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REVIEW

A model of blood-ammonia homeostasis based on a quantitative analysis of nitrogen metabolism in the multiple organs involved in the production, catabolism, and excretion of ammonia in humans

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Abstract: Increased blood ammonia (NH_3) is an important causative factor in hepatic encephalopathy, and clinical treatment of hepatic encephalopathy is focused on lowering NH_3. Ammonia is a central element in intraorgan nitrogen (N) transport, and modeling the factors that determine blood-NH_3 concentration is complicated by the need to account for a variety of reactions carried out in multiple organs. This review presents a detailed quantitative analysis of the major factors determining blood-NH_3 homeostasis – the N metabolism of urea, NH_3, and amino acids by the liver, gastrointestinal system, muscle, kidney, and brain – with the ultimate goal of creating a model that allows for prediction of blood-NH_3 concentration. Although enormous amounts of NH_3 are produced during normal liver amino-acid metabolism, this NH_3 is completely captured by the urea cycle and does not contribute to blood NH_3. While some systemic NH_3 derives from renal and muscle metabolism, the primary site of blood-NH_3 production is the gastrointestinal tract, as evidenced by portal vein-NH_3 concentrations that are about three times that of systemic blood. Three mechanisms, in order of quantitative importance, release NH_3 in the gut: 1) hydrolysis of urea by bacterial urease, 2) bacterial protein deamination, and 3) intestinal mucosal glutamine metabolism. Although the colon is conventionally assumed to be the major site of gut-NH_3 production, evidence is reviewed that indicates that the stomach (via *Helicobacter pylori* metabolism) and small intestine and may be of greater importance. In healthy subjects, most of this gut NH_3 is removed by the liver before reaching the systemic circulation. Using a quantitative model, loss of this "first-pass metabolism" due to portal collateral circulation can account for the hyperammonemia observed in chronic liver disease, and there is usually no need to implicate hepatocyte malfunction. In contrast, in acute hepatic necrosis, hyperammonemia results from damaged hepatocytes. Although muscle-NH_3 uptake is normally negligible, it can become important in severe hyperammonemia. The NH_3-lowering actions of intestinal antibiotics (rifaximin) and lactulose are discussed in detail, with particular emphasis on the seeming lack of importance of the frequently emphasized acidifying action of lactulose in the colon.

Keywords: ammonia, encephalopathy, urease, shunt, urea, glutamate, glutamine, lactulose, cirrhosis

Introduction

Pavlov et al described a link between NH_3 and hepatic encephalopathy (HE) in 1893, but detailed investigation into the biochemistry of NH_3 and its alterations in liver disease did not begin until the 1950s.[1,2] Despite enormous research efforts, the multifaceted pathophysiology of HE, including the role played by NH_3, remains poorly understood.[3,4]

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In our experience, clinicians commonly believe that HE results in large part from the defective hepatic removal of NH_3 produced from bacterial deamination of protein in the colon.[5] As a result, therapeutic interventions have been directed toward attempts to limit this putative source of NH_3 via colectomy (1960s)[5,6] and more recently via medical interventions, such as administration of inabsorbable disaccharides and antibiotics.[7] While this medical therapy clearly has efficacy, it is equally clear that multiple processes in addition to protein deamination in the colon play major roles in the production of NH_3. This review peripherally discusses the connection between NH_3 and HE, but rather focuses on analyzing available data concerning the quantitative influence of the multiple reactions in various organs that determine blood-NH_3 level. Our ultimate objective was the creation of a quantitative model describing the interaction of the multiple factors involved in NH_3 homeostasis, a model that makes possible estimation of the influence of individual pathophysiological processes, such as portosystemic shunting, on blood-NH_3 concentration.

Ammonia is ionizable with equilibrium between the gaseous (NH_3) and ionized (NH_4^+) forms:

$$NH_3 + H^+ \rightleftharpoons NH_4^+ \qquad K_a = \frac{[NH_3][H^+]}{NH_4^+} \qquad (1)$$

The ratio of NH_3:NH_4^+ is described by the Henderson–Hasselbalch relation:

$$\log_{10}(NH_3 / NH_4^+) = pH - pK_a \qquad (2)$$

where $pK_a = -\log_{10}(K_a)$. Substituting the pK_a of 8.95 (at 35°C)[8,9] and pH 7.4 into Equation 2, $NH_3/NH_4^+ = 0.028$, or 2.8% of NH_3 is in the form of NH_3. It had been generally assumed that the high cell-membrane permeability of the gaseous form (NH_3) allowed NH_3 to equilibrate rapidly between blood and tissue via nonionic diffusion. However, it has recently been recognized that there are cell-membrane transporters for both NH_4^+ and NH_3,[10,11] and hence the assumption of nonionic equilibrium may not be valid. Nonionic equilibrium clearly does not occur in the kidney, where acid–base balance is maintained via the concentration of NH_4^+ in the urine (discussed in detail in the "Renal N balance" section).[12] This review focuses primarily on factors determining blood NH_3, and is not concerned with the details of blood–tissue exchange.

The factors determining blood NH_3 (the term "NH_3" is used herein to refer to both the gaseous and ionized forms) are extremely complicated, because NH_3 is a central element in multiple transfer processes involving nitrogen (N) within the body, with blood-NH_3 concentration being the resultant of all these N metabolic processes. While a number of recent reviews have focused on various aspects of this complex process,[3,13–15] we attempt to provide a complete examination of all the factors determining blood NH_3, with an emphasis on their relation to human clinical pathophysiology.

Table 1 summarizes the quantitative contribution of the main N-metabolizing organs (gastrointestinal [GI] tract, liver, kidney, brain, and muscle) to the four primary N metabolites (urea, NH_3, glutamine, and alanine). Figure 1 summarizes the influence of the various reactions occurring in the different organs and the resultant arterial and venous concentrations of N compounds, based on the organ blood flows listed in the second column of Table 1. The rapid turnover of N compounds results in a pseudo-steady state, with the net rate of organ uptake equal to organ production. For example, plasma glutamine has a turnover half-life of 25 minutes.[16] The values listed for "muscle" in Table 1 are theoretical (not experimental) rates that have been adjusted to produce this steady state. Although it is obviously difficult to measure some of these values, eg, the total body-muscle glutamine production, it will be shown that the theoretical values in Table 1 are in rough agreement with experimental measurements.

The results in Table 1 and Figure 1 should be regarded as highly simplified, first-order attempts to quantify the role played by various organs in the metabolism of N compounds, nitrogen balance, and the contribution of these organs to plasma-NH_3 concentration. Measurements of human-organ N fluxes are difficult to perform, limited in number, and have large experimental variability, especially for the GI tract, which requires simultaneous sampling of portal and hepatic vein blood. We have limited the analysis to the four major N metabolites: urea, NH_3, glutamine, and alanine. The attention paid to glutamine and alanine to the exclusion of the other amino acids (AAs) is in part related to the fact that the arterial blood concentrations (in units of micromole/liter) of glutamine (650 μM) and alanine (300 μM) are appreciably higher than those of other AAs, most of which have concentrations <100 μM.[17] More importantly, as discussed herein, glutamine and alanine are unique among the AAs in having important functions in interorgan N transport. Finally, we have only considered N balance in five organs (GI tract, liver, kidney, brain and muscle) and neglected the contributions of other major organs, such as adipose or lungs. Although the lungs may be involved in N exchange in rats,[18] they do not seem to be important in humans.[19] Although the brain is included because of its obvious clinical importance in HE,

Table 1 Postprandial human blood-flow rates

Organ	Blood flow (L/min/70 kg)	Organ balance (M) (μm/min/70 kg; +, net output; –, net uptake)			
		Urea	Glutamine	Alanine	Ammonia
GI tract	1.1 (F_{PV})	–61	–50	+21	+66
Liver	0.45 (F_{HA})	+305	+2.3	–82	–75
Kidney	1.24	–244	–30	+13	15
Brain	0.78	0	–20	0	0
Muscle	1	0	+97.67	+48	–6

Notes: In GI tract (portal vein), liver (hepatic artery), kidney, brain, and muscle and net organ balance M (+, production; –, uptake)[27,48,98,164] of urea, glutamine, alanine, and NH_3.[20–23] Urea organ balances are based on total nitrogen balance, assuming that 20% of the urea formed in the liver is metabolized by GI tract bacterial urease and the other 80% excreted in the kidney. The NH_3 balance for the kidney is from van de Poll et al,[27] GI tract and liver from portal vein and hepatic vein measurements in patients with idiopathic portal hypertension from Nomura et al[110] or during cholecystectomy from McDermott et al.[111] Renal NH_3 balance refers to the net NH_3 added to the systemic circulation. An additional 32 μm/min/70 kg of NH_3 is produced in the kidney and excreted in the urine. Muscle balances are theoretical (not experimental) rates that have been adjusted to produce a steady state (total solute production = total solute uptake). Organ flow rates are from Levitt.[43]
Abbreviation: GI, gastrointestinal.

