic and other purposes. Amino acids are the key providers of these one-carbon groups, which are generated during the catabolism of glycine, serine, histidine and tryptophan. The catabolism of serine and glycine probably supplies the bulk of the one-carbon pool by the reactions catalysed by serine hydroxymethyltransferase (reaction 1) and the glycine cleavage enzyme (reaction 2):



(Tetrahydrofolate is abbreviated as THF). In addition, labile methyl groups are provided directly in the diet as methionine and choline. The one-carbon pool provides one-carbon groups for functions such as the synthesis of purine nucleotides and thymine. However, it is the abundance and variety of methylation reactions that best illustrate the importance of one-carbon metabolism.

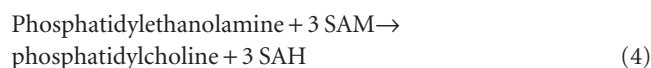
Figure 2 provides an outline of methionine metabolism (also see Chapter 2.3.9). The key first step is the conversion of methionine to *S*-adenosylmethionine (SAM). SAM is the universal methyl donor for a remarkably large number of methylation reactions. Indeed, a recent bioinformatic analysis of a number of genomes, including human, suggests that between 0.6% and 1.6% of all genes may code for methyltransferases.



**Fig. 2** Methionine metabolism in liver. MAT, methionine adenosyltransferase; SAHH, S-adenosylhomocysteine hydrolase; BHMT, betaine:homocysteine methyltransferase; SHMT, serine hydroxymethyltransferase; MTHFR, methylenetetrahydrofolate reductase; CBS, cystathionine  $\beta$ -synthase; CGL, cystathionine  $\gamma$ -lyase; MS, methionine synthase.

Methyltransferases catalyse the transfer of methyl groups from SAM to an acceptor to produce the methylated acceptor and S-adenosylhomocysteine (SAH) [11]. These methyltransferases include enzymes involved in the synthesis of small molecules (e.g. creatine, carnitine, phosphatidylcholine, epinephrine), methylation of nucleic acids (DNA methylation is a major mechanism for the regulation of gene expression, chromosome inactivation and genomic imprinting), RNA methylation (e.g. RNA capping), methylation of specific amino acid residues of proteins (e.g. protein repair, intracellular localization) and detoxification (e.g. methylation of arsenite and xenobiotic compounds).

Although methylation reactions occur in all cells of the body, they are of the greatest quantitative significance in the liver. Creatine synthesis (reaction 3) and phosphatidylcholine synthesis (reaction 4) account for about 70% of SAM utilization in the body:



Reaction (3) is catalysed by guanidinoacetate methyltransferase (GAMT) and reaction (4) by phosphatidylethanolamine methyltransferase (PEMT). The liver is the dominant site of both GAMT and PEMT expression.

SAH, once produced by methylation reactions, is hydrolysed to adenosine and homocysteine [11]. Some of this homocysteine is released into the plasma. We now appreciate that the liver is the principal source of plasma homocysteine because of the quantitatively dominant role played by the liver in methylation reactions. This is significant because elevated plasma homocysteine has been implicated as a risk factor for cardiovascular disease, Alzheimer's disease and fractures. Metabolism of homocysteine, rather than its release into the circulation, is the fate of most of the homocysteine that arises within the liver. Homocysteine may be metabolized in two different ways – by trans-sulphuration and by remethylation. The trans-sulphuration pathway consists of two enzymes, cystathionine  $\beta$ -synthase and cystathionine  $\gamma$ -lyase. This sequence of reactions is irreversible, which means that methionine can be converted to cysteine but cysteine cannot be converted to methionine. This, in turn, provides the biochemical basis for the well-known nutritional observation that methionine is an essential amino acid but that cysteine is not essential, provided that an adequate supply of methionine is available.

Homocysteine metabolism also occurs via remethylation to methionine [11]. In the liver, this can occur via two quite separate enzymes. Methionine synthase, one of only two vitamin B12-requiring enzymes in mammals, employs  $\text{N}^5$ -methylTHF as a methyl donor. This  $\text{N}^5$ -methylTHF arises as a result of the reduction of  $\text{N}^5, \text{N}^{10}$ -methyleneTHF by reduced nicotinamide

adenine dinucleotide phosphate (NADPH), a reaction catalysed by methylenetetrahydrofolate reductase (MTHFR). Methionine synthase occurs ubiquitously throughout the body. However, a second enzyme, betaine:homocysteine methyltransferase (BHMT), which is found in the liver and kidneys of humans, can also remethylate homocysteine to methionine. In this case, the methyl group is provided by betaine, which arises during choline catabolism and is also a minor dietary constituent.

