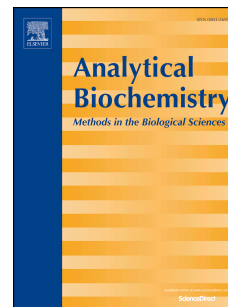# Journal Pre-proof

Amino acids, ammonia, and hepatic encephalopathy

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Amino Acids, Ammonia, and Hepatic Encephalopathy

Cirrhotic patients have elevated protein requirements

Ingested protein → Ammonia

Protein intake may precipitate hepatic encephalopathy

Safe method of protein intake is needed
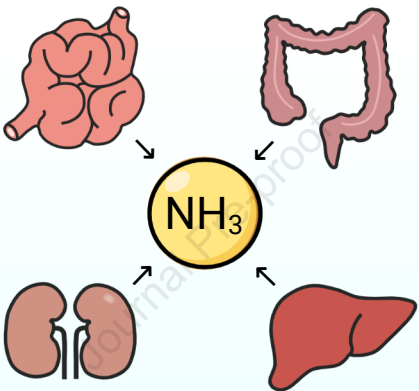
Ammonia is generated from the catabolism of amino acids in various organs

Glutamate Dehydrogenase

Glutaminase
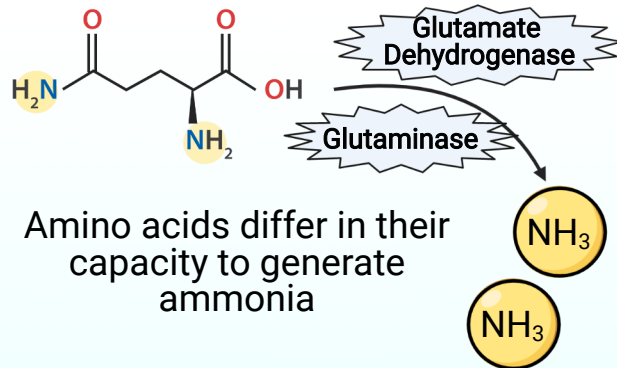
Amino acids differ in their capacity to generate ammonia

Arg, Asn, Gln, His, Lys, Met have increased ammoniagenic potential

Conclusion: Modifying the intake distribution of amino acids may be a solution to provide proper protein intake while decreasing hyperammonemia and risk of hepatic encephalopathy in cirrhotic patients

Amino Acids, Ammonia, and Hepatic Encephalopathy

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Abstract

Hepatic encephalopathy (HE) is a decline in brain function arising due to liver insufficiency. The liver's diminished capacity to clear ammonia, and the subsequent accumulation of it, is highly implicated in pathogenesis of HE. Ammonia is endogenously generated from the catabolism of amino acids derived from dietary protein intake. Therefore, a conflict arises in cirrhosis where dietary protein intake may increase ammonia and precipitate HE, and at the same time, cirrhotic patients require high daily protein intake due to altered nutrient metabolism. A nutritional solution is needed to deliver sufficient doses of protein to patients without increasing the risk of HE. In order to address this issue, this review will discuss the catabolism of individual amino acids with a special focus on ammonia-generation steps and highlight a subset of amino acids that have the potential to generate multiple equivalents of ammonia. Following, studies investigating the effects of individual amino acids in cirrhosis on blood ammonia levels as well as development of HE will be reviewed.

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List of Abbreviations

BCAA: branched-chain amino acid

GDH: glutamate dehydrogenase

GS: glutamine synthetase

HE: hepatic encephalopathy

IAAO: indicator amino acid oxidation

LOLA: L-ornithine L-aspartate

OP: ornithine phenylacetate

TCA: tricarboxylic acid

1. Introduction

In response to liver failure, brain function changes. Hepatic encephalopathy (HE) is a devastating neuropsychiatric complication that arises due to liver insufficiency, affecting up to 80% of patients with cirrhosis [1]. It manifests as a wide spectrum of symptoms ranging from subclinical impairments in attention, memory, motor coordination, and personality changes, to severe symptoms including debilitating disorientation and confusion, asterixis, lethargy, and stupor. HE immensely impacts the quality of life of not only patients [2] but also their caregivers [3]. HE can require hospitalization and represents a significant economic burden with the 2014 national charges related to HE in the United States totalling $11.9 billion [4]. Furthermore, HE is a significant strain on the healthcare system with 31, 182 patients with HE requiring hospitalization in the United States in 2014 [4]. HE can progress to coma and death, representing a 64% one-year mortality rate in patients with alcoholic liver disease [5].

Although the pathophysiology of HE is incompletely understood and contributing factors such as systemic inflammation and reactive oxygen species have been identified, elevated ammonia remains central to pathogenesis [6]. While the association between the severity of HE and blood ammonia levels is controversial [7,8], blood ammonia levels have been shown to be an important factor in determining mortality, with higher ammonia levels associated with increased risk of mortality in patients with acute-on-chronic liver failure [9], acute liver failure [10] and chronic liver disease [11]. Additionally, it has been found that fasting ammonia levels predict not only the risk but also the frequency of HE episodes [12]. In line with this, the reduction of serum ammonia is associated with better outcomes [13] and increased ammonia is associated with deterioration [14]. Therefore, the prognostic role of ammonia in patients with cirrhosis has been well defined.

Ammonia is a natural, endogenous molecule generated as a by-product of protein ingestion via the catabolism of amino acids, small nitrogenous components of proteins. Ammonia, NH_3, and ammonium NH_4^+, are collectively referred to as ammonia throughout the text. The structure of amino acids consists of an amine group (NH_2), a carboxylic acid group (COOH), and a variable R group, or side chain, that is unique to each amino acid (Figure 1). The chemistry of the side chain defines an amino acid with-specific properties such as charge, alkalinity, and solubility. Amino acids generated within the body are non-essential, in contrast to essential amino acids that need to be ingested through diet since they cannot be endogenously synthesized. Some non-essential amino acids can also be categorized as semi-essential or conditionally essential. These amino acids can typically be synthesized in sufficient quantities in health, but during

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distinct conditions such as infancy, illness, injury, or post-surgery, their capacity to be synthesized may fall short in fulfilling daily requirements.

Once ingested, proteins are catabolized to peptides and amino acids via proteases in the stomach and small intestine, the latter in which they are subsequently absorbed. The cells of the intestine drain to the portal vasculature; the venous system that connects the gastrointestinal system with the liver. Therefore, nutrient- and amino acid-rich blood from the gut is carried directly to the liver for metabolic processing before joining the systemic circulation.
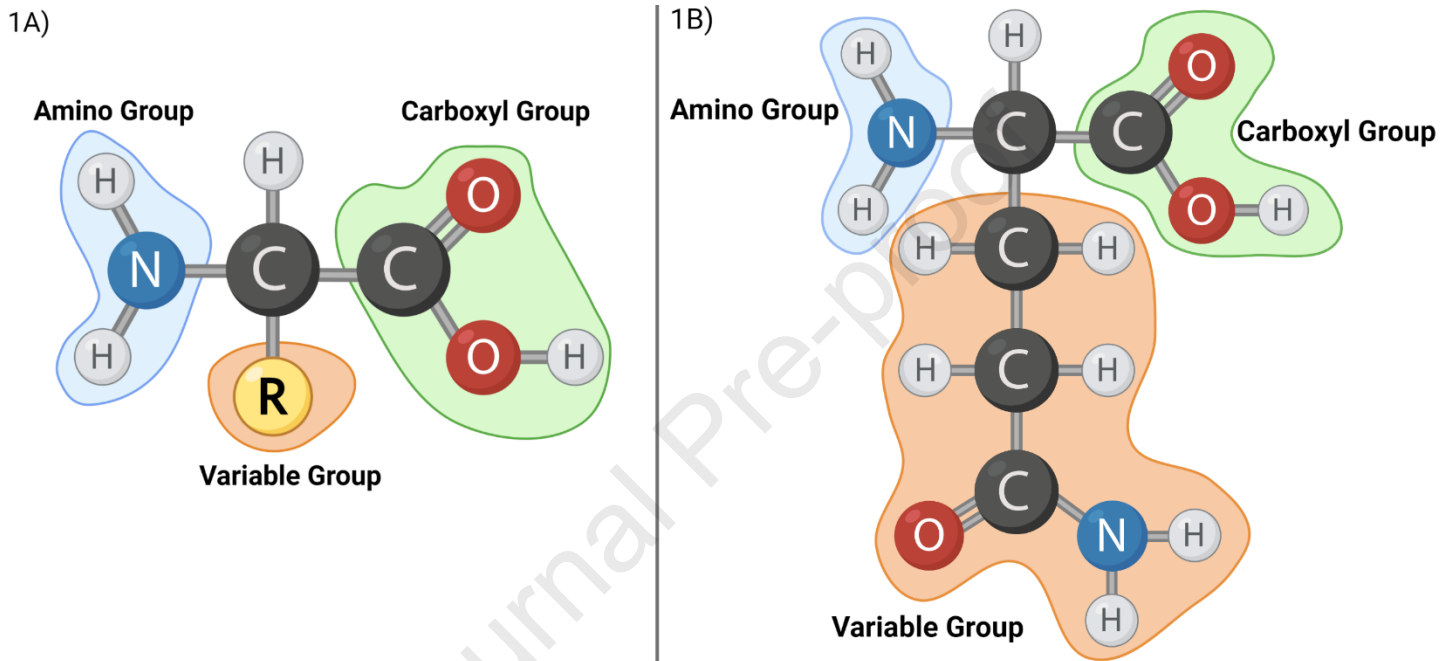
Figure 1. The Structure of Amino Acids. 1A) The general structure of an uncharged amino acid. The charge of the amino acid will vary depending on the pH of its environment. 1B) The structure of glutamine, an example of an amino acid with multiple nitrogen atoms.

Plasma ammonia levels can rise to 0.2–1 mM in patients with liver damage and beyond 1mM in patients with inborn errors of the urea cycle [15]. Ammonia at elevated concentrations is neurotoxic, contributing to altered brain energetics [16], morphological changes in astrocytes [17], and disrupted glutamate-glutamine cycling [18]. Its homeostasis is therefore highly controlled in the body. Typically, ammonia generated from amino acid catabolism is regulated in the liver via two clearance mechanisms housed in separate hepatic zones [19] (Figure 2). The urea cycle is the main ammonia detoxification pathway and consists of a series of five enzymes that together are exclusively expressed in the liver. Urea synthesis takes place in the periportal zone, an area that is characterised by a high capacity for uptake and catabolism of

amino acids and gluconeogenesis. The urea cycle represents a low-affinity high-capacity pathway for ammonia excretion. Ultimately, two amino groups (one from free ammonia, one from aspartate) are incorporated into urea. Urea is subsequently excreted by the kidneys in the urine.