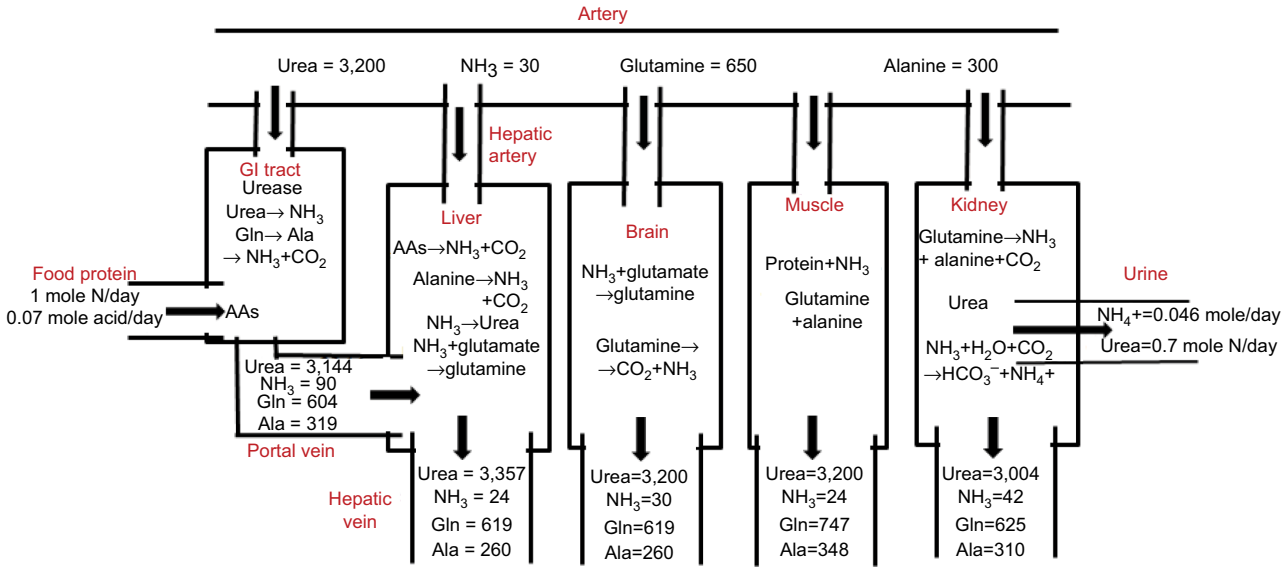

Figure 1 Total human N balance.
Notes: Concentrations (μM) of urea, glutamine, NH_3, and alanine entering each organ in the artery and portal vein and leaving in the veins of each organ resulting from N-balance values and organ flows in Table 1. Processes acting on these N compounds in each organ are briefly outlined.
Abbreviations: GI, gastrointestinal; AAs, amino acids, Gln, glutamine, Ala, alanine.

its contribution to the systemic N balance is small with negligible NH_3 production[20,21] and small glutamine uptake.[22,23] This analysis focuses on the net organ balance of NH_3 and other N solutes. Studies following tracer N-labeled NH_3 are not useful, because the N in NH_3 exchanges rapidly (in seconds) with many AAs.[22,24]

Branched-chain AAs (BCAAs; leucine, isoleucine, and valine) have unique metabolic properties that have made them the subject of intense study.[25] Their total normal plasma concentration is about 215 μM,[26] less than that of alanine, and they make only minor direct contributions to organ-NH_3 balance.[27] However, they have important indirect contributions through their influence on the muscle metabolism of glutamine and alanine. In chronic liver disease (CLD), plasma-BCAA concentrations are significantly reduced. Holeček[28–32] reviewed their influence on muscle-protein metabolism and plasma NH_3 and the therapeutic implications of nutritional BCAA supplementation. This is discussed in more detail in the following sections.

Brief overviews of the factors determining the steady state balance for urea, glutamine and alanine, and NH_3 are presented next, followed by in-depth discussions of N metabolism in the GI tract, kidney, muscle, and liver. Then, we focus on the pathophysiology of the hyperammonemia associated with chronic and acute liver disease. Following a brief review of the hyperammonemia accompanying genetic urea-cycle disorders, we discuss the mechanistic basis of therapeutic GI interventions that have been used in the treatment of hyperammonemia in CLD. Finally, we discuss the clinical implications of blood-NH_3 measurements.

Urea balance

The data for urea in Table 1 and Figure 1 are based on simple metabolic balance estimates. Renal urea excretion is the primary N-excretory mechanism in mammals. The standard human diet contains about 85 g protein/day,[33] corresponding to about 13.6 g N[34] or about 1 M N/70 kg/day. In a steady state, this intake must be balanced by the excretion of 1M/day of N, which occurs via urine (~82%), feces (~15%), and skin (~3%).[35] Urine N is primarily urea (~87%), NH_3 (~6%), and creatinine (~7%).[36] Therefore, about 0.7 M urea N or 350 mm of urea is excreted per day (Figure 1).

The normal renal urea clearance of 75 mL/min implies an arterial urea (U_{Art}) concentration of 3,200 μM (Figure 1). Urea is produced only in the liver by the urea-cycle system. As illustrated in Figure 2, the liver removes AAs via transamination to L-glutamate, which is transported into liver mitochondria and deaminated by glutamate dehydrogenase. This process releases NH_3, which is immediately converted to urea and cycled to the cytosol. The AAs metabolized by the liver represent both those newly absorbed from the intestinal tract and AAs released systemically during normal protein turnover. It should be emphasized that the NH_3 produced by liver mitochondrial AA metabolism is completely captured and converted to urea by the urea cycle, and thus does not directly contribute to systemic NH_3 (the focus of this review). The best evidence in support of this concept is that an intravenous (IV) AA infusion at a rate roughly three times the normal protein intake increased the urea-excretion rate fourfold, raised blood-urea N concentration by 10,000 μM, but had no appreciable effect on blood-NH_3 concentration (normal, about 30 μM).[37]

Studies in humans of the kinetics of ^{15}N- or ^{14}C-labeled urea have found that only about 75% of IV labeled urea was recoverable in the urine (no urea is present in feces).[38–40] Since mammalian cells lack urease and cannot metabolize urea, this metabolism must be the result of bacterial urease activity that presumably takes place in the GI tract:

$$(NH)_2\ CO + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3 \qquad (3)$$

Direct support for this concept comes from the observation that hydrolysis of urea is negligible in germ-free rats[41] and dogs.[42] In Table 1, it is assumed that GI urea metabolism is 25% of renal urea excretion (or 20% of the total renal plus GI), corresponding to normal GI urea clearance of 18.75 mL/min or $M_{GI} = -61$ μm/70 kg/min (Table 1, where the negative sign indicates net urea extraction), which produces 122 μm/70 kg/min of NH_3. A fraction of this NH_3 is incorporated into bacterial protein and trapped in the GI tract (not well quantified in humans), while the remainder is absorbed into the portal circulation (Figure 1). The portal vein urea concentration (U_{PV}) in Figure 1 is determined from the net GI urea balance:

$$[U_{Art} - U_{PV}]\, Q_{PV} = 61\,\mu m/70\,kg/min \qquad (4)$$

where Q_{PV} is the portal vein flow rate. Assuming a normal Q_{PV} of 1.1 L/min/70 kg[43] and solving Equation 4, $U_{PV} = U_{Art} - 55$ μM = 3,144 μM. The total body urea production is the sum of the urine secretion (350 mm/day) plus GI metabolism (87.5 mm/day) = 437 mm/day = 305 μm/70 kg/min. Since only the liver can synthesize urea, the corresponding hepatic vein urea concentration (U_{HV}) is determined from the balance equation:

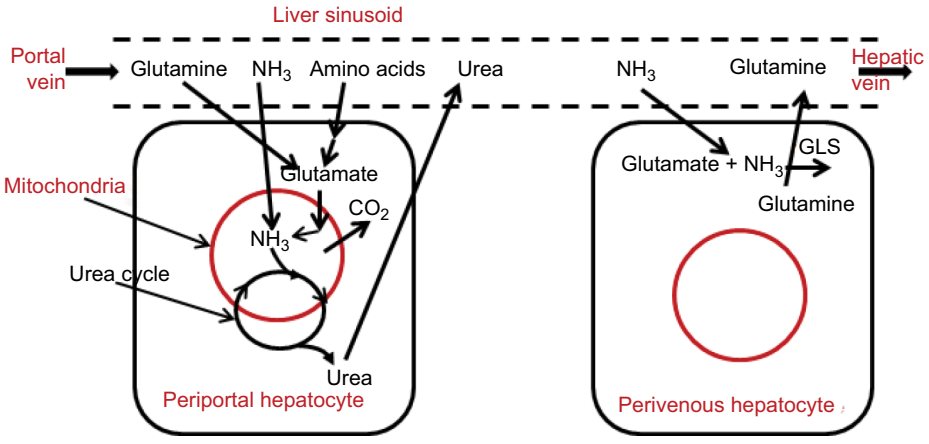

Figure 2 Processes involved in N balance in the liver.