Methionine metabolism provides a fascinating example of the interplay between amino acid metabolism and vitamin status. Deficiencies of a number of B vitamins are known to cause elevated plasma homocysteine. As the two enzymes of the trans-sulphuration pathway contain pyridoxal phosphate as prosthetic groups, pyridoxal deficiency impairs this pathway and increases homocysteine levels. As methionine synthase contains methylcobalamin as its prosthetic group and employs N<sup>5</sup>-methylTHF as a substrate, it is hardly surprising that deficiency of either vitamin B12 or folic acid results in elevated plasma homocysteine [12].

Deeper analysis reveals more sophisticated interactions. Methionine synthase can be viewed as playing two critical roles. On the one hand, homocysteine retains the carbon skeleton of methionine, and its methylation conserves this essential amino acid. Support for this function of methionine synthase is afforded by findings that a higher proportion of homocysteine is remethylated to methionine on feeding a diet low in methionine. However, methionine synthase also plays a crucial role in folate metabolism. This is best exemplified by the methyl trap hypothesis, which accounts for the fact that cobalamin deficiency often results in a functional folate deficiency [13]. This hypothesis postulates that cobalamin deficiency, which leads to decreased methionine synthase activity, will result in an accumulation of much of a cell's folate as N<sup>5</sup>-methylTHF, with consequent depletion of the other THF coenzymes. The methyl trap is exacerbated by the fact that N<sup>5</sup>-methylTHF is the principal folate form that is provided to cells from the circulation and must be metabolized by methionine synthase to provide the other THF coenzymes that are required by the cell. Clearly, there is a very close interrelationship between the metabolism of methionine, homocysteine and folate.

### Creatine synthesis

Although the liver does not employ the creatine kinase system for its energy metabolism, it is very much involved in creatine synthesis. Creatine and creatine:phosphate break down spontaneously to creatinine, which is excreted in the urine. In young adults, some 1.5–2 g of creatinine are excreted each day, which means that a comparable quantity of creatine must be replaced. Replacement occurs by a combination of diet and synthesis in omnivores, but strict vegetarians need to synthesize all their creatine. Creatine synthesis involves a very simple metabolic pathway, but it requires no fewer than three amino acids. The first step, catalysed by the glycine:L-arginine amidinotransferase

(AGAT), involves the transfer of an amidino group from arginine to glycine to produce guanidinoacetate and ornithine (reaction 5):



The second reaction involves the methylation of guanidinoacetate to produce creatine (reaction 3). This reaction is predominantly found in the liver, which is the principal site of creatine synthesis. However, in humans, both liver and kidney can produce guanidinoacetate. A consideration of the quantitative aspects of creatine synthesis is revealing. After accounting for the 0.5 g of creatine that may be obtained in the diet, it is calculated that an 18- to 29-year-old man will synthesize about 1.5 g of creatine per day. This requires 2 g of arginine, which is about 40–50% of all the arginine ingested by an individual with a typical North American protein intake. In addition, as noted above, creatine synthesis consumes approximately 35% of SAM-derived methyl groups. Creatine synthesis is clearly a major pathway in amino acid metabolism.

### Glutathione synthesis and function

(see also Chapter 2.3.9)

Glutathione ( $\gamma$ -glutamyl-cysteinyl glycine) plays important roles in many tissues but is particularly important in the liver. It exists in both reduced (GSH) and oxidized glutathione disulphide (GSSG) forms, which are interconverted by the enzyme glutathione reductase (reaction 6):



In cells, the great bulk of glutathione (~ 95%) occurs in the reduced form.

A detailed review of glutathione metabolism is found in Chapter 2.3.9.

### Regulatory functions of amino acids in liver

In addition to their roles as substrates for key biosyntheses (e.g. protein, glutathione and creatine synthesis) and in intracellular metabolism (urea cycle, malate–aspartate shuttle), amino acids are important regulatory molecules that act through signalling pathways. In the liver, this is particularly apparent in the control of protein synthesis and proteolysis, as both are very active processes. Although the liver only accounts for about 2% of body weight, it is responsible for about 18% of total body protein synthesis (53 g/day in a 70-kg man) [14]. This rate of hepatic protein synthesis can increase substantially during inflammation and infections when the synthesis of acute-phase proteins is greatly increased. In healthy individuals, the liver produces about 15–20 g of albumin per day; this is very sensitive to nutritional status, in particular to protein intake. Hepatic proteolysis, which is a highly active process, is also sensitive to nutritional status, being markedly increased early in starvation. Amino