Another ammonia clearance mechanism in the liver is the ATP-dependent enzyme glutamine synthetase (GS, EC 6.3.1.2.), also called glutamate ammonia ligase. GS catalyses the reversible addition of ammonia to glutamate to generate glutamine. GS is housed in the perivenous hepatocytes where glycolysis, liponeogenesis, and glutamine formation are the main metabolic processes. GS acts as an ammonia scavenger, with a high affinity for ammonia but low capacity. Taken together, the urea cycle and GS serve to regulate and maintain blood ammonia levels between 35-50 μM. However, in the disease state of cirrhosis, the liver is not adequately functional due to high levels of hepatocyte death and therefore the capacity to clear ammonia is drastically reduced. Consequently, this results in elevated systemic and cerebral concentrations of ammonia, causing neurological deficits and HE.

Since amino acids are the primary source of ammonia generation, a treatment strategy arose in the 1950s wherein a low-protein diet was recommended to patients with cirrhosis in order to decrease HE risk by decreasing ammonia generated
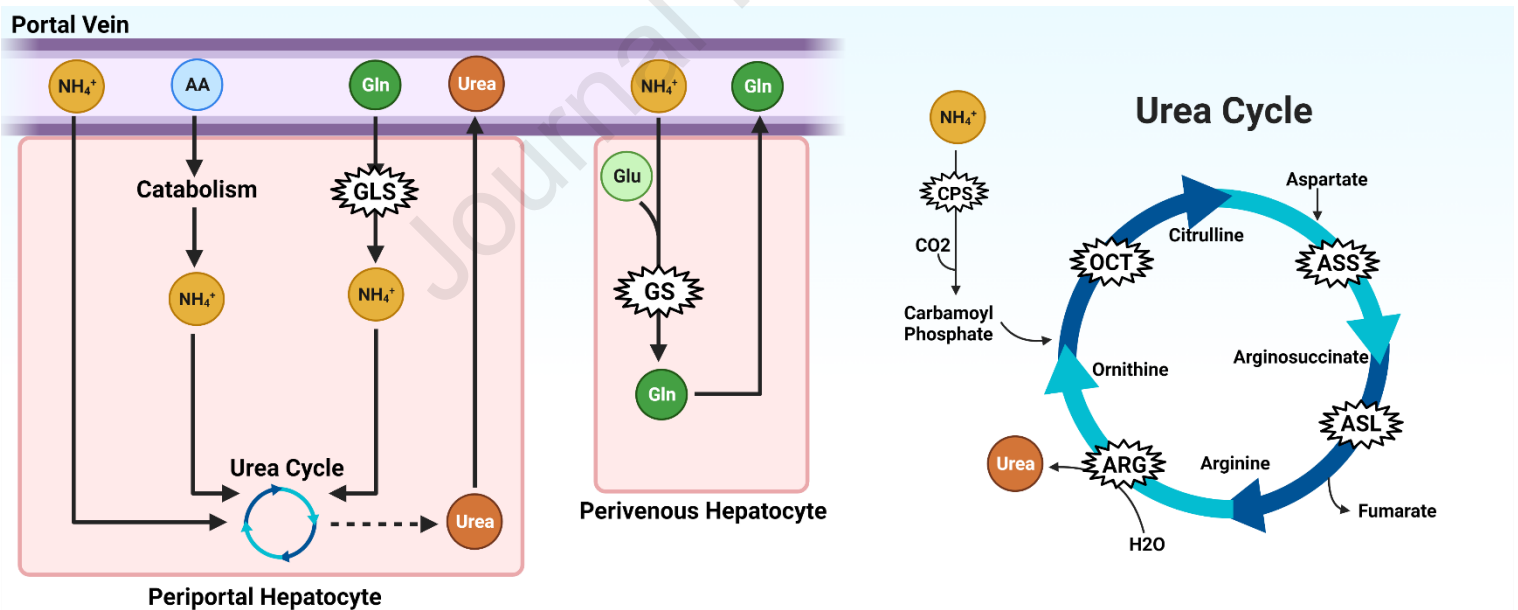
Figure 2: Ammonia Clearance in Hepatocytes. Ammonia and amino acids are taken up from the portal vein into periportal hepatocytes. Here, amino acids are catabolized and generate one or more molecules of ammonia. The ammonia absorbed from the portal vein and generated from amino acid catabolism is cleared through the enzymes of the urea cycle to generate urea, which goes into systemic circulation. In perivenous hepatocytes, the ammonia absorbed from the portal vein is cleared through the enzyme glutamine synthetase to generate a molecule of glutamine, that is deposited back into the venous system. Abbreviations: NH4+; ammonia, AA; amino acid, Gln; glutamine, GLS; glutaminase, Glu; glutamate, GS; glutamine synthetase, CPS; carbamoyl phosphate synthetase, OCT; ornithine carbamoyl transferase, ASS; arginosuccinate synthetase; ASL; arginosuccinate lyase; ARG; arginase.

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from amino acid catabolism [20]. However, the low-protein diet nutritional strategy does not confer any benefits to patients and increases protein breakdown when compared with a regular protein diet [21]. Furthermore, low protein intake is an independent risk-factor for malnutrition in liver disease [22], which in turn increases mortality [23]. Therefore, protein restriction is no longer recommended and current nutritional recommendations for cirrhotic patients have been updated to include a diet with protein levels increased to 1.2-1.5g/kg daily from 0.8g/kg daily recommended for healthy adults [24]. These recommendations aim to correct the high risk of sarcopenia in cirrhotic patients. Sarcopenia is defined as a significant loss of muscle mass and affects up to 90% of cirrhotic patients, primarily due to protein-calorie malnutrition [25]. In alcoholic liver disease this issue of malnutrition may be exacerbated by the consumption of alcohol, a calorie-rich but nutrient-poor substance. Patients are often not meeting their daily dietary needs due not only to poor intake but also hypermetabolism (increased nutritional requirements) and malabsorption of nutrients [26]. Furthermore, increased ammonia toxicity negatively impacts muscle mass as well [26]. Hyperammonemia results in both decreased muscle protein synthesis as well as increased muscle catabolism, further contributing to the loss of muscle mass [27]. Sarcopenia in patients with cirrhosis is associated with mortality risk both pre- and post-liver transplantation, poor quality of life, and increased HE risk [28].

The consequences of sarcopenia in cirrhosis go beyond malnutrition as muscle tissue plays an important compensatory role in the removal of circulating ammonia during liver disease [29]. This occurs through the enzyme GS, the same deaminating reaction found in perivenous hepatocytes. While GS is active across many tissues such as the brain, the capacity of ammonia clearance by the muscle is significant due to its volume and mass; Skeletal muscle represents up to 38.4% of body weight [30]. Therefore, the presence of sarcopenia in cirrhosis represents a further impairment in the capacity to clear ammonia. As a result, reducing the prevalence of sarcopenia and supporting the maintenance of muscle mass through increased protein intake is incredibly important. However, the effects of a high protein diet in cirrhosis remain unknown. While patients with cirrhosis have a clear metabolic need for elevated protein intake, an increased intake may trigger HE. Increasing protein intake may result in increased ammonia production by providing more amino acids to catabolize to ammonia, causing hyperammonemia. Though current clinical AASLD guidelines for HE do not recognize a high protein meal/diet as a precipitating factor [1], several clinical studies have documented diet as a precipitant, reporting up to 58% of HE cases to be precipitated by food [31, 32, 33, 34, 35]. High quality studies are required to thoroughly

understand the effects of diet on HE since the possible detriment of a high protein diet cannot be ignored but is not yet confirmed. Furthermore, the diagnosis of HE has a high rate of unidentifiable precipitating factors, with some studies finding as high as 55.8% of cases to be unprecipitated or spontaneous [36]. It is plausible that high protein diet may be accountable for a portion of these spontaneous cases of HE.

As a result, protein intake in cirrhotic patients may seem like a double-edged sword where a low protein diet cannot be recommended, but a high protein diet carries the risk of elevating blood ammonia levels. Therefore, adding sufficient protein to the diet to support muscle mass and nutritional status without increasing HE risk becomes challenging. Ingested amino acids have differing metabolic pathways as well as number of nitrogen atoms, and as a result generate unpredictable number of ammonia molecules from their catabolism. Consequently, some amino acids have high potential to generate ammonia and therefore may be more detrimental to the health of cirrhotic patients when compared to those that generate less ammonia. Therefore, the safety of protein intake may depend not only the quantity of protein intake but also the amino acid composition and metabolic state of cirrhotic patients.

2. Cellular Amino Acid Catabolism

The complete catabolism of amino acids to their respective energy intermediates generates free ammonia. Since human cells lack many deaminating enzymes that other organisms such as bacteria possess, most amino acids are not directly deaminated, but instead lose their amine group through a transamination-deamination reaction that can occur in virtually all organs. In the first step of this reaction, the transamination, the amine group is transferred from an amino acid to an α-ketoacid, usually α-ketoglutarate. The products of this reaction are glutamate (from the amination of α-ketoglutarate) and the resulting α-keto acid of the original amino acid with no free ammonia generated. The α-keto acid can then enter energy producing pathways. Amino acids that generate gluconeogenesis intermediates are called glucogenic. Glucogenic amino acids can enter the gluconeogenic pathway as pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate. Those that can be metabolised into acetoacetate or acetyl-CoA are referred to as ketogenic. While there are amino acids that are both glucogenic and ketogenic, most amino acids are solely glucogenic. Table 1 shows the energy intermediates each amino acid can create.

Following its generation through transamination, glutamate can then be deaminated by the enzyme glutamate dehydrogenase (GDH, EC 1.4.1.2). GDH catalyses the deamination of glutamate to produce α-ketoglutarate and in this

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step, a free molecule of ammonia is generated. This is significant because many amino acids are transaminated to form glutamate in the process of catabolism and therefore, indirectly, generate a molecule of ammonia through the deamination of glutamate. Consequently, all amino acids have the capacity to form at least one molecule of ammonia by the formation of glutamate. While a small subset of amino acids have the capacity to be directly deaminated, this is a less common catabolic pathway and does not result in additional generation of free ammonia. On the contrary, some amino acids contain more than one nitrogen atom and therefore have the potential to generate more than one equivalent of ammonia.

A summary of catabolic pathways for the standard twenty amino acids that make up proteins in the human body can be found in Table 1. It is interesting to note that the number of possible ammonia molecules generated from the catabolism of individual amino acids varies; fourteen of amino acids have the capability to produce one free molecule, whereas six have the potential to generate two molecules of ammonia. However, not all amino groups are catabolised to generate free ammonia.