Notes: In periportal hepatocytes, amino acids (including glutamine) are metabolized to NH_3 and CO_2 in the mitochondria, and all this NH_3 is captured and converted to urea by the urea cycle. About half of the NH_3 removed by the liver diffuses to the urea cycle and is converted to urea. In perivenous hepatocytes, the other half of the NH_3 that is removed is stoichiometrically converted to glutamine by glutamine synthetase (GLS), with the glutamine leaving in the hepatic vein.

$$U_{Art}\,Q_{HA} + U_{PV}\,Q_{PV} - U_{HV}(Q_{HA} + Q_{PV}) = -305\,\mu m/70\,kg/min \quad (5)$$

where Q_{HA} is the hepatic artery flow rate (~0.45 L/min/70 kg).[43] Using the previously determined values for U_{Art} (3,200 μM) and U_{PV} (3,144 μM), solving Equation 5 indicates U_{HV} = 3,357 μM. The only other organ that alters urea concentration is the kidney, which excretes 244 μm/70 kg/min of urea:

$$[U_{Art} - U_{RV}]Q_{Ren} = 243\,\mu m/70\,kg/min \quad (6)$$

where U_{RV} is the renal vein urea concentration and Q_{Ren} the renal blood flow. Assuming a Q_{Ren} of 1.24 L/min[43] and U_{RV} = 3,004 μM. These arterial and venous urea concentrations are illustrated in Figure 1, and organ-urea uptake or output and blood flow are summarized in Table 1.

Glutamine, alanine, and branched-chain amino-acid balance

Glutamine has a major role in N transport, and knowledge of its rate of synthesis and metabolism in different body organs is crucial to understanding NH_3 physiology. Skeletal muscle provides a large carbon, N, and energy reservoir, and glutamine is its primary transfer agent, acting, for example, as a major source for gluconeogenesis.[44,45] Skeletal muscle has an extremely high free-glutamine cytosolic concentration of about 20,000 μM[46] that allows maintenance of relatively constant plasma glutamine of about 650 μM in response to rapid changes in utilization. In the postprandial state, skeletal muscle is the major endogenous source of glutamine.[47] As discussed herein, muscle glutamine and alanine synthesis is linked to BCAA metabolism. Estimates for the normal postprandial glutamine balance of the GI tract, liver kidney, brain, and muscle are listed in Table 1, and corresponding venous plasma concentrations are shown in Figure 1. As discussed, it is assumed in Table 1 that muscle production rates must balance other organ uptake. This theoretical muscle glutamine-production rate of rate of 113.67 μm/min/70 kg (Table 1) is of the same order as the experimental measurement of 95 μm/min/70 kg of Damink et al[48] for the lower legs in patients with stable cirrhosis.

As shown in Table 1, there are high rates of glutamine utilization in the kidney and GI tract, organs for which glutamine is used as a source for energy production. The first step in glutamine metabolism is enzymatic conversion by glutaminase (GA) to NH_3 and glutamate, which is then further metabolized to alanine, NH_3, CO_2, and minor amounts of citrulline and proline:

$$\text{Glutamine} + H_2O \xrightarrow{GA} \text{Glutamate} + NH_3 \rightarrow\rightarrow \text{alanine} \rightarrow\rightarrow CO_2 + NH_3 \quad (7)$$

Most of the alanine produced by this process in the kidneys and GI tract (Table 1) is then metabolized by the liver during gluconeogenesis. In the kidneys, the NH_3 produced has an important role in the regulation of acid–base balance and is discussed in more detail in the "Renal N balance" section. Glutaminase is located primarily in kidney, GI tract, liver, and brain tissue, and is notably absent from skeletal muscle.[49] Therefore, though muscle cannot produce NH_3 by GA, NH_3 is produced in muscle via another pathway, the purine nucleotide system (discussed in detail in the "Muscle N balance" section).[50]

In the liver, there are two competing processes affecting glutamine (see Figure 2). In periportal hepatocytes, glutamine is metabolized by GA and the AA mitochondrial system to CO_2, with the NH_3 converted to urea. In perivenous hepatocytes, glutamine is synthesized from glutamate and catalyzed by glutamine synthetase (GLS):

$$\text{Glutamate} + ATP + NH_3 \xrightarrow{GLS} \text{Glutamine} + \text{ADP} + \text{phosphate} \quad (8)$$

In mammals, all glutamine synthesis involves the amination of glutamate via the action of GLS. As such, Equation 8 depicts the necessary last step in glutamine synthesis, an important mechanism for clearing NH_3 produced in the GI tract. These two hepatic processes involving glutamine are regulated in response to the body's metabolic and acid–base needs. Because GLS is at the venous end of the sinusoid, the newly synthesized glutamine cannot be metabolized by the periportal GA.[51] Based on the arterial, portal, and hepatic vein glutamine measurements of van de Poll et al[27] and the estimated flow rates, these two effects roughly cancel each other out, so there is negligible hepatic glutamine uptake.

Alanine has a similar but smaller function than glutamine, serving as a muscle carbon and N reservoir that is tapped during the postprandial and fasting state. Some of the glutamine released from muscle is converted to alanine by the kidney and GI tract. Most of this alanine is consumed by the liver for gluconeogenesis, in marked contrast to glutamine, for which the liver has negligible uptake (Table 1).

Most AAs are metabolized in the liver via cytosolic aminotransferase to glutamate, which is then converted to urea. The BCAAs are unique among the AAs in that, since cytosolic branched-chain aminotransferase (BCAAT) is

absent in the liver, the liver cannot metabolize BCAAs to urea, and BCAAs do not have hepatic first-pass metabolism.[25] The primary site of BCAAT is muscle, with about half the muscle AA uptake consisting of BCAAs.[25,31,32] As discussed in the "Muscle N balance" section, this conversion of BCAAs to glutamate provides the major source of muscle glutamine and alanine production.

Ammonia balance

Systemic NH_3 is a byproduct of the urea, glutamine, and alanine metabolism discussed. Normal arterial plasma NH_3 ranges 12–40 μM, with significant variation obtained by various laboratories using different methodologies.[52] We have assumed a normal value of 30 μM in Table 1. Plasma NH_3 is relatively constant, eg, it does not change significantly following a meal.[53] Since human lung NH_3 metabolism is insignificant, arterial concentration should be equal to central venous concentration.

As shown in Table 1, the main sites of systemic NH_3 production are the kidneys and GI tract. In kidneys, 30 μm/min/70 kg of glutamine is removed and 13 μm/min/70 kg of alanine and 15 μm/min/70 kg of NH_3 added to the systemic circulation. One can calculate a net N balance for this glutamine (2 N/molecule), assuming that the only N products of glutamine metabolism are alanine and NH_3:

$$30 \text{ Glutamine} \rightarrow 13 \text{ Alanine} + 47 \text{ } NH_3 \quad (9)$$

Of the 47 μm/min/70 kg NH_3 produced, 15 μm/min/70 kg (Table 1) enters the systemic circulation and the other 32 μm/min/70 kg is secreted in the urine as NH_4^+ (Figure 1). Equation 9 is an overestimate of the NH_3 produced, because there are also significant amounts of proline and citrulline produced, in addition to alanine.