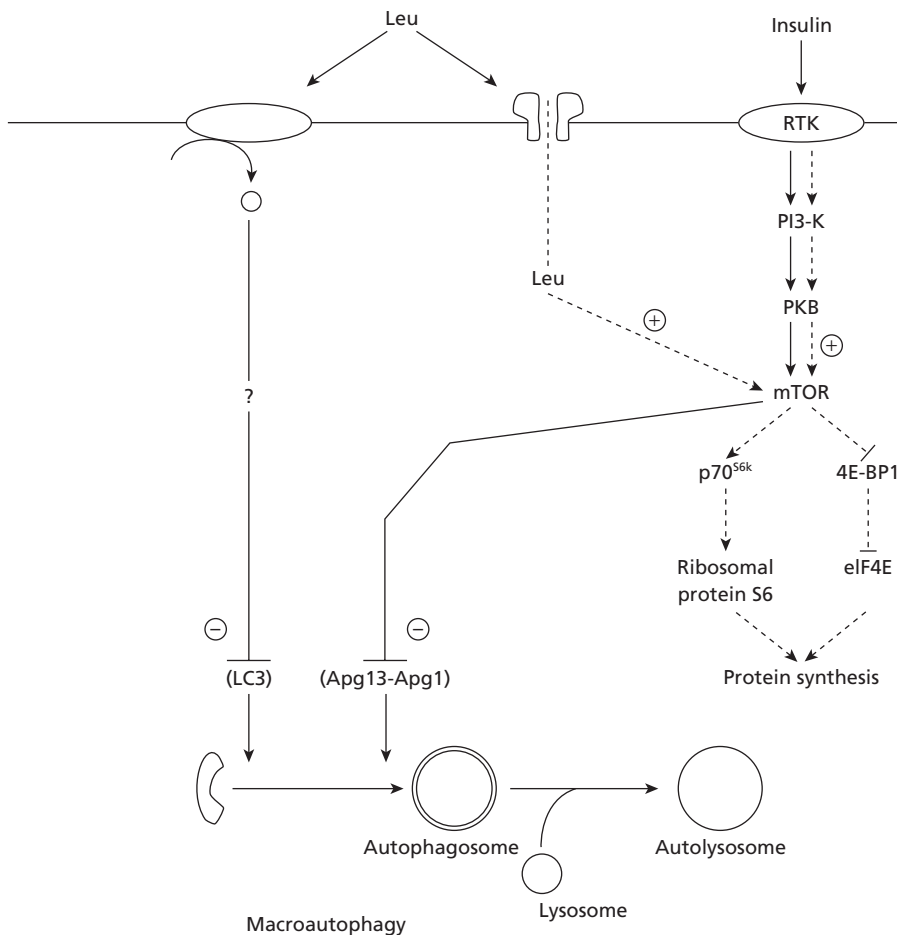
acids, together with insulin, are major controllers of both hepatic protein synthesis and proteolysis.

The synthesis of individual proteins is generally regulated by rates of gene expression, which affect the abundance of individual mRNAs. In addition to this primarily transcriptional control of the synthesis of specific proteins, regulation also occurs at the translational level and can affect the synthesis of classes of proteins or even of all proteins. Amino acids and insulin increase hepatic protein synthesis through partly shared signalling networks. Leucine is the principal amino acid that promotes protein synthesis in a variety of tissues, including the liver. Its effects in liver are not as global as in muscle, although it does increase the synthesis of a number of strategically important proteins. An outline of the mechanism of this effect has become apparent through the work of Kimball and Jefferson [15], although much remains to be learned.

Provision of leucine to hepatocytes results in enhanced phosphorylation of 4E-BP1 (the binding protein for the eukaryotic initiation factor, eIF4E) and S6K1, the ribosomal protein S6 kinase. Enhanced phosphorylation of 4E-BP1 releases eIF4E, which is required for the binding of mRNA to the 43S preinitiation complex. Phosphorylation of S6K1 results in its activation and, subsequently, in the phosphorylation of ribosomal protein

(rp) S6. In turn, the phosphorylation of rpS6 results in the enhanced translation of mRNAs that contain an uninterrupted stretch of pyrimidine bases adjacent to the 5' cap. Proteins encoded by these mRNAs include ribosomal proteins, elongation factors and polyA-binding proteins. Both 4E-BP1 and S6K1 are downstream targets of the serine/threonine protein kinase, mTOR, which is viewed as a key target of leucine's action. Amino acids that increase cell volume (*vide infra*) can also stimulate hepatic protein synthesis.