Below are the common catabolic pathways of coded amino acids that can generate multiple molecules of ammonia. However, amino acids can be used in a plethora of reactions, including direct incorporation into enzymes or structural proteins. Therefore, they are not necessarily fully degraded into metabolic intermediates but instead can be kept intact, in which case no ammonia is generated. Consequently, the below analysis of ammonia generation by the catabolism of amino acids is one of many possible pathways and describes the maximal possible ammonia generation rather than a certainty.

Arginine

Arginine can maximally generate two molecules of ammonia, despite having four nitrogen atoms. Arginine is a versatile amino acid which is involved in a number of metabolic pathways. Focusing on the generation of energy intermediates, arginine is catalysed by the enzyme arginase (EC 3.5.3.1), cleaving arginine into ornithine and urea, each product taking two of the original nitrogen atoms. Urea is excreted by the kidneys in the urine and therefore no free ammonia is generated. Ornithine is used in multiple pathways, including that of the urea cycle and within the brain [37]. When metabolised to an energy intermediate, ornithine generates glutamate and proline through transamination of the terminal amine by ornithine aminotransferase in a multi-step pathway (EC 2.6.1.13). The further metabolism of proline generates a molecule of glutamate through oxidoreduction (EC 1.5.5.2, EC 1.2.1.88). As discussed above, glutamate can further be

metabolised to generate a molecule of ammonia via GDH. Therefore, arginine has the potential to generate two ammonia molecules from its full metabolism, both resulting from the deamination of glutamate.

Asparagine

The first step of the main pathway for asparagine metabolism is catalysed by the enzyme asparaginase (EC 3.5.1.1). It catabolizes the breakdown of asparagine to aspartate through the release of a free ammonia molecule. The further metabolism of aspartate proceeds through the enzyme aspartate aminotransferase (EC 2.6.1.1) to generate oxaloacetate and a molecule of glutamate by transamination of α-ketoglutarate. Therefore, asparagine can form two molecules of free ammonia from its two amino groups; one through the deamidating activity of asparaginase and one through the deamination of glutamate by GDH.

Glutamine

Glutamine contains two nitrogen atoms as one amine and one amide. Glutamine can be metabolized to its energy intermediate, α-ketoglutarate, through two pathways. In the glutaminase I pathway, one ammonia molecule is generated through the deamidation of glutamine to glutamate, catalysed by glutaminase (EC 3.5.1.2). The second nitrogen is removed through the deamination of glutamate to α-ketoglutarate by GDH. Accordingly, the complete metabolism of glutamine via the glutaminase I pathway generates two molecules of free ammonia. The glutaminase II pathway consists of a glutamine aminotransferase (EC 2.6.1.-) coupled with an ω-amidase (EC 3.5.1.3). In the first step, glutamine is transaminated with an α-keto acid acceptor to generate α-ketoglutamarate and the corresponding amino acid of the α-keto acid. Following, the deamidation of α-ketoglutamarate by ω-amidase generates α-ketoglutarate and a molecule of ammonia. By the glutaminase II pathway, glutamine generates one ammonia molecule directly and has the potential to indirectly produce an additional two ammonia molecules. First, is an ammonia molecule potentially generated from the metabolism of the amino acid generated by the glutamine aminotransferase. Second, the generated α-ketoglutarate can participate in a second aminotransferase reaction and generate another glutamate molecule that can be deaminated by GDH. It should be noted that while the glutaminase I pathway can be reversed by the activity of GS, the glutaminase II pathway is largely irreversible [38].

Histidine

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Histidine can maximally generate two molecules of ammonia, despite containing three nitrogen atoms. Histidine is primarily metabolised through the enzyme histidase (EC 4.3.1.3), a deaminating enzyme that generates a molecule of free ammonia and urocanate [39]. Through multiple steps of catabolism, the two nitrogen atoms in urocanate are incoorporated into glutamate and formamide (EC 2.1.2.5). Glutamate can further be degraded to α-ketoglutarate and ammonia, rendering the total ammonia generated from histidine to be two molecules.

Lysine

Lysine, comprised of two amino groups, can be catabolised by several pathways including the pipecoline pathway in the brain, but the following saccharopine pathway described predominates in the liver [40]. In mammals, the degradation of lysine generates two molecules of glutamate. In the first steps, reacting with α-ketoglutarate, lysine is converted to alpha-aminoadipate semialdehyde and glutamate through the activity of two enzymes (EC 1.5.1.8, EC 1.5.1.9). Subsequently, alpha-aminoadipate semialdehyde is metabolised to 2-oxoadipate in a multi-step pathway and generates a molecule of glutamate in the process via the enzyme 2-aminoadipate transaminase (EC 2.6.1.39). 2-oxoadipate is then catabolised further to generate two molecules of acetyl-CoA through the intermediate glutaryl-CoA (R01360). The two molecules of glutamate have the capacity to generate two molecules of free ammonia via GDH.

Methionine

Methionine is a unique amino acid in that it produces more ammonia molecules than it contains; It has only one amino group but produces two free ammonia molecules during its metabolism. This occurs because in the first step of methionine degradation, it is converted to S-adenosylmethionine by the enzyme S-adenosylmethionine synthethase (EC 2.5.1.6). It is important to note that S-adenosylmethionine contains two amine groups, and multiple nitrogen atoms. Consequently, an additional amine has been added to methionine in the process of its catabolism and due to this, methionine produces two ammonia molecules despite originally containing only a single amine. The metabolism of S-adenosylmethionine continues to generate cystathionine, which is broken down to cysteine through the enzyme cystathionine gamma-lyase (EC 4.4.1.1). This produces a free ammonia molecule, cysteine, and α-ketobutyrate, that generates succinyl-CoA. Cysteine can then further be catabolised to generate pyruvate through several steps and the transamination of α-ketoglutarate to form glutamate via aspartate aminotransferase (EC 2.6.1.3). Therefore, methionine can potentially form two ammonia molecules, one via the deamination of S-adenosylmethionine and the second through the deamination of glutamate.

3. Ammoniagenesis in organs from amino acids

Ammonia is a small and mobile molecule whose metabolism involves many organs. The main removal organ for ammonia in health is the liver through the activities of the urea cycle and GS. However, in cirrhosis the muscle takes over as the main ammonia clearance mechanism through the activity of GS [41]. Ammonia is primarily generated in the small and large intestines through the metabolism of ingested amino acids and in the kidneys through the catabolism of glutamine.

3.1 Small and Large Intestine

The main site of protein metabolism is the small intestine. From the stomach, proteins denatured by hydrochloric acid and hydrolysed by pepsin enter the small intestine as polypeptides. The pancreas secretes the protease enzymes trypsin, chymotrypsin, and elastase into the small intestine, and the enterocytes secrete the proteases aminopeptidase and dipeptidase for further cleavage [42]. Under normal conditions, up to 91-95% of dietary proteins are absorbed within the cells of the small intestine, the enterocytes [43, 44] in the form of free amino acids as well as di- and tri-peptides [45]. Absorbed amino acids are directly passed through the enterocytes to the portal vasculature, whereas a large proportion of glutamine appears to be retained for catabolism [46] (Figure 3).
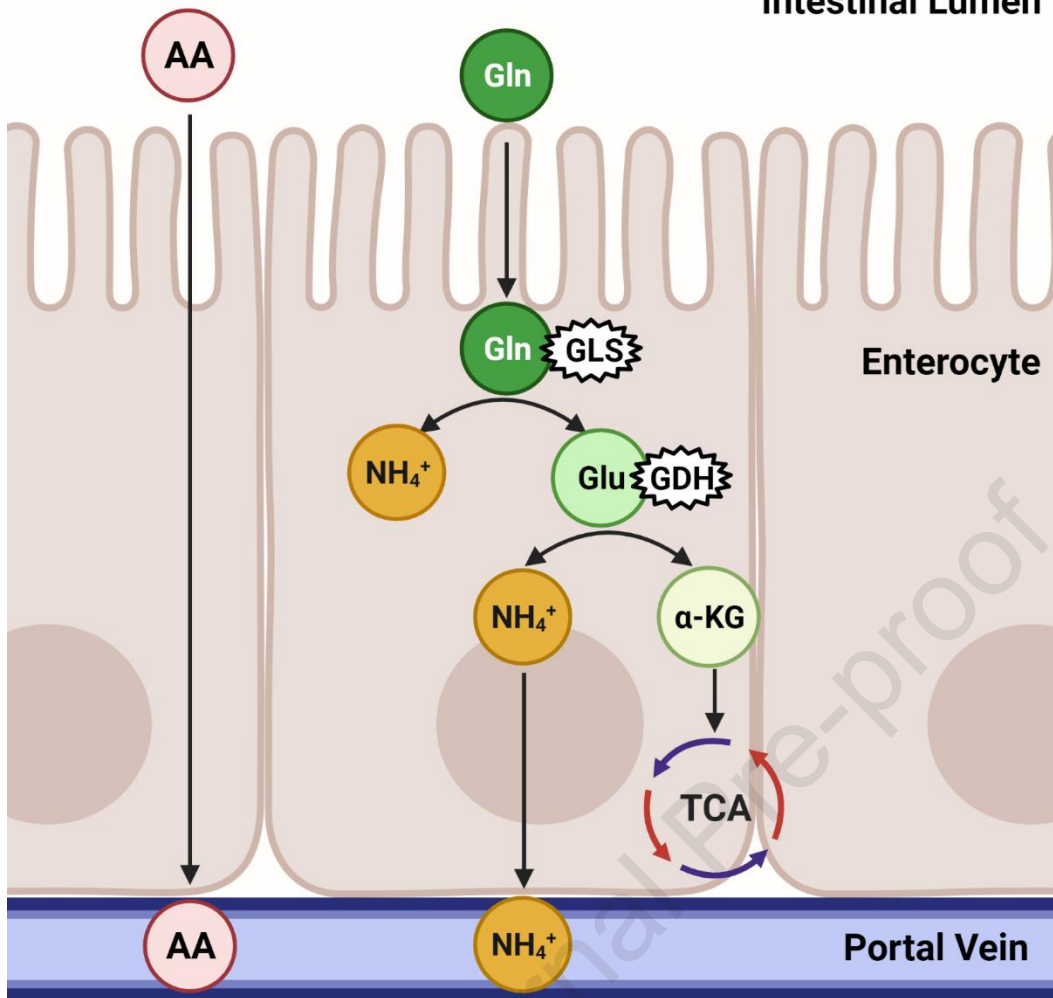
Figure 3. Metabolism of amino acids in the small intestine. The enterocytes preferentially metabolise glutamine to generate glutamate and a molecule of ammonia. Glutamate can further be metabolised to generate α-ketoglutarate for energy generation and another molecule of ammonia. Ammonia can be incorporated into cellular functions or released to the portal vein for transport to the liver. Abbreviations: AA; amino acid, Gln; glutamine, GLS; glutaminase, Glu; glutamate, GDH; glutamate dehydrogenase, NH4+; ammonia, α-KG; α-ketoglutarate, TCA; tricarboxylic acid cycle.