The GI tract is the major site of NH_3 production, adding 66 μm/min/70 kg (Table 1) to the portal vein, resulting in an NH_3 concentration in the portal vein of about three times the arterial concentration (Figure 1). GI NH_3 is formed by three distinct processes: bacterial urease hydrolysis of circulating urea (Equation 3), metabolism of glutamine (Equation 7) extracted from circulating blood, and bacterial deamination of luminal protein. The first two processes can be quantified from urea and glutamine arterial–venous concentration differences across the GI tract (Table 1; Figure 1). As listed in Table 1, 61 μm/min/70 kg urea and 50 μm/min/70 kg glutamine are metabolized and 66 μm/min/70 kg NH_3 and 21 μm/min/70 kg alanine added to the portal circulation. An N balance on these three solutes (units in μm/min/70 kg) indicates:

$$61 \text{ Urea} \rightarrow 122 \text{ } NH_3$$
$$50 \text{ Glutamine} \rightarrow 21 \text{ Alanine} + ? \text{ } NH_3 + ? \text{ citrulline} + ? \text{proline} \quad (10)$$

As indicated in Equation 10, the NH_3 produced from glutamine metabolism is uncertain, because significant (but unknown in humans) amounts of proline and citrulline (with their attendant N) are produced in addition to alanine.[54] The third process (NH_3 release from bacterial protein deamination) is difficult to quantify, because it represents net NH_3 release and NH_3 uptake by bacteria and cannot be determined simply from arterial–venous differences. As discussed in the "GI-tract N balance" section, this process could potentially produce another 29 μm/min/70 kg NH_3. Since only 66 μm/min/70 kg NH_3 enters the systemic circulation via the portal vein, less than the 122 μm/min/70 kg NH_3 produced just from urea, much of this NH_3 is presumably converted to bacterial protein.

Since postprandial skeletal muscle conversion of protein to glutamine requires additional N (Figure 1), muscle is a potential site of NH_3 uptake. In the normal postprandial state, this NH_3 uptake is small (~6 μm/min/70 kg) compared to liver uptake (~75 μm/min/70 kg) (Table 1). However, as discussed herein, in liver failure this muscle contribution increases and becomes of major importance.

The liver is the major organ responsible for NH_3 removal. As shown in Figure 2, there are two distinct liver pathways for NH_3 metabolism. Periportal hepatocytes represent the major mass of the liver, responsible for most of the metabolism, including the breakdown of AAs and the very efficient mitochondrial conversion of NH_3 to urea by the urea-cycle system. A fraction of the NH_3 entering the liver via the portal vein diffuses to this site and is converted to urea. The second NH_3-metabolic pathway is the conversion of NH_3 to glutamine by cytosolic GLS (Equation 8) that is localized in a small ring of cells encircling the terminal hepatic venules: perivenous hepatocytes.[51,55] Although there are no quantitative human measurements, in mouse[56] and pig[57] liver, about half of the removed NH_3 is converted to urea and the other half to glutamine.

GI-tract N balance

As discussed, it is clear from arterial–venous concentration measurements that the GI tract is the major source of the NH_3 that determines plasma concentration. Based on a variety of earlier studies,[58,59] the current (2016) textbook description is that NH_3 ". . . is produced primarily in the colon, where bacteria metabolize proteins and other nitrogen-based products into ammonia".[60] Therefore, it is generally assumed that the main source of GI-NH_3 production results from two distinct

colonic bacterial processes: urea hydrolysis (Equation 7) and protein deamination of malabsorbed protein. More recently, a third mechanism, glutamine metabolism (Equation 7), which occurs throughout the GI tract, has been proposed as another major contributor to GI-NH_3 production.[14] These three processes are illustrated schematically in Figure 3 and discussed in detail herein. One emphasis of this discussion will be demonstrating that there are serious questions about the conventional view that bacterial urea hydrolysis occurs in the colon. The location of bacterial urea metabolism has important clinical significance, because one of the primary forms of HE treatment is directed at decreasing this GI-NH_3 production and it is important to understand the location and types of bacteria involved.

While bacteria are commonly recognized to produce NH_3 in the gut, it should be noted that bacteria in the process of replicating must synthesize protein. A sizable fraction of the requisite nitrogen may be supplied by NH_3, and hence bacteria may serve as a mechanism of eliminating intestinal NH_3. Such bacterial uptake of NH_3 is supported by the observation that enteric bacteria rapidly proliferate in a medium in which NH_3 is the sole source of nitrogen.[61] The relatively low bacterial counts of the small bowel would seemingly relegate the importance of enteric bacterial accumulation of nitrogen to the colon. However, Miner-Williams et al[62] found that bacterial protein accounted for >50% of the roughly 0.6 g nitrogen passing the terminal ileum of healthy controls over an 8-hour period following a casein meal.

The commonly accepted idea that AA deamination by colonic bacterial metabolism is a primary net producer of gut NH3 has little direct experimental support. The massive proliferation of bacteria in the colon to counts of $>10^{11}$/g feces could obviously be a major NH_3 sump if most of the bacterial N was derived from NH_3. The best (but indirect) argument for this concept that colonic bacteria are a net producer of NH_3 is that the fecal NH_3 estimated using swallowed dialysis bags is relatively high – about 14 mM (1%–2% of total fecal N) – and protein deamination is a likely source of this NH_3.[40] A rough estimate of the balance between colonic bacterial production minus bacterial removal is provided by the difference between total N entering and leaving the large intestine, assuming that all this difference reflects net bacterial NH_3 production. Gibson et al[63] compared the total N in ileostomy drainage (1.8 g/day) versus that in normal subject feces (1.2 g/day). Assuming that this difference (0.6 g/day) was all converted to NH_3, it would be equivalent to 29 µm/min/70 kg NH_3, about 16% of the total GI-NH_3 production from all sources: protein deamination (~29 µm/min), urea hydrolysis (~122 µm/min/70 kg), and glutamine metabolism (~20 µm/min/70 kg).

Intestinal glutamine metabolism (Equation 7) is now generally accepted to be a major source of GI NH_3.[14] The glutamine extraction across the entire GI tract (arterial–portal vein NH_3) varies from about 33% in rats[54] and dogs[54] to 12% in monkeys[54] and 7.6% in humans (Figure 2). Glutamine has a major role in maintaining normal gut function. It is an oxidative fuel source for the intestine. In the perfused rat intestine, glutamine was the source of 32% of the CO_2 produced, even though the perfusate had a high glucose concentration.[54] This is species-dependent. In dogs in the 24-hour fasted state, glutamine provided only about 15% as much fuel as glucose.[64] The alanine produced by glutamine metabolism (Equation 7) is used by the liver for gluconeogenesis during the postprandial period. These two functions are regulated in response to the body's nutritional needs. In dogs, the fraction of glutamine converted to alanine falls from 67% in the

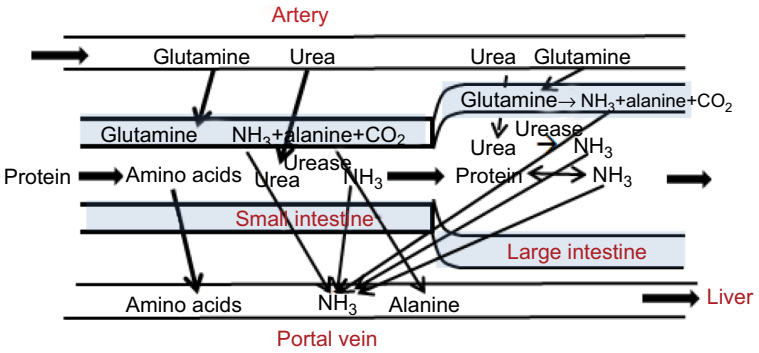

Figure 3 Processes involved in N balance in the gastrointestinal tract.
Notes: There are three distinct processes producing NH_3. Urea diffuses from the blood to the intestinal lumen, where it is hydrolyzed to NH_3 and CO_2 by bacterial urease. As indicated by the dashed arrow, mucosal permeability is very low in the large intestine, limiting the rate at which urea can enter the lumen. The second process is the metabolism of glutamine by the intestinal mucosa. This produces varying amounts of NH_3 and alanine. The third process is bacterial deamination of the malabsorbed protein in the large intestine.

postprandial period (24-hour fast) to 20% after a 96-hour fast.[64] This fraction is important for NH_3 balance, because for each alanine produced there is one less NH_3 released than is the case with complete oxidation of glutamine as a fuel source (Equation 10). Glutamine is important for a number of other intestinal functions, including regulation of protein synthesis and as a precursor of nucleic acid biosynthesis.[65]

Although there is unequivocal evidence from urea-tracer studies that in humans, about 20% of the urea produced is metabolized to NH_3 in the GI tract, there are important questions about the actual location where this occurs. The large intestine has been assumed to be the main site of urea catabolism because the concentration of colonic bacteria exceeds that of the more proximal gut by roughly three orders of magnitude. However, there is strong evidence that the large intestinal mucosa is impermeable to urea, preventing systemic urea from reaching luminal bacteria. The measured rate of transport of blood urea to colonic saline perfusions is very small (clearance of about 0.2% colonic urea blood flow), and even this very small transport may be artifactual, because the absolute appearance rate in the lumen is not altered when plasma-urea concentration was doubled by IV urea infusion.[66] Similarly, there is negligible urea absorption when the human colonic lumen is perfused with high urea concentrations.[67] In marked contrast, urea readily permeates tight small-intestine junctions,[68–70] and the urea concentration in human ileostomy fluid is nearly identical to plasma.[71] Additional support for the small-intestine site of urea metabolism is the observation of Gibson et al[71] that the absolute rate of GI-urea metabolism in patients with ileostomies and nonfunctioning colons is similar to that in normal subjects.