Hepatic protein turnover is mediated both by proteasome-catalysed proteolysis and by macroautophagy; the latter process is dominant in the liver and is regulated by both insulin and amino acids [16]. Macroautophagy is a complex process in which cytoplasmic material is sequestered in autophagosomes, followed by acidification of these organelles and their fusion with lysosomes. It is a non-specific process in that most constituents of the cytosol are engulfed and degraded. However, proteins constitute the great bulk of cytoplasmic material, so that macroautophagy is rightly characterized as a means of effecting protein turnover. There seem to be two parallel means whereby amino acids suppress macroautophagy. Amino acids that are transported into cells via sodium-linked, concentrative transporters tend to increase cell hydration and inhibit



**Fig. 3** Proposed signalling pathways for leucine (Leu) and regulatory amino acids in the control of macroautophagy and protein synthesis. One pathway for macroautophagy is independent of mTOR, does not affect protein synthesis and may involve leucine's interaction with a plasma membrane sensor. The other signalling pathway involves an intracellular leucine sensor, requires mTOR and both stimulates protein synthesis and suppresses macroautophagy; it shares common elements with insulin's signalling pathway. Reproduced, with permission, from ref. 18.

macroautophagy via a mitogen-activated protein (MAP) kinase-mediated mechanism. The other mechanism involves amino acids (e.g. leucine, phenylalanine and tyrosine) that are not concentrated within the liver but appear to exert their actions via an mTOR-mediated pathway.

Recent studies have clarified the mechanism by which glutamine and system A substrates affect macroautophagy. The accumulation of these amino acids within cells, together with the cotransported Na<sup>+</sup> (which is partly exchanged for K<sup>+</sup>), is followed by a flux of water into the cell to equilibrate the osmotic pressure on both sides of the cell membrane. The resulting increase in cell volume (cell hydration) results in an immediate activation of MAP kinases, in particular ERK and p38<sup>MAPK</sup>. This latter kinase brings about the inhibition of macroautophagy at the level of the formation of the autophagosome [17]. More work is needed to elucidate the complete mechanism, but the key role of cell swelling is confirmed by experiments that demonstrated a comparable degree of inhibition of proteolysis when cells were swollen in hypo-osmotic media.

Amino acids such as leucine, phenylalanine and tyrosine are agonists for another mechanism whereby amino acids inhibit macroautophagy [16,18]. The mechanism appears to involve mTOR, as rpS6, which is downstream from mTOR, becomes rapidly phosphorylated with the same kinetics as the inhibition of proteolysis. Rapamycin, an inhibitor of mTOR, could partly overcome the inhibition of autophagic proteolysis by amino acids. Again, much work remains before we will have a complete picture of the mechanism. The phosphorylation of rpS6 does not implicate this protein in the inhibition of macroautophagy; rather, it should be taken as indicating an efficient regulatory system in which mTOR activation plays a role in both the stimulation of protein synthesis and the suppression of proteolysis. There is also evidence for an mTOR-independent mechanism whereby these amino acids can suppress macroautophagy. This appears to be another example of redundant cell signalling systems. Figure 3 summarizes these mechanisms.

These new insights into amino acid-mediated cell signalling have considerable physiological significance. Amino acids signal their availability for anabolic processes, not only by being available as substrates, but also by more sophisticated mechanisms. Simultaneously, they signal the need to suppress catabolic processes. mTOR and cell hydration emerge as important 'interpreters' of amino acid availability. Recent studies also suggest that leucine may act via mTOR-independent mechanisms. It is clear that mTOR is not only an amino acid-responsive kinase but also a more general nutrient sensor; it can be antagonized by the adenosine monophosphate (AMP)-stimulated kinase which responds to the AMP/ATP ratio. The interaction and synergy of amino acid signalling with that of insulin remains to be fully elucidated. Finally, a crucial unsolved issue is the identification of the precise molecule(s) that interact(s) with regulatory amino acids such as leucine. However, it can be confidently asserted that recent work on amino acid signalling has revealed a new vista in amino acid metabolism that is likely to become even more important in the future.

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### 2.3.4 Mitochondria and energy formation

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Mitochondria are double-membrane organelles derived evolutionarily from bacteria. They have their own DNA and reproduce asexually, but only women transmit mitochondrial DNA. Mitochondria represent remarkable biochemical machines that are able to harness the energy produced by the oxidation of fat and other energetic substrates in a form usable by the cell.