Enterocytes preferentially use glutamine as their source of fuel and nitrogen [47, 48]. Given that enterocytes have the

highest turnover rate of any cell in the body, with a lifespan of approximately 3.48 days [49], it has been suggested that

the high glutamine intake of these cells is reflective of their high energy needs and high rate of nucleotide synthesis due to

rapid cell turnover [48]. Glutamine metabolism in the enterocyte takes place through the enzyme glutaminase, generating

ammonia and glutamate. Glutamate can further be metabolized to generate α-ketoglutarate, an energy precursor, and

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another molecule of free ammonia. However, enterocyte glutamine catabolism generates more nitrogen in the form of ammonia than is needed to fulfill cellular requirements, resulting in a net production of ammonia. It was found that the jejunum and ileum of anesthetized dogs were responsible for 28% and 22%, respectively, of ammonia released into the venous system from the gastrointestinal tract, in correspondence with the uptake of glutamine [50]. A similar study evaluated the ammonia production from the small intestine of canine subjects in response to perfused amino acids and found that glutamine resulted in a 106% increase of ammonia within the venous blood [51]. This increase was by far the highest observed, with the next greatest increase being attributed to leucine, with a 42% increase in ammonia. Therefore, the small intestine is a significant source of ammonia production with the main contributor being glutamine.

Despite the fact that the majority of dietary proteins are absorbed within the small intestine, a significant quantity of nitrogenous products still descend into the large intestine [43, 52]. The source of nitrogen is both of dietary and endogenous origin, such as dead bacterial and epithelial cells from within the intestinal tract [52, 53]. Since absorption of amino acids across the colonic epithelium is limited [54], the primary fate of amino acids and proteins entering the large intestine is catabolism by proteolytic microbiota [55] (Figure 4). Metabolism of amino acids occurs through one of four main pathways: oxidation, reduction, hydrolysis, direct deamination [56]. The *Clostridium* genus of proteolytic bacteria follows a unique fifth pathway for protein metabolism, Stickland fermentation. This reaction proceeds through the mutual deamination of two amino acids to generate two ammonia molecules [57]. Asparagine, serine, lysine, and glutamate were found to be preferentially used by the colonic bacteria [58] whereas arginine, lysine, and histidine metabolism was found to result in more ammonia production, presumably due to their additional nitrogen atoms [56].
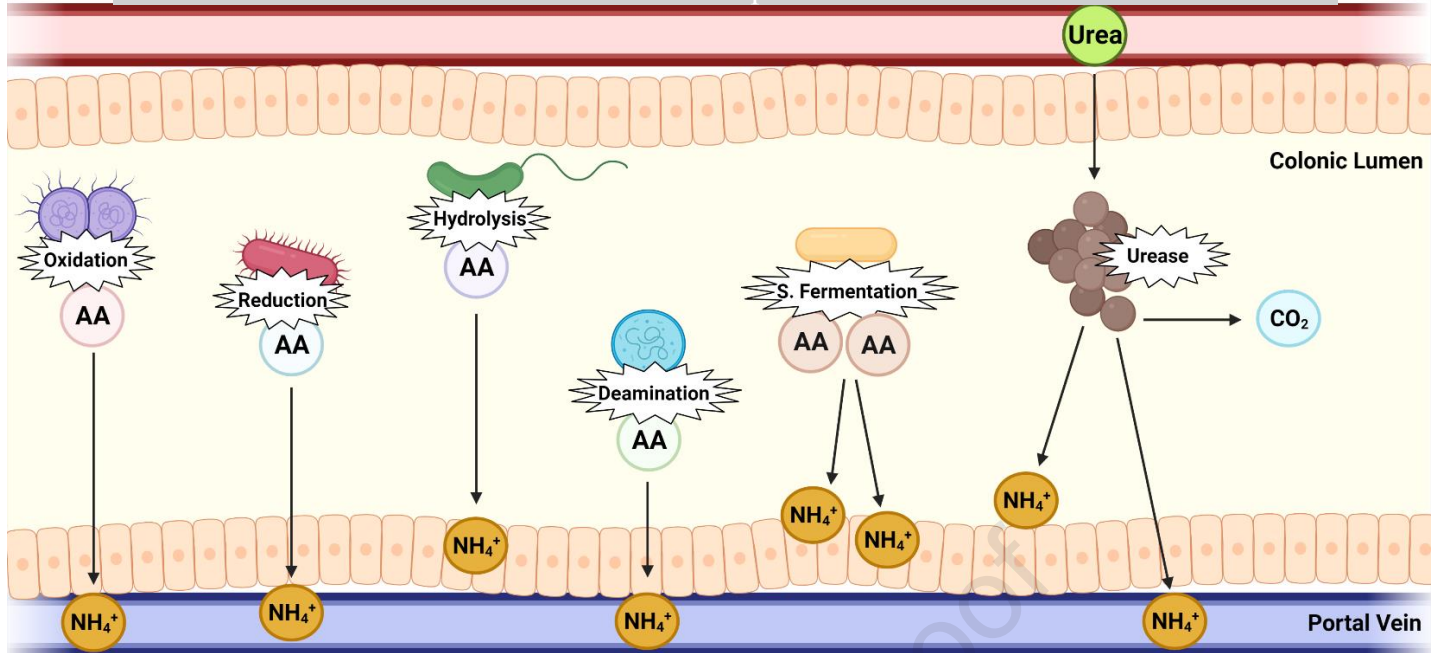
Figure 4. Metabolism of amino acids in the large intestine. Proteinaceous debris entering the colon may be acted upon by the microbiota to generate ammonia through various enzymatic pathways (oxidation, reduction, hydrolysis, deamination, deamidation, and Stickland fermentation). Urea is absorbed from the blood into the large intestine, where it is metabolised by urease-producing bacteria to generate two molecules of ammonia and one molecule of carbon dioxide. Ammonia can be incoorporated into bacteria as a source of nitrogen, excess is absorbed into the portal venous system for transport to the liver. Abbreviations: AA; amino acid, NH4+; ammonia, S. Fermentation; Stickland Fermentation, CO2; carbon dioxide.

In addition to generating ammonia from the catabolism of colonic protein, the microbiota also generates a significant quantity from urea catabolism [59]. Ammonia is converted to urea in the liver, but it has been found in healthy participants that only 30-50% of urea filtered from the blood is excreted [60]. The remaining urea load is reabsorbed into systemic circulation in the kidneys. As a result, urea reabsorbed into systemic circulation passes through the intestinal vasculature where it can be absorbed into the large intestine and utilized by the microbiota. The human microbiota houses particular strains of bacteria that express the enzyme urease (EC 3.5.1.5) that catalyses the breakdown of urea into two molecules of ammonia and a carbon dioxide molecule [61]. Therefore, the large intestine generates ammonia not only from the catabolism of proteinaceous debris but also from the catabolism of urea through urease-producing bacteria. An interesting consideration in the context of cirrhosis is that the urea cycle, the main producer of urea in the body, is less active in the ailing hepatocytes during cirrhosis. Therefore, with less urea production in cirrhosis, the production of ammonia from urease-producing bacteria should be reduced. However, the proportion of ammonia generated in the gut from amino acids vs urea in both the context of cirrhosis and health remains to be thoroughly evaluated.

Another consideration is though the liver is the only organ in which the urea cycle can take place, urea synthesis can occur in any organ that contains the enzyme arginase (EC 3.5.3.1), which catalyzes the conversion of arginine to ornithine and urea. The synthesis of urea outside of the urea cycle is not associated with ammonia clearance, as the arginase reaction is a hydrolysis and does not involve free ammonia. The interplay between urea generation from the urea cycle, urea generation from other tissues, and urease-producing bacteria remains to be elucidated.

Together, the small and large intestines are significant producers of ammonia in cirrhosis. It has been shown in cirrhotic patients with a transjugular intrahepatic portosystemic stent that the portal drained viscera are the major producers of ammonia [41]. The production of ammonia from the portal drained viscera is significantly correlated with the uptake of glutamine into enterocytes, suggesting that the small intestine has a more important role in the production of ammonia than proteolysis by the microbiota in the colon. This is supported by the finding that hyperammonemia production is not prevented in germ-free animals that are lacking a microbiome [62].

3.2 Liver

Following the breakdown of protein in the intestines, the resulting amino acids are brought to the liver via the portal vein. The liver is the main site of amino acid metabolism (Figure 5). Intact amino acids can be used for protein synthesis or released into the systemic circulation to satisfy the needs of other organs. Excess amino acids may be stored within a small amino acid pool within cells, but contrary to carbohydrates and lipids, no specialised storage organ for proteins exists. Due to this, excess amino acids are instead catabolised to energy intermediates that can enter the pathways of gluconeogenesis or tricarboxylic acid (TCA) cycle, depending if the amino acid is glucogenic or ketogenic. Glucogenic amino acids are those that form energy intermediates that enter gluconeogenesis, whereas ketogenic amino acids generate energy intermediates that will enter the TCA cycle for energy generation. Amino acids are broken down in the liver by the pathways described above. In times of fasting, the intermediates can be used for immediate energy whereas in the fed state when energy is abundant, they can be converted to either glucose or fatty acids for longterm storage. As discussed above, ammonia generated in the catabolism of amino acids within the liver is cleared via the urea cycle and enzymatic GS activity. However, in cirrhosis when liver functioning is reduced, it is unclear to what degree the activities of the ammonia generating processes (amino acid catabolism) and ammonia clearing processes (urea cycle and GS) are altered.