Using ingested cellophane dialysis bags to sample stool-fluid concentrations, Wilson et al[72] found that there was no detectable fecal dialysate urea in normal subjects. Supporters of the argument that the colon is the main site of urea metabolism have argued that this implies that bacteria catabolized 100% of the urea that diffused into the lumen.[73] Several observations argue against this interpretation. First, if colonic bacteria actually catabolized urea as rapidly as it enters the colon, the rate of urea metabolism would be diffusion-limited and directly proportional to plasma-urea concentration. However, the absolute rate of GI urea metabolism remains normal in patients with chronic renal failure, even though their plasma-urea concentration is up to six times normal.[74] Consistent with this is the important observation that blood NH_3 is not elevated in chronic renal failure patients with urea levels six times normal.[75] Second, if colonic bacteria were consuming most of the urea, one would predict that a large fraction of the colonic NH_3 production should be derived from urea metabolism. However, Wrong et al[76] labeled the plasma urea with ^{15}N and found that the majority of the fecal NH_3 was not derived from urea, but from some other source, such as plasma glutamine metabolism (Equation 7) or protein deamination.

These arguments indicate that only a minor fraction of urea metabolism occurs in the large intestine, and thus implies that most bacterial urea metabolism must occur in the stomach and small intestine. This would provide an explanation for the unexpected observation that the absolute rate of urea metabolism is independent of plasma-urea concentration. Because of high small-intestine urea permeability,[70] the luminal intestinal urea concentration is approximately equal to plasma urea (~3.2 mM). This indicates that the rate of small-intestine bacterial urea metabolism is maximal at normal serum urea concentrations and further increases in plasma urea (eg, in renal failure) would not be expected to increase the urea metabolic rate.[74] For example, *Helicobacter pylori* urease has a K_m of 0.48 mM,[77] sixfold lower than normal plasma urea (3.2 mM). If the rates of gastric and small-intestine bacterial urea metabolism were saturated at normal plasma-urea concentrations, it would also explain the "surprising" result that administering urea by mouth did not increase plasma-NH_3 levels in cirrhotic liver patients.[78] Administering the same amount of urea by rectum increased plasma NH_3 by a factor of about 4.[78] The increased gastric and small-intestine urea produced by oral dosing of urea does not increase NH_3 production from bacteria that are already consuming urea at a maximum rate, and unmetabolized urea is absorbed in the small intestine before reaching colonic bacteria.

Even if the colonic mucosa were impermeable to urea, a small amount of urea would be delivered to the colon in the ileal effluent. Assuming a 1 L/per day ileal flow containing a urea concentration equal to that of plasma would make possible a colonic urea clearance of 1 L/day. This colonic clearance is negligible compared to the total GI urea clearance, which is 25% of renal urea clearance or about 27 L/day. One would expect this colonic clearance to increase as plasma (and thus ileal) urea increases in patients in renal failure. But even the sixfold increase in plasma urea observed in the studies of Walser[74] would only increase colonic clearance to about 22% of normal gut clearance, and this increase probably would not produce a recognizable increase in serum-NH_3 levels in renal failure patients.

Although these arguments that most bacterial urea metabolism occurs in the stomach and small intestine seem

convincing, surprisingly little attention has been directed at their implications. The only major urea-splitting bacteria that have been clearly identified in the upper gut are *H. pylori*. There is a surprising divergence of opinion in regard to the importance of *H. pylori* metabolism as a source of systemic NH_3. The largest study was that of Chen et al[79] of 457 cirrhotic Chinese patients. They found that blood NH_3 was significantly higher in *H. pylori*-positive (78 μM) versus *H. pylori*-negative (54 μM) patients, and that following successful eradication of *H. pylori*, blood NH_3 declined to 54 μM, the value observed in the initially *H. pylori*-negative subjects. No such decline was observed in subjects in whom eradication efforts had failed. As might be expected, the authors concluded that "*H. pylori* is an important factor for inducing high blood-NH_3 concentration in cirrhotic patients". Other smaller studies have found no relationship between the existence of *H. pylori* infection and serum-NH_3 concentrations.[80,81] A meta-analysis published in 2013[82] came to the unusual conclusion that *H. pylori* infection increases serum NH_3, but only in subjects of Asian ethnicity. While the explanation for this putative ethnic difference may be attributable to unclear technical differences among studies carried out in different countries, *H. pylori* organisms appear to be more aggressive in Asian subjects. It seems possible that *H. pylori* in these subjects produces more NH_3. As discussed, only a fraction (50% or less) of the total NH_3 produced in the GI tract appears in the portal vein, and presumably the remainder is converted into bacterial protein. This is supported by in vitro studies of *H. pylori* urea metabolism, where only about 12% of the NH_3 produced was recovered as NH_3 in the medium.[83] If the fraction of the NH_3 converted into protein declined with increasing NH_3 production, an influence on serum NH_3 might only be observed at very high *H. pylori* NH_3-production rates.

In contrast to the intensive study of urea catabolism by *H. pylori*, there has been limited research on other urea-splitting organisms that might inhabit the small bowel. In an older study (1966), before it was recognized that only bacteria could metabolize urea, Aoyagi et al[84] determined urease activity in mucosal biopsy samples. Not surprisingly, they found especially high gastric activity (presumably *H. pylori*), but also found jejunum mucosal urease activity that was about threefold that of the colon. Although the relationship between this mucosal activity and total luminal activity is uncertain, these results clearly indicate that there are urea utilizing bacteria in the human small intestine. The finding of higher bacterial urease activity in the small bowel versus colonic biopsies is surprising, given the likelihood of much greater luminal bacteria contamination in colonic biopsies. Presumably, small-bowel bacteria are either more closely associated with mucosal tissue than is the case with colon tissue or have higher urease activity. Exclusively of *H. pylori*, there has been surprisingly little study of human urease containing intestinal bacteria, with most attention directed to the presence of such organisms in urinary tract infections. Suzuki et al[85] studied the urease activity of 120 strains of human intestinal bacteria, and found evidence of urease activity in a sizable fraction of *Bifidobacterium* and *Proteus* spp. and a small fraction of *Lactobacillus*.

These arguments have focused on the evidence supporting a small-intestine or gastric site of urea metabolism. However, it should be emphasized that there is a body of contradictory evidence favoring the large intestine as the primary site of urea metabolism.[58,59] Probably the strongest evidence favoring the large-intestine site in humans is that unabsorbable disaccharides (eg, lactulose), which are assumed to act primarily via acidification of colonic contents, are nearly as effective as antibiotics in reducing blood NH_3 in patients with CLD.[86,87] The potential mechanisms by which lactulose reduces blood NH_3 are discussed in more detail in the "Gastrointestinal therapeutic approaches to lowering blood ammonia " section.

As the preceding discussion demonstrates, there is uncertainty about the important clinical question of the site of NH_3 production in the GI tract. This question could be definitively answered by determining NH_3 concentration and flow rate in veins draining the different GI-tract segments. We are aware of only two preliminary reports. From measurements of NH_3 concentration and flow rates in the portal and superior mesenteric veins of the pig, van Berlo et al[88] estimated that " . . . more than 75% of total intestinal" NH_3 was produced in the small intestine. van der Hulst et al[89] reported concentrations of 65, 33, and 30 μM in veins draining the jejunum, ileum, and colon, respectively, in patients during GI-cancer surgery. Although they did not measure flow rates, since total small-intestine blood flow is about fivefold that of the large intestine,[90] these NH_3-concentration measurements suggest that about 80% of GI-tract NH_3 is produced in the small intestine. Both of these results are consistent with arguments that the conventional view that GI tract NH_3 is produced primarily by colonic bacteria is questionable.

Renal N balance

Ammonium (NH_4^+) urinary excretion (Figure 4) is the most important factor in the control of acid–base balance. Normal human net endogenous acid production (NEAP) depends on dietary protein composition, and we have assumed in

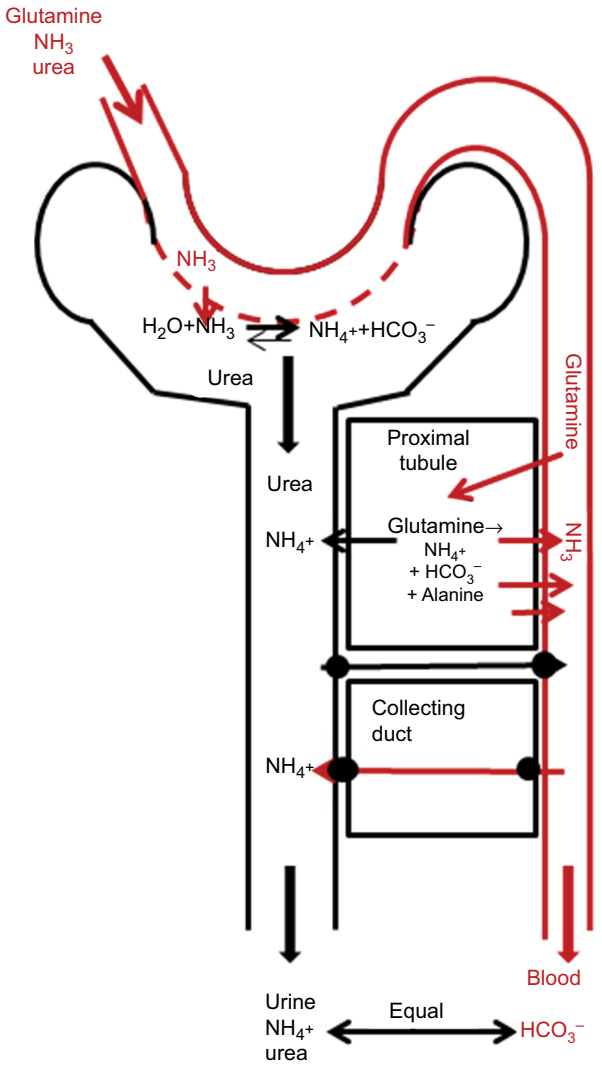

Figure 4 Schematic diagram of processes involved in N balance in the kidney.
Notes: Urinary excretion of NH_4^+ is the most important factor in the control of acid–base balance. NH_4^+ is derived primarily from the metabolism of blood glutamine, which is metabolized in the proximal tubule. A complex set of NH_4^+, NH_3, and HCO_3^- tubule- and collecting duct-transport processes regulate NH_4^+ excretion. For each NH_4^+ molecule excreted, an HCO_3^- molecule is added to the systemic circulation. In addition, some of the glutamine is either used as an energy source or converted to alanine.

Figure 1 that NEAP is 70 mEq/70 kg/day.[91,92] In the steady state, this is balanced by the renal net acid excretion, which consists of about a third titratable acids (eg, phosphate) and two-thirds NH_4^+,[92] or 46 mm/70 kg/day NH_4^+ excreted in urine (Figure 1). This urine NH_4^+ cannot come solely from plasma-NH_3 clearance, because with arterial plasma NH_3 of only 30 µM, it would require renal plasma clearance of about 1.5 L/min/70 kg, which is greater than the total Q_{Ren}. Most of the NH_4^+ excreted in the urine is provided by glutamine metabolism (Equation 7), which releases more NH_3, than is excreted in the urine; therefore, the kidney is a net producer of systemic NH_3 (Table 1; Figure 1).

Not surprisingly for something as important as acid–base balance, the processes involved in renal NH_4^+ excretion are complicated.[92,93] NH_3 is synthesized from glutamine metabolism (Equation 7), primarily in the proximal tubule, and the amount of NH_4^+ excreted in the urine is controlled by a complex set of collecting-duct NH_4^+ and HCO_3^- transporters (Figure 4). In addition to providing NH_3, the renal metabolism of glutamine provides fuel (eg, ATP) and is used for gluconeogenesis[94] or alanine synthesis.[95] In the normal postprandial state, the overall renal N balance assumed in Table 1 (in units of µm/min/70 kg) is:

$$30\ \text{Glutamine} \rightarrow 13\ \text{Alanine} + 15\ NH_3 + 32\ NH_4^+ \quad (11)$$

where the alanine and 15 µm/min/70 kg NH_3 are added to the systemic circulation and the 32 µm/min/70 kg NH_4^+ (0.046 M/day, Figure 1) is excreted in the urine. This balance depends on the metabolic acid–base state. In mild experimental acidosis in which total acid intake is increased by factor of about 3, urine NH_4^+ excretion increases threefold, renal glutamine uptake doubles, and NH_3 added to the systemic circulation is relatively unchanged.[96] It should be emphasized that the NH_3 added to the systemic circulation by the kidney does not undergo the "first-pass" type of metabolism of intestinal NH_3 (about 70% efficient). Therefore, NH_3 delivered to the serum via the kidney has a roughly threefold-greater effect on serum NH_3 than is the case with intestinally produced NH_3.

Muscle N balance

As discussed, the release of AAs by skeletal muscle (Figure 5) is a major source of both energy and N in the postabsorptive state. Muscle N balance has been comprehensively reviewed by Damink et al,[14] and this section summarizes the main features. Although muscle releases all AAs, alanine and glutamine dominate, accounting for >50% of the total.[97] Glutamine is utilized predominantly by the kidney and GI tract, while alanine is used by the liver, primarily for gluconeogenesis (Table 1). The major source for muscle glutamine and alanine synthesis is BCAAs, which are transaminated by BCAAT to glutamate.[28] Glutamate can then either pick up an NH_3 molecule and be converted to glutamine by GLS (Equation 8) or react with pyruvate and be converted to alanine.[28] As such, BCAA metabolism plays a central role in controlling muscle glutamine and alanine synthesis and NH_3 uptake (Figure 5).

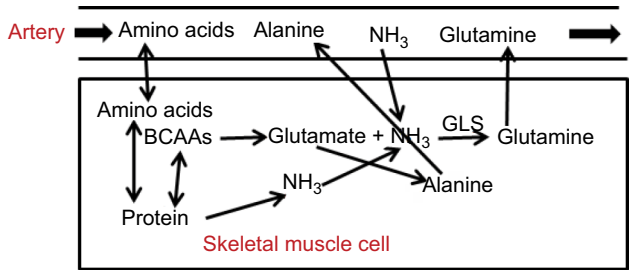

Figure 5 Schematic diagram of processes involved in N balance in the skeletal muscle.
Notes: During the postprandial state, muscle provides a reservoir for N, carbon, and energy stores. Glutamine and alanine are produced primarily by transamination of BCAAs. Normally, only a small fraction of the NH_3 required for glutamine synthesis comes from blood NH_3, with the majority provided by muscle-protein metabolism. During states of hyperammonemia, the amount of NH_3 removed by muscle may increase roughly linearly with arterial NH_3 concentration.
Abbreviations: BCAAs, branched-chain amino acids, GLS, glutamine synthetase.

The NH_3 required for glutamine synthesis can be provided either by uptake from the plasma or muscle AA metabolism. Muscle-NH_3 uptake is much lower than glutamine production, and in a number of studies no significant muscle-NH_3 uptakes have been detected.[14] In Table 1, the standard postprandial muscle-NH_3 uptake is about 6 μm/min/70 kg, $^1/_{16}$ the glutamine release of 97 μm/min/70 kg. Muscle clearly cannot rely on NH_3 uptake from plasma for glutamine synthesis, because the rate of glutamine synthesis may be greater than the total rate of NH_3 supplied by arterial blood flow (Figure 1). Therefore, most of the additional nitrogen released in the form of glutamine must come from muscle AA breakdown (Figure 5). Muscle utilizes the purine-nucleotide cycle to produce N from protein breakdown.[50]

Human muscle-tissue samples indicate that there is a large free muscle-NH_3 concentration of about 150 μM,[98] roughly similar to the 300 μM[99] NH_3 K_m of GLS, the rate limiting enzyme of glutamine production. Since this is fivefold normal arterial NH_3, it is presumably supplied by muscle-protein catabolism. Although we have emphasized the role of muscle-glutamine release as a fuel and gluconeogenesis source during the postprandial period, another function of muscle glutamine emphasized by Chang et al[100] is as a means of "nitrogen disposal" during AA breakdown that is less toxic than NH_3.