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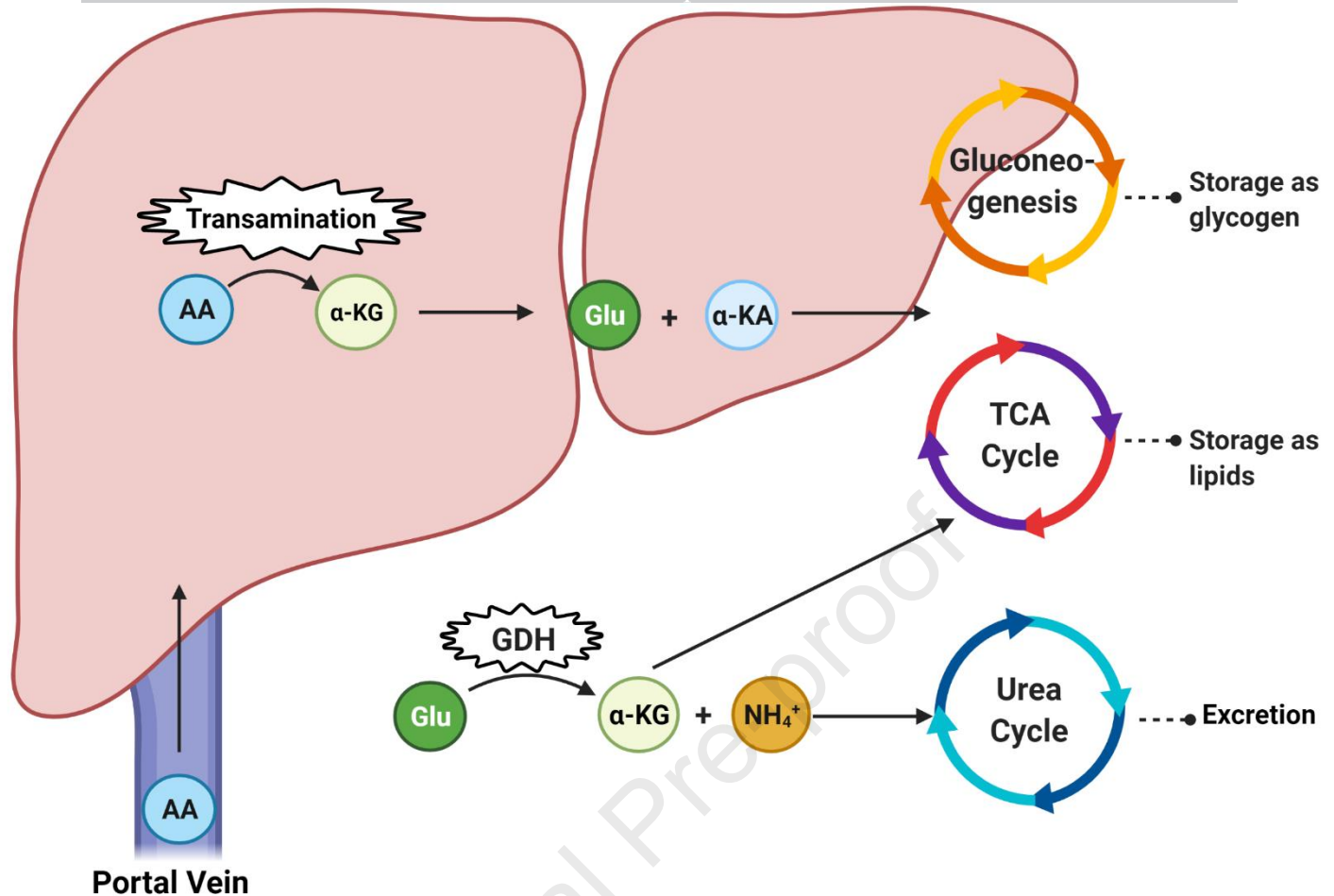
Figure 5. Metabolism of amino acids in the liver. The liver is the main site of amino acid catabolism. Amino acid absorbed from the gut are carried to the liver via the portal vein. Most amino acids are deaminated via transamination to generate glutamate and the corresponding α-ketoacid of the original amino acid. Depending on whether the amino acid is glucogenic or ketogenic, the α-ketoacid will enter gluconeogenic or TCA cycle pathways. Glutamate is then deaminated to generate a free molecule of ammonia and α-KG. Ammonia enters the urea cycle for clearance from the body and α-KG enters TCA for energy production. Abbreviations: AA; amino acid, α-KG; α-ketoglutarate, Glu; glutamate, α-KA; α-ketoacid, TCA; tricarboxylic acid, GDH; glutamate dehydrogenase, NH4+; ammonia.

3.3 Kidney

While the main function of the kidney is the regulation of blood solutes, it also serves to replenish glucose stores through de novo gluconeogenesis using amino acids as precursors and maintains acid-base balance. Glutamine is an important substrate for both functions, with its catabolism generating the energy intermediate α-ketoglutarate and the weak base bicarbonate [63]. Its importance is highlighted by the fact that renal glutamine uptake accounts for as much as 58% of total renal amino acid uptake [64] and assessments of renal arteriovenous differences consistently demonstrate a large absorption of glutamine [65, 66].

In the post-absorptive state (12-16 hour fast), the kidney is responsible for approximately 28% of systemic glucose appearance [67]. In the post-prandial state (4-6 hours of fasting), liver gluconeogenesis rates decrease, while kidney gluconeogenesis unexpectedly increases two-fold, accounting for approximately 60% of glucose appearance [68]. Renal gluconeogenic precursors have been shown to be lactate, glycerol, and glutamine, with the importance of glutamine exceeding the others [69].

The metabolism of glutamine for both glucose generation and pH regulation proceeds through the same pathway (Figure 6). Renal glutamine is metabolised by the glutaminase I or II pathway [70]. The glutaminase I pathway utilizes the kidney-type glutaminase I enzyme whereas the glutaminase II pathway proceeds via a glutamine transaminase coupled to ω-amidase. The resulting glutamate derived from the glutaminase I pathway is further deaminated by GDH. The metabolism of α-ketoglutarate derived from glutamate can generate both gluconeogenic precursors and bicarbonate for acid-base balance.
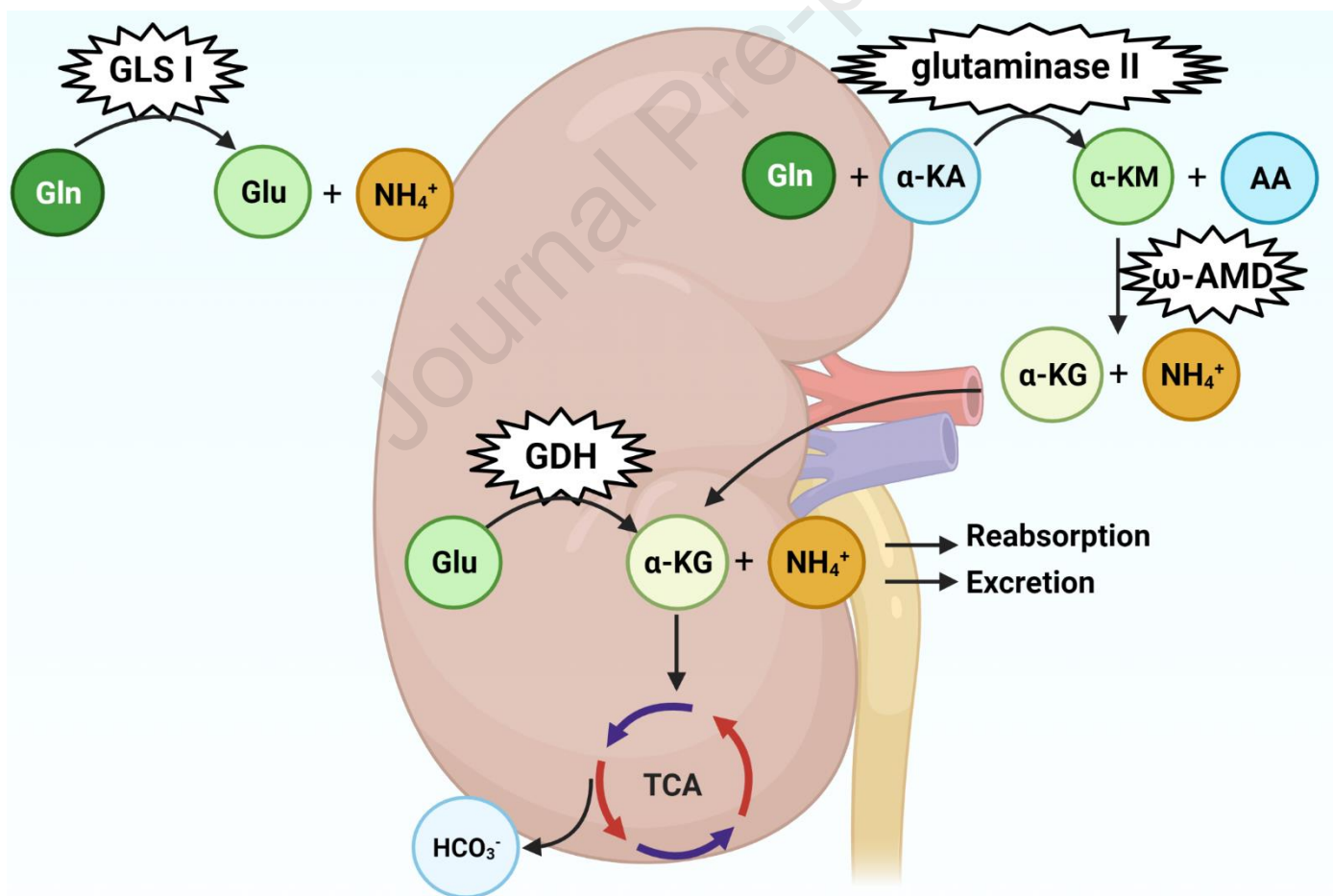
Figure 6. Metabolism of amino acids in the kidney. The kidney preferentially uses glutamine as a source of energy and for acid-base homeostasis. Glutamine is metabolised through the glutaminase I or glutaminase II pathway. Through the glutaminase I pathway (left), glutamine is metabolized to glutamate and ammonia. Glutamate is further metabolised to

generate α-KG and another molecule of ammonia. Through the glutaminase II pathway (right), glutamine first generates α-ketoglutamarate, which is then hydrolyzed to α-KG by ω-amidase. The complete metabolism of α-KG via the TCA cycle generates energy as well as bicarbonate for acid-base homeostasis. The reabsorption of excretion of ammonia is dependant on the pH of blood. Abbreviations: Gln; glutamine, GLS I; kidney-type glutaminase I, α -KA; α-ketoacid, α -KM; α-ketoglutamarate, AA; amino acid, ω-AMD; ω-amidase, NH4+; ammonia, Glu; glutamate, GDH; glutamate dehydrogenase, TCA; tricarboxylic acid cycle, HCO3-; bicarbonate.

3.4 Other Organs

While the organs central to amino acid catabolism have been discussed, other organs may participate in this process as well, though little is known regarding their ammonia contribution in cirrhosis. It is known that the heart is capable of amino acid catabolism. However, the breakdown of amino acids typically contributes a very little to cardiac ATP stores in health [71]. Therefore, despite the ability to produce ammonia, the heart presumably contributes very little ammonia to systemic circulation, though this remains to be validated. However, in the case of ischemia, it appears that amino acid catabolism increases in the heart, though the effects on systemic ammonia are not well described [71]. Additionally, urocanic acid found in the skin is derived from the catabolism of histidine, catalyzed by histidase (EC 4.3.1.3) and generating a molecule of ammonia [72]. However, the skin directly excretes ammonia so it is unclear whether the generated ammonia enters systemic circulation or to what extent. Interestingly, it was found that patients with liver disease had an increased ammonia output from the skin compared with healthy controls and the levels emitted from the skin correlated well with that found in the blood [73]. Additionally, the deaminating enzyme GDH discussed above is not specific to the liver and shows activity across any other tissue types including (but not limited to) pancreas, heart, and brain [74]. There is also evidence using animal tissues that the retina may participate in ammonia metabolism as well [75]. Evidently, organs outside of the gastrointestinal tract may also be producers of ammonia. However, the contribution of these peripheral organs and sense organs to ammonia generation in health as well as in cirrhosis is not yet well characterised.

4. Amino acid metabolism in liver disease

It is known that cirrhotic patients have altered metabolism. It is well established that cirrhotic patients have reduced respiratory quotients when compared to healthy controls [76]. This is indicative of increased utilization of protein and fat for energy as opposed to glucose. This could be due to inadequate liver glycogen stores; without sufficient glycogen, the body must turn to other sources for energy metabolism [77]. This is supported by the finding that cirrhotic patients have increased protein requirements and altered protein metabolism [77]. In particular, it has been shown that cirrhotic patients

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metabolise leucine differently from healthy controls [78]. It was demonstrated that nonoxidative leucine degradation was decreased in cirrhotic patients [78]. Therefore, the pathways above may be altered in cirrhosis and more research is needed to elucidate where changes occur and their effects on whole-body metabolism.

It is clear that all amino acids have the potential to generate ammonia from their transamination to glutamate, but what remains undetermined is under what circumstances ammonia is produced. In the setting of liver disease, there is evidence that amino acid metabolism differs depending on the amino acid where some amino acids have shown beneficial effects through increasing ammonia clearance, while others appear to cause deficit.

4.4 Branched-chain amino acids:

It is well described that branched-chain amino acids (BCAAs) [79] and L-ornithine-L-aspartate (LOLA) [80] are beneficial in cirrhotic patients and exert positive effects on mental status and disease progression. Isoleucine, leucine, and valine comprise the three BCAAs and they are unique in that they are largely metabolised in the skeletal muscle rather than the liver [81]. BCAAs have been found to confer positive effects on immune function, hepatocyte proliferation, and support of skeletal muscle [82]. As mentioned above, the skeletal muscle has the capacity to clear ammonia and increasing muscle mass may have the added benefit of increasing ammonia clearance. BCAAs have the capacity to directly drive ammonia clearance as well [83] though the efficacy of BCAAs as an ammonia-lowering strategy remains debated. The first step in BCAA metabolism is the transamination by branched-chain aminotransferase (EC 2.6.1.42). This reaction removes the amino group from the BCAA and transfers it to another α-keto acid, typically α-ketoglutarate. Therefore, the branched-chain aminotransferase reaction generates a molecule of glutamate. This glutamate molecule can then interact with GS to utilize a molecule of free ammonia and generate a molecule of glutamine. As discussed above, the GS reaction is an important ammonia clearance mechanism and by driving the generation of glutamate, BCAAs can also drive the clearance of ammonia via its incorporation into glutamine.

4.2 L-Ornithine L-Aspartate

L-Ornithine L-Aspartate (LOLA) is a combination amino acid treatment strategy to help reduce ammonia. The constituents of LOLA, ornithine and aspartate, readily dissociate to individual amino acids when administered and serve to drive the urea cycle forward [84]. Aspartate serves as a nitrogen donor for the enzymes of the urea cycle whereas ornithine is an important substrate. By administering these amino acids, the urea cycle is stimulated in hepatocytes with

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the intention of driving the clearance of ammonia and therefore reducing HE risk. In addition, LOLA can also reduce ammonia through driving GS activity. Both ornithine and aspartate are also substrates for glutamate generation through a transamination reaction with α-ketoglutarate. Therefore, by increasing the availability of these amino acids, they can more readily generate glutamate that can clear a molecule of ammonia through its incorporation into glutamine via GS [85].

In addition to its use in LOLA, ornithine is also used as a treatment of hyperammonemia in the form of ornithine phenylacetate (OP). To date, OP has primarily been used in animal models of cirrhosis and has been found to decrease serum ammonia and improve symptoms of HE [86]. In patients with acute liver failure as well as those with chronic liver disease, OP has been shown to be safe for use and a reduction in serum ammonia was observed, with increased glutamine excretion [87, 88]. Similar to its use in LOLA, ornithine in OP indirectly clears ammonia through increasing muscle GS activity and through its transamination to glutamate. Thus, ornithine as part of OP can drive ammonia clearance through its conversion to glutamate, which can clear a molecule of ammonia by generating glutamine. However, the condensation of glutamate and ammonia to glutamine is reversible through the enzyme glutaminase, that generates glutamate and ammonia from glutamine. Therefore, the generation of glutamine as a strategy to clear ammonia may not be sufficient due to the activities of glutaminase. OP is unique in its method of action in that it not only drives ammonia clearance through the generation of glutamine but phenylacetate subsequently traps glutamine as phenylacetylglutamine to be excreted from the body [89]. In this way, glutamate generated from ornithine may clear a molecule of ammonia through the generation of glutamine, and destine this ammonia to be excreted as phenylacetylglutamine, eliminating the potential to re-generate ammonia through glutaminase.

4.3 Simulated Hemoglobin

An upper gastrointestinal bleed in cirrhotic patients is a common precipitating factor of HE and known to contribute to hyperammonemia. Simulating an upper gastrointestinal bleed via the administration of an amino acid blend (Table 3) replicating the hemoglobin molecule has been found to have similar effects to a real upper gastrointestinal bleed with increases in ammonia and alterations in mental status (Table 2).

Hemoglobin, and its simulated amino acid blend, completely lacks isoleucine. This is significant because isoleucine may act as limiting factor for protein synthesis, since it is involved in many anabolic processes. Simulated hemoglobin blends administered to patients resulted in decreased levels of hepatic protein synthesis and hypoisoleucinemia that were

attenuated when isoleucine was included in the simulated bleed mixture [90]. As discussed above, excess amino acids not used for protein synthesis are oxidised. Therefore, if hemoglobin cannot be used for protein synthesis due to a lack of isoleucine, it is possible that a large proportion of the administered amino acids are oxidised, thereby creating ammonia in the process. However, adding isoleucine to a simulated bleed did not attenuate ammonia production [91]. Therefore, UGI bleed-induced hyperammonemia must be multifactorial and not simply due to limited protein synthesis.

Additionally, hemoglobin consists of approximately 22% amino acids that have the capacity to generate multiple ammonia equivalents from their metabolism (Arg, Asn, Gln, His, Lys, Met). Therefore, an amino acid mixture that is high in these ammoniagenic amino acids could more readily generate ammonia compared to an amino acid mixture of equivalent weight but comprised of low ammoniagenic amino acids. It would be interesting to see how the composition of hemoglobin compares to other sources of protein to determine if the content of ammoniagenic amino acids is higher than the norm. However, it is difficult to compare this value to other sources of protein since glutamine and asparagine are rarely reported, presumably due to their rapid breakdown in solution [92].

4.4 Glutamine

An amino acid that has shown to induce hyperammonemia and HE is glutamine. When cleaved, glutamine leads to ammonia production in the small intestine, liver, and kidneys. Moreover, it has the potential to form an additional molecule of ammonia with its full catabolism to α-ketoglutarate when compared to most amino acids that generate only one molecule of ammonia. It is therefore an amino acid with high ammoniagenic potential. Clinical studies in cirrhotic patients confirm this, with evidence showing that glutamine administration increases blood ammonia and, in some cases, results in a deterioration of mental status (Table 2). This occurs at doses as low as 10 g of glutamine. Evidently, glutamine is highly detrimental to mental status and ammonia, especially when considering the low dose at which this occurs. Comparatively, the lowest dose of amino acids simulating the composition of hemoglobin that was found to have a detrimental effect was 54 g, significantly higher than the 10 g dose of glutamine needed to induce deficit. Furthermore, BCAA's can be administered at doses as high as 60 g per day long-term (56 weeks) with an improvement in mental status rather than a deterioration [93]. Therefore, glutamine elicits a detrimental response in patients at a lower dose than other amino acids and amino acid blends. The detrimental effect of glutamine may be synergistic between its capacity to generate two molecules of ammonia and its prominent catabolism in multiple organs.

The importance of glutamine breakdown by glutaminase in the development of HE is further evidenced by an apparent genetic propensity for HE. As mentioned above, glutaminase catalyses the hydrolysis of glutamine to glutamate and ammonia. This ammonia producing process occurs across many organs, including the small intestine, liver, kidneys, brain, and lymphocytes [94]. It has been found that there is a genetic variant in which the glutaminase enzyme is upregulated and is a predisposing factor for the risk of HE [95, 96]. This link between upregulated glutaminase and increased risk of HE suggests that there are cirrhotic patients in which not only is ammonia clearance impaired, but ammonia generation is also increased.

4.5 Arginine

Arginine is another amino acid that is capable of producing multiple molecules of ammonia with its full catabolism. However, when administered intravenously at a dose of 30 g, no significant changes in ammonia levels were found. Therefore, the quantity of ammonia produced from amino acid catabolism is not the sole factor to consider when looking at which amino acids are detrimental to mental status in cirrhosis. Since glutamine catabolism is the preferred source of cellular energy for both the small intestine and the kidneys, glutamine catabolism occurs at a high rate within the body and this is likely to contribute to its effects.

4. Conclusion

The full catabolism of an amino acid to energy intermediates generates one or two molecules of ammonia, depending on the amino acid. However, not all dietary amino acids derived from protein will be fully catabolised to generate ammonia, as some may follow other metabolic pathways or be incorporated into proteins. In chronic liver disease, it has been shown that amino acid metabolism is altered [97]. Therefore, despite all amino acids possessing the potential for ammonia generation, in the setting of cirrhosis, some amino acids (glutamine and simulated hemoglobin) increase ammonia and precipitate HE whereas others (BCAAs, ornithine, and aspartate) can confer benefits to disease progression and reduce ammonia. Unfortunately, what propels a particular amino acid to follow one pathway of metabolism over another (such as production vs. clearance of ammonia) is currently unknown in detail. However, elucidating the mechanism may be complex as an interplay between hypermetabolism, hyperammonemia, and protein-calorie malnutrition may affect the end products of amino acid ingestion. Moreover, the microbiota is shown to be altered in cirrhotic patients [98]. Therefore, the end products of bacterial metabolism may be altered as well and further impact the fate of ingested amino acids.