The basal muscle-NH_3 uptake of 6 μm/min/70 kg is negligible relative to normal liver-NH_3 uptake (75 μm/min/70 kg). However, in a variety of animal studies, it has been shown that the rate of muscle-NH_3 uptake increases roughly linearly with arterial NH_3.[14] As discussed herein, in patients with increased NH_3 secondary to cirrhosis[101,102] or acute liver failure (ALF),[103] this increased muscle-NH_3 uptake becomes important in limiting hyperammonemia.[104] Holeček et al[105] showed that in an in vitro rat-muscle model, increased bathing NH_3 concentration increased BCAA oxidation and glutamine synthesis, suggesting that the increased plasma NH_3 in cirrhosis may directly activate BCAA metabolism.

During exercise, muscle-protein catabolism increases the rate of muscle-NH_3 production, with muscle becoming a net supplier of systemic NH_3.[98,106] In studies of Katz et al,[98] muscle NH_3 balance went from a net NH_3 uptake (for two legs) of about 6 μm/min/70 kg (identical to the value listed Table 1) during rest, to a small net release of about 4 μm/min/70 kg during submaximal exercise, rising markedly to 178 μm/min/70 kg during maximal exercise. This is associated with a small increase in arterial plasma NH_3 from 21 μM at rest to 27 μM during submaximal exercise and a dramatic fivefold increase to 112 μM during maximal exercise, when the rate of NH_3 production is so high that it overwhelms the GLS system. Since submaximal exercise (50% VO_{2max}) produces only minor changes in muscle-NH_3 balance, it is assumed that the values in Table 1 are applicable to the human postprandial state, even during the normal daily exercise regimen. It should once again be emphasized that NH_3 released by muscle does not undergo first-pass metabolism in the liver, and hence has a disproportionate effect on blood NH_3 relative to intestinally produced NH_3.

As this discussion indicates, muscle GLS has at least two important functions related to NH_3: it converts the muscle NH_3 produced during postprandial protein catabolism to glutamine, and during hyperammonemia, it is an important factor in removing systemic NH_3. A direct confirmation of these two muscle-GLS functions was provided by He et al,[45] in which GLS was selectively knocked out in mouse skeletal muscle. The knockout (KO) mice were healthy and fertile, with normal weight gain and organ histology. Fed KO mice had relatively normal blood chemistry, including normal arterial glutamine. However, major differences became apparent during fasting, when presumably muscle-protein catabolism becomes important. At 20 hours of fasting, the rate of muscle (actually, hindquarter)-glutamine production increased more than fourfold over the fed state in wild-type mice, but was unchanged from the fed state in the KO mice. The second GLS function was tested by IV administration of NH_4HCO_3 at varying rates. In control mice, blood NH_3 remained relatively normal until the rate exceeded 40 μm/h, after which it rose rapidly, increasing eightfold at rates of 50 μm/h. This indicates that normal NH_3-metabolizing functions in such tissue as liver and muscle become saturated and have a maximal removal rate of 40 μm/h/mouse. In the KO mouse, this maximal removal rate was reduced by half to 20 μm/h/

mouse. Since the only difference between the normal and KO mice was the lack of muscle GLS, this implies that muscle contributes 50% to mouse NH_3-metabolizing potential during hyperammonemia. It may seem antithetical to state that muscle is a major site of NH_3 removal when it was emphasized in the "Urea balance" section that renal urea secretion is the only N-excretory process in humans. In actuality, the NH_3 taken up by muscle is used to synthesize AAs (primarily glutamine and alanine), which are then converted to urea by the liver. To the extent that some of this glutamine is metabolized to NH_3 in such organs as kidneys or intestines, this concept of net muscle-NH_3 removal is incorrect.

Liver N balance

As previously discussed and summarized in Table 1, the liver is the major organ responsible for normal NH_3 removal (ie, detoxification). There are two pathways involved: periportal hepatocyte conversion of NH_3 to urea by the urea-cycle system, and perivenous hepatocyte conversion of NH_3 to glutamine by GLS (Equation 8). These processes are illustrated in Figure 2. Resection of up to 90% of the liver in the pig surprisingly results in no significant change in serum NH_3 levels,[107] indicating that liver-NH_3-detoxifying capacity is far in excess of normal requirements. It also suggests that liver-NH_3 metabolism is responding to some sort of arterial blood NH_3 set point and is independent of absolute liver mass. This raises the possibility that the NH_3 normally presents in blood has a physiological function yet to be demonstrated.

A significant fraction of glutamine entering the liver is catabolized to urea by the standard AA-metabolic process (transamination to glutamate, which then enters urea cycle) in periportal hepatocytes. However, this fraction cannot be determined simply from the net liver balance in Table 1, because glutamine is also synthesized from glutamate in perivenous hepatocytes (Equation 8), and liver-glutamine balance is the difference between the rate of glutamine production and glutamine catabolism:

$$\text{Net glutamine output} = \text{Glutamine production} - \text{Glutamine catabolism} \quad (12)$$

Since glutamine output is approximately zero (Table 1), glutamine production and catabolism are about equal. Of the total liver-NH_3 removal rate (75 μm/min/70 kg, Table 1), about 50% results from glutamine production, implying that liver-glutamine production is about 37 μm/min/70 kg. Therefore, from Equation 12, liver-glutamine catabolism must also be about 37 μm/min/70 kg. This is a major component of glutamine metabolism (37% of total muscle-glutamine production, Table 1), and the liver is primarily responsible for controlling steady-state glutamine arterial concentration. Knocking out this glutamine catabolism produces a 25-fold increase in arterial glutamine.[108]

The importance of the hepatic GLS mechanism was clarified by a recent (2017) interesting study in which GLS was selectively knocked out in mouse liver.[56] The KO mice had about doubled elevation of arterial NH_3 and a third decrease in arterial glutamine. In the postabsorptive state when there is muscle protein breakdown and increased NH_3 production, there was a marked decrease in the rate of glutamine production by the liver in the KO mice. It was also shown that the GLS system had relatively high NH_3 affinity compared to the urea-cycle system, and in wild-type mice it accounted for about 50% of total NH_3 detoxification. It was concluded that in wild-type mice, 70% of the NH_3 delivered to the liver was cleared: 35% by the GLS system and 35% by the urea-cycle system. Again, as was discussed in the "Muscle N balance" section, the glutamine produced must eventually be converted to urea for net N removal.

In the same study, GLS KO mice had a 50% decrease in muscle mass and a threefold increase in fat mass.[56] Although the mechanisms responsible for this are unknown, one possibility is that arterial glutamine concentration is a signal controlling muscle and fat contributions to postprandial fuel supply. The decreased arterial glutamine (ie, fuel) in the KO mice may be a signal for increasing muscle breakdown and increasing adipose-energy stores.

As illustrated in Figure 1, the GI tract is the major producer of systemic NH_3, with portal vein-NH_3 concentration three times the arterial. Normally, the rate that the liver removes NH_3 exceeds the rate that NH_3 is added to portal blood flow by the gut, such that the hepatic vein-NH_3 concentration is only 80% that of arterial blood. As is discussed herein, one of the major causes of hyperammonemia in cirrhotic patients is the shunting of portal blood around the liver, and we try to relate quantitatively the degree of shunting to hyperammonemia. There is some confusion in the literature about how to quantify this portal NH_3 metabolism. The standard pharmacokinetic term is "first-pass metabolism", which is defined as the fraction of an orally absorbed drug that does not reach systemic circulation.[109] Since this cannot be applied to an endogenous solute, such as NH_3, an alternative definition is necessary. Nomura et al[110] define "hepatic extraction" simply in terms of portal vein (C_{PV}) and hepatic vein (C_{HV}) NH_3 concentration:

$$\text{Hepatic extraction} = (C_{PV} - C_{HV})/C_{PV} \quad (13)$$

This simple definition is only approximate, because it neglects the hepatic artery-NH_3 contribution to C_{HV}. In our analysis of the effect of portal shunting, we use another definition – fraction metabolized (F_{Met}) – which is the fraction of total NH_3 reaching the liver that is metabolized:

$$\text{Fraction metabolized} = F_{Met} = \frac{Q_{PV}C_{PV} + Q_{HA}C_{Art} - Q_{HV}C_{HV}}{Q_{PV}C_{PV} + Q_{HA}C_{Art}} \quad (14)$$

where Q_{PV}, Q_{HA}, and Q_{HV} are portal vein, hepatic artery, and hepatic vein blood-flow rates, respectively. For the data in Table 1, the hepatic extraction (Equation 13) is 73%, similar to that found experimentally in a series of patients with idiopathic portal hypertension[110] or during cholecystectomy.[111] Using the flow rates and concentrations listed in Table 1, the fraction metabolized (Equation 14) is 67%, similar to values reported in mice, cows, lambs, and rats.[57]