Determining what drives an amino acid to clear rather than produce ammonia may be a key step in improving nutritional guidelines for patients and elucidating new treatments.

Though the determinants of the fate of an individual amino acid may be unknown, amino acids in general are typically only catabolized to energy intermediates when energy levels are low or when they are in excess so storage as energy intermediates can occur. Future nutritional strategies could determine the specific amino acid needs of a cirrhotic patient and recommend a diet that is tailored to provide only the amino acids that are needed for the maintenance of health and avoid excess that would be catabolised to ammonia. Such a highly regulated and meticulous nutritional plan may be difficult and time-consuming to implement but as analytic methods improve, this may become more feasible. Indicator amino acid oxidation (IAAO) is a new methodology, originally designed to determine amino acid requirements in pigs, that shows promise in the development of personalised nutrition (See [99] for a comprehensive review). In brief, this technique is based on the premise that when one indispensable amino acid is deficient for protein synthesis, then all other amino acids will be oxidized. A limiting amino acid is administered, and oxidation of an indicator amino acid is determined. By administering increasing amounts of the limiting amino acid, oxidation of the indicator amino acid will decrease which is indicative of amino acids being incorporated into proteins. This minimally invasive technique has already been used in adults to define essential amino acid requirements [100]. Furthermore, this method has been used in children with liver disease to determine their daily BCAA requirements [101]. Though this method is relatively new, its wide implementation may provide a method of quantifying individualized amino acid needs.

However, the evaluation of amino acid requirements for all twenty coded amino acids may be a costly and lengthy process. Above, a subset of amino acids (Arg, Asn, Gln, His, Lys, Met) have been identified as having higher ammoniagenic potential due to the capacity to generate multiple ammonia molecules rather than solely one. Thus, monitoring the dietary requirements of only these ammoniagenic amino acids may provide a more realistic starting point for personalised nutrition.

A simpler prevention strategy could be the global reduction of the ammoniagenic amino acids in the diet. However, five of the six high ammoniagenic amino acids are essential; They must be consumed from the diet since they cannot be endogenously synthesized. Determining the baseline dietary requirements through a method such as IAAO for these essential ammoniagenic amino acids would be paramount in safely reducing intake. While this may complicate the

nutritional intervention, this treatment strategy is not new. Phenylketonuria is an inborn error of metabolism that results in the accumulation of phenylalanine and neurological damage [102]. A lifelong low-phenylalanine diet is a well-established and effective prevention to neurological damage, despite phenylalanine being an essential amino acid [102]. Adherence to the low-phenylalanine diet is difficult for patients, as they need to consume enough of this amino acid to support optimal growth and development, while preventing its accumulation. Recommendations for intake are individualized and monitored through the assessment of blood phenylalanine levels [102]. Likewise, a lysine-free arginine-fortified diet has been evaluated in the treatment of glutaric aciduria type I. This genetic syndrome results from glutaryl-CoA dehydrogenase deficiency, resulting in cerebral entrapment of glutaryl-CoA and its derivatives that cause striatal damage and motor delay [103]. It was found that the essential amino acid lysine is an important source of carbon for the generation of glutaryl-CoA, with the growing brain producing large amounts of glutaryl-CoA from lysine [103]. Therefore, a lysine-free diet was evaluated in children suffering from glutaric aciduria type I, with results showing neuroprotection [103]. Thus, in some disease states such as the ones discussed above, the exclusion of an amino acid from the diet is not harmful and can be favourable if done with correct monitoring.

However, the effects of all the ammoniagenic amino acids in cirrhosis are unknown and as demonstrated with arginine, simply because an amino acid has high ammoniagenic potential does not mean it will cause detriment. Therefore, the modification of nutritional recommendations to limit the intake of all six ammoniagenic amino acids may not be necessary. Instead, the focus may be placed on glutamine, an ammoniagenic amino acid that is proven to effect cirrhotic patients negatively and has the benefit of being non-essential in healthy patients, reflecting the ease with which daily glutamine requirements can be met through endogenous generation in healthy adults. Glutamine can be generated from glutamate and ammonia by the enzyme GS, and this can occur in many organs beyond the liver [104]. These include, but are not limited to, the skeletal muscle, brain, brown adipose tissue, and intestine [104]. Due to glutamine's confirmed negative impact on mental status and ammonia generation from multiple organs, avoidance of high glutamine foods may be appropriate for consideration in cirrhotic patients to mitigate HE risk, especially in patients with the genetic predisposition for upregulated glutaminase activity [105].

It should be noted that glutamine is an important energy substrate for enterocytes [106] and therefore full elimination of glutamine from the diet may pose a risk to gut health. Moreover, glutamine is also an essential nutrient for immune functioning including lymphocyte proliferation and cytokine production, macrophage activities, and neutrophil

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functioning [107]. Since cirrhotic patients are immunocompromised and at increased risk of bacterial infection [108], the maintenance of immune functioning is important and another factor to consider in discussing a low glutamine diet. Furthermore, in times of critical illness or injury, glutamine requirements are increased, exceeding the quantity that can be endogenously produced, which renders glutamine a conditionally essential amino acid during some illnesses [109]. It has not been determined yet whether the state of cirrhosis alters glutamine's importance in the body and renders this amino acid essential. A balance should be found between limiting ammonia production from glutamine and inducing glutamine deficiency. With this in mind, an alternative option to limiting daily ammoniagenic amino acid intake, would be to limit the intake at each meal. It is currently recommended for cirrhotic patients to spread their daily food intake into five to seven small but frequent meals [110]. The primary reason for this recommendation is to aid in the maintenance of muscle mass and combat malnutrition, as cirrhotic patients are often in hypercatabolic states. Moreover, this prevents patients from having an acute protein overload and spike in blood ammonia [110]. Therefore, it is possible that regular daily intake of ammoniagenic amino acids can be maintained if their intake is spread out over several meals.

Unfortunately, evaluating the safety and efficacy of a low-glutamine diet in HE may be a difficult task. Canadian [111], United States [112], and European Union [113] nutrition labels are required to disclose the amount of protein a product contains but are not required to break down the protein content by amino acid. Moreover, fresh produce as well as raw meats and seafood typically do not carry nutrition labels. To compound this issue, Canada's Nutrient File resource that provides the nutritional content of foods, does not include data on glutamine. Therefore, it would not only be difficult to ask patients to avoid high glutamine foods when there is little data available on the glutamine content in foods, but it could also be difficult to administer such a diet in a clinical research setting.

A relatively recent publication was the first of its kind to evaluate glutamine using DNA sequencing and provided some insightful data on dietary sources of glutamine [114]. Through DNA sequencing of foods, the authors were able to determine the quantity of glutamine in a variety of different food sources. For example, it was found that beef is high in glutamine (1.231 g glutamine per 100 g beef) whereas white rice is low (0.275 g glutamine per 100 g white rice). When used for the determination of common amino acids, the data from this novel DNA sequencing technique highly agreed with the conventional methods, indicating its reliability. Unfortunately, the data for glutamine cannot be confirmed with other sources since glutamine is so rarely quantified. Additionally, the protein quality and preparation of food will have an impact on the true bioavailability of glutamine, something that was not considered in this study.

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An interesting consequence of the theory that ammoniagenic amino acids may be detrimental in cirrhosis is that it may provide explanation for why vegetable protein diets seem to be more beneficial to cirrhotic patients when compared to animal-sourced protein diets. A consistent trend is seen in cirrhotic patients where vegetable-sourced protein is found to confer more benefits than animal-sourced protein [115, 116, 117]. Furthermore, a study observing the relationship between mortality and diet in cirrhosis found that animal protein was more significantly related to cirrhosis death rates than vegetable protein [118]. These findings can possibly be explained by the elevated fibre content in vegetable protein diets compared to animal protein diets [119]. Fibre increases intestinal motility, preventing constipation (a precipitating factor of HE) thereby reducing the amount of ammonia that is absorbed from material in the colon. For this reason alone it is possible that a plant-based diet may be preferred for cirrhotic patients.

However, an additional explanation for this difference could be in the amino acid content of the foods. It appears that vegetable-sourced proteins may contain less ammoniagenic amino acids compared to animal-sourced proteins. It has been found that the ammoniagenic amino acid lysine is consistently at a lower concentration in all plant-based proteins than in animal-sourced proteins. In addition, the sulfur-containing amino acids (including methionine) are distinctly lower in legumes and fruits, compared with animal-sourced proteins [120]. A more recent amino acid evaluation of various vegetable and animal proteins confirms these findings. It was found that lysine, histidine, and methionine were generally higher in animal sources of protein compared to vegetable sources [121]. Unfortunately, this study did not evaluate asparagine or glutamine. Additionally, these studies only evaluated the raw amino acid contents of the foods and did not take digestibility into account. Since some vegetable proteins are less digestible when compared to animal sources [122], it is possible that this reduced digestibility further contributes to lesser ammonia generation. This would mean that vegetable proteins with lower digestibility may undergo less proteolysis within the digestive system and therefore result in less amino acid metabolism and less ammonia generation. It should also be noted that the authors found large variations within the vegetable sources of protein, suggesting not all sources of vegetable proteins may be beneficial in cirrhosis. While these studies have found that some vegetables tend to contain lesser quantities of ammoniagenic amino acids when compared to animal protiens, this is not universally true for all vegetables. In the curation of a low-ammoniagenic diet, vegetables high in ammoniagenic acids should be considered for exclusion. Thus, a plant-based diet in cirrhosis has some support but more research is needed to not only determine clinical efficacy but also to determine a method of action.

5. Summary

The catabolism of amino acids is a significant source of ammonia in the body, largely occurring in the gut. While hyperammonemia is highly implicated in the progression of HE in cirrhosis, protein restriction is not a viable solution to mitigate risk. Protein intake has increased importance in cirrhotic patients due to the prevalence of hypermetabolism, protein-calorie malnutrition, and sarcopenia. Furthermore, maintaining muscle mass can increase ammonia clearance through enzymatic GS activity. Therefore, modifying nutritional recommendations to support the maintenance of muscle mass and allow sufficient protein intake while reducing ammoniagenesis from amino acids is crucial in the management of HE.

A subset of six amino acids (Arg, Asn, Gln, His, Lys, Met) from the common protein amino acids have been identified as ammoniagenic due to their capacity to generate maximally two equivalents of ammonia during their catabolism to energy intermediates, either directly or indirectly. Of these ammoniagenic amino acids glutamine is proven to cause detriment in cirrhotic patients. Conversely, ornithine and aspartate, and BCAAs have been shown to be beneficial. As a result of the varied ammoniagenesis of amino acids, modifying the intake distribution may be an interesting solution to strike a balance between maintaining proper protein intake while decreasing hyperammonemia and HE risk. The restriction of one or several key ammoniagenic amino acids paired with the increased intake of ammonia-clearing amino acids may be appropriate in the place of global restriction of protein. While much is known about ammonia metabolism in the body, many considerations remain to be investigated in the state of cirrhosis. Future research should investigate the efficacy of personalised nutrition and modification of amino acid intake for the prevention of HE.

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Table 1. Catabolic Pathways of Amino Acids. The main catabolic pathways for the common protein amino acids are described; Other pathways are possible but not discussed in detail.

Amino Acid	Essential	Nitrogen Quantity	Max. NH_4 Produced	Nitrogen Removal Reaction	Terminal Metabolite
Ala	No	1	1	Transamination (EC 2.6.1.2)	Pyruvate; Glucogenic
Arg	No	4	2	Deamination (EC 3.5.3.1),	α-ketoglutarate; Glucogenic
Asn	No	2	2	Deamination (EC 3.5.1.1), Transamination (EC 2.6.1.39)	Oxaloacetate; Glucogenic
Asp	No	1	1	Transamination (EC 2.6.1.1)	Oxaloacetate; Glucogenic
Cys	No	1	1	Transamination (EC 2.6.1.1)	Pyruvate; Glucogenic
Glu	No	1	1	Deamination (EC 1.4.1.2)	α-ketoglutarate; Glucogenic
Gln	No	2	2	Deamidation via glutaminase I pathway (EC 3.5.1.1); Deamination via glutaminase II pathway (EC 3.5.1.3)	α-ketoglutarate; Glucogenic
Gly	No	1	1	Deamination (EC 2.1.2.10)	Pyruvate; Glucogenic
His	Yes	3	2	Deamination (EC 4.3.1.3), Transamination (EC. 2.1.2.5)	α-ketoglutarate; Glucogenic
Ile	Yes	1	1	Transamination (EC 2.6.1.42)	Succinyl-CoA; Glucogenic and Ketogenic
Leu	Yes	1	1	Transamination (EC 2.6.1.42)	Acetyl-CoA; Ketogenic

Lys	Yes	2	2	Oxidoreduction (EC 1.5.1.9), Transamination (2.6.1.39)	Acetyl-CoA; Ketogenic
Met	Yes	1	2	Deamination (EC 4.4.1.1), Transamination (EC 2.6.1.1)	Pyruvate, Succinyl-CoA; Glucogenic
Phe	Yes	1	1	Transamination (EC 2.6.1.5)	Fumarate, Acetoacetate; Glucogenic and Ketogenic
Pro	No	1	1	Oxidation (EC 1.2.1.88)	α-ketoglutarate; Glucogenic
Ser	No	1	1	Deamination (EC 4.3.1.17)	Pyruvate; Glucogenic
Thr	Yes	1	1	Deamination (4.3.1.19)	Pyruvate, Succinyl-CoA, Acetyl-CoA; Glucogenic and Ketogenic
Trp	Yes	2	1	Transamination (EC 2.6.1.2)	Pyruvate, Acetyl-CoA; Glucogenic and Ketogenic
Tyr	No	1	1	Transamination (EC 2.6.1.5)	Fumarate, Acetoacetate; Glucogenic and Ketogenic
Val	Yes	1	1	Transamination (EC 2.6.1.42)	Succinyl-CoA; Glucogenic

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Table 2. Clinical Evidence of Amino Acids with Detrimental Effects in Cirrhotic Patients.

This table provides a summary of the currently available clinical research exploring the administration of amino acids to cirrhotic patients and finds that the administered amino acid results in a detriment, either through the increase of serum ammonia or through the worsening of HE. Research articles were included if they were published after 1955, administered an amino acid to patients with liver dysfunction through any route, observed worsening of mental status and/or increase in serum ammonia, were written in English, and were available as an online source. For additional details on simulated hemoglobin, see Table 3.

Effects of Simulated Hemoglobin on Hepatic Encephalopathy in Cirrhotic Patients						
Reference	N	Control	Dose	Trial Period	Route	Outcome
[123]	8	Baseline	75g	Single dose	Oral	Significant deterioration in performance in immediate (p<0.01) and delayed (p<0.01) story recall test 4 hours following amino acid load. Ammonia was increased 2 (p<0.001) and 4 (p<0.01) hours post amino acid load.
[124]	41	Baseline, Healthy Patients	54g	Single Dose	Oral	180 minutes post amino acid load, blood ammonia was significantly higher in cirrhotic patients classified as CP-B (p<0.01) and CP-C (p<0.02). Patients classified as CP-B (p<0.01) and CP-C (p<0.03) performed more poorly in reaction time compared to baseline assessment.
[125]	30	Baseline, Healthy Patients	108g	Single Dose	Oral	120 minutes after an amino acid load, ammonia levels were significantly increased compared to baseline (p<0.004) and compared to healthy controls (p<0.001).
[126]	18	Baseline	54g	Single Dose	Oral	Amino acid load induced a significant increase in ammonia 4 hours after administration (p=0.0038) and an increase in subjective sleepiness (p=0.001). Meanwhile, PHES scores improved following amino acid load (p<0.001).
[127]	8	Baseline	60% of daily nitrogen intake (estimated using 3-day food history and dietetic consultation)	Single Dose	IG	Arterial concentrations of ammonia increased significantly (p=0.008), particularly from the kidney (p=0.008).
[91]	16	Baseline	60% of daily nitrogen intake (estimated using 3-day food history and dietetic consultation)	Single dose over 4 hours	IG	Administration of amino acid load resulted in increased arterial ammonia compared to baseline (p=0.018).
[128]	48	Placebo, Baseline	75g	Single dose	Oral	Amino acid load increased ammonia compared to baseline 4 hours after administration (p<0.001). Placebo group's performance improved (p<0.03) on Trails B Test whereas amino acid load group's performance remained the same (p=0.77). Placebo group exhibited improved performance on Digit Symbol Substitution Test (p<0.02) whereas amino acid load group had no

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						changes in performance (p=0.78). Amino acid load resulted in poorer performance in the Immediate Story Recall Test compared to baseline (p<0.03). The placebo group significantly improved in Reaction Time (p<0.03) whereas the amino acid load group did not have a change in performance (p=0.49).
[129]	84	Placebo, Baseline	75g	Single Dose	Oral	Amino acid administration resulted in a significant increase in plasma ammonia (p<0.0001). Placebo group performed better than amino acid group in Trails B Test, Digit Symbol Substitution Test, Reaction Time, and Immediate Story Recall.
[130]	14	Baseline	75g	Single Dose	Oral	Amino acid load resulted in in increase in ammonia (p<0.001). Immediate Story Recall Scores were lower following amino acid load compared to before (p=0.001). No significant differences in Trails B Test (p=0.06), Digit Symbol Substitution Test (p=0.06) and Reaction Time (p=0.72) following amino acid load compared to baseline.
Effects of Glutamine on Hepatic Encephalopathy in Cirrhotic Patients						
[131]	18	Baseline, Placebo	20g	Single Dose	Oral	Plasma ammonia increased at 30 (p=0.006) and 60 minutes (p=0.001) after oral glutamine challenge.
[132]	70	Baseline, Healthy Control	20g	Single Dose	Oral	Oral glutamine load increased arterial ammonia in cirrhotic patients compared to baseline (p<0.0001) meanwhile remaining unchanged in healthy controls (p=0.064). Cirrhotic patients performed more poorly on psychometric testing following the oral glutamine load compared to baseline (p<0.0001), meanwhile healthy controls had no change in performance.
[133]	70	Baseline, Healthy Control	20g	Single Dose	Oral	Cirrhotic patients exhibited an increase in capillary blood ammonia 30 (p<0.05) and 60 (p<0.05) minutes after oral glutamine load whereas healthy controls had no change in ammonia. Cirrhotic patients performed more poorly in Trail Test A (p=0.04) and Trail Test B (p=0.001) psychometric tests 60 minutes after oral glutamine load, whereas healthy controls had no change in performance.

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[134]	6	Baseline	20g	Single Dose	Oral	Venous blood ammonia increased significantly after glutamine load (p=0.0004).
[135]	80	Baseline, Healthy Control	10g	Single Dose	Oral	Oral glutamine load significantly elevated ammonia in cirrhotic patients compared to healthy controls (p<0.001).
[136]	27	Baseline, Healthy Control, Placebo	10g or 20g	Single Dose	Oral	Oral glutamine challenge (both 10g and 20g) induced a significant increase in blood ammonia (p<0.01), poorer performance in Reaction Time test (p<0.01), which were not seen in healthy controls or in cirrhotic patients receiving water.
[137]	8	Baseline	20g	Single Dose	Oral	Venous blood ammonia increased after oral glutamine load (p=0.006).
Effects of Arginine on Hepatic Encephalopathy in Cirrhotic Patients						
[138]	32	Baseline, Placebo	30g	Single Dose	IV	No significant changes in blood ammonia. 5.9% of patients improved in their mental status following infusion, 23.5% improved minimally, and 53% of patients had no change, and 17.6% deteriorated.
ABBREVIATIONS: CP: CHILD-PUGH, PHES: PSYCHOMETRIC HEPATIC ENCEPHALOPATHY SCORE, IG: INTRAGASTRIC, IV: INTRAVENOUS						

Table 3. Simulated Hemoglobin Composition

Leucine	99.8	Methionine	8.3
Isoleucine	0	Arginine	16.6
Valine	85.9	Tyrosine	16.6
Glycine	55.4	Proline	38.8
Tryptophan	8.3	Aspartate	41.6
Threonine	44.4	Alanine	99.8
Lysine	61.0	Cysteine	8.3
Glutamate	33.3	Serine	44.4
Asparagine	27.7	Phenylalanine	41.6
Glutamine	11.1	Histidine	52.7
Expressed as mmol per 100-gram amino acid solution. Adapted from [127].			

Highlights

(3-5 bullet points, each 85 characters max with spaces included, help increase the discoverability of your article via search engines)

- The metabolism of dietary protein generates ammonia
- Arg, Asn, Gln, His, Lys, Met have high ammoniagenic potential
- Modifying amino acid may be a strategy to prevent/treat hepatic encephalopathy