Hyperammonemia associated with CLD and acute liver failure

Hyperammonemia is commonly observed in CLD, and the potential for this elevated NH_3 to cause HE is the primary reason for the strong interest in the pathophysiology of blood NH_3. Plasma NH_3 increases with severity of CLD classified by the Child–Pugh score, rising from a normal value of about 30–45 μM (high normal) for Child–Pugh A to 66 μM in Child–Pugh B and 108 μM in Child–Pugh C[112] (assignment of Child–Pugh score does not depend on plasma NH_3). In the most severe forms of CLD, with marked HE, arterial NH_3 can increase to 250 μM or higher.[113]

Although multiple factors influence the degree of hyperammonemia, it has long been recognized that most important is the shunting of portal blood past the liver. As discussed, the GI tract is the major producer of systemic NH_3, with portal vein-NH_3 concentration three times arterial concentration. Normally, all the portal blood passes through the liver, which extracts NH_3 more quickly than it is produced in the gut, thus maintaining low levels of NH_3 in the systemic circulation. A portal to systemic shunt allows NH_3 produced in the gut to circumvent the liver and enter the systemic circulation. Since the liver clears about 70% of the NH_3, a portal shunt effectively increases the delivery of NH_3 to the systemic blood about threefold. The classic experimental model of such shunting is the "Eck fistula"[114] described by Eck in 1877,[115] in which all the portal blood flow is shunted to the inferior vena cava in the dog. In 1893, Hahn et al carried out detailed and extended studies of Eck fistulae and described that these dogs developed severe HE.[1,2] It was not until the 1950s that it was recognized that the GI tract was a major source of NH_3 production and that the portacaval shunt allowed this NH_3 to bypass the liver.[116–118] In monkeys on a normal protein diet, plasma NH_3 levels increase fourfold after creating a portacaval shunt, and rapidly fall back to normal levels if the shunt is closed.[119]

Qualitatively, the increase in blood NH_3 is correlated with the severity of the portosystemic shunt.[112] However, there are only a small number of quantitative measurements of portosystemic shunt flow in humans, and no measurements that have directly correlated this shunt flow with arterial NH_3. Moreno et al[120] reported that the portal vein flow rate entering the liver in patients with advanced CLD is on average only about 32% of normal. If the total GI-tract flow remained constant, this would imply that about 68% of the normal GI flow is shunted to the systemic circulation. This is directly supported by the measurements of Groszmann et al[121] who found that on average 62% of mesenteric flow and 60% of splenic flow were shunted past the liver in CLD. In severe CLD, the portal flow can be reduced to zero,[120] and for a small fraction (8%), reverse flow (hepatofugal) has been observed in portal vein Doppler-flow measurements.[122]

The following highly simplified model can be employed to make a quantitative estimate of the effect of a portosystemic shunt on arterial NH_3. It will be assumed that in patients with portal shunts: 1) The "Fraction Metabolized" (F_{Met}, eq.) of the NH_3 that enters the liver is unchanged from the normal value of 67% determined above; 2) the rates of NH_3 production by the kidney (M_{Ren}= 15 μm/min/70kg) and GI tract (M_{GI} = 66 μm/min/70kg), and utilization by muscle (M_{Mus}= –6 μm/min/70kg) are unchanged from the normal values in Table 1; and 3) the total portal vein (Q_{PV}) and hepatic artery (QHA) blood flow rates are unchanged from the normal values (Table 1). The change in steady-state NH_3 arterial concentration as a function of the fraction of portal vein blood that is shunted (f_{shunt}) can then be calculated using the following analysis.

In the steady state, the NH_3 metabolized by the liver must balance the total amount made by the kidney, GI tract, and muscle:

$$F_{Met}[(1 - f_{shunt})Q_{PV}C_{PV} + Q_{HA}C_{Art}] = M_{Ren} + M_{GI} + M_{mus} \quad (15)$$

and C_{PV} can be related to arterial concentration (C_{Art}) by Equation 4:

$$Q_{PV}(C_{PV} - C_{Art}) = M_{GI}$$
$$\Rightarrow C_{PV} = C_{Art} + M_{GI} / Q_{PV} \quad (16)$$

Upon solving Equations 15 and 16, one can determine the steady-state NH_3 C_{Art} as a function of f_{shunt}:

$$C_{Art} = \frac{F_{Met} M_{GI}(1 - f_{shunt}) - M_{GI} - M_{Mus} = M_{Ren}}{F_{Met}(Q_{PV} f_{shunt} - Q_{HA} - Q_{PV})} \quad (17)$$

Noiret et al[123] described a similar, but much more detailed modeling of portosystemic shunting using a somewhat different set of parameters. It should be emphasized that because of the uncertainty in the parameters in Table 1 and how they vary in patients with CLD, these models are very approximate and useful primarily in a heuristic manner to illustrate the effect of a portosystemic shunt on arterial NH_3.

A plot of arterial NH_3 (C_{Art}) as a function of f_{shunt} (Equation 17) is shown in Figure 6 (black line). It can be seen that as f_{shunt} increases from 0 to 1, C_{Art} increases from 30 μM to about 250 μM. This latter value is similar to the C_{Art} levels that are reached in patients with severe CLD.[113] For the average advanced CLD shunt value of 65%,[120] C_{Art} is 107 μM, similar to what is observed in Child–Pugh class C patients.[112] As such, this analysis shows that most of the hyperammonemia observed in CLD can be accounted for simply by portosystemic shunting, without invoking any other pathophysiology, such as poor hepatocellular function.

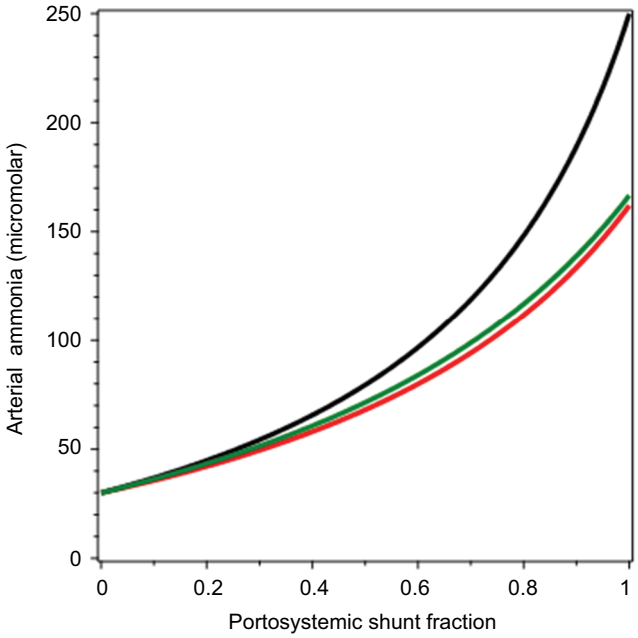

Figure 6 Arterial NH_3 concentration as a function of the fraction of portal blood shunted to systemic veins, bypassing the liver.

Notes: The black line represents rates of NH_3 production by the kidney and gastrointestinal tract and utilization by muscle, unchanged from the normal values listed in Table 1. The red line represents muscle NH_3 utilization in proportion to arterial NH_3, increasing as arterial NH_3 increases. The green line represents hepatic artery blood increasing to compensate for decreased portal liver flow, increasing linearly by a factor of 1.5 as the shunt fraction goes from 0 to 1.

The fundamental assumption in the derivation of Equation 17 is that the normal NH_3-balance parameters listed in Table 1 are unchanged in CLD. Actually, there are at least two adjustments in CLD that would be expected to blunt the increase in C_{Art} resulting from portosystemic shunting. The first is an increased rate of muscle-NH_3 consumption (M_{Mus}) as C_{Art} increases. Bessman and Bradley[101] reported that in CLD patients, the increase in M_{Mus} was roughly proportional to C_{Art} (there was also a smaller and more variable increase in brain uptake[101] that we neglect). We can modify this derivation by assuming that M_{Mus} is directly proportional to C_{Art}:

$$M_{Mus} = M^0_{Mus} C_{Art} / C^0_{Art} \quad (18)$$

where M^0_{Mus} is the normal value of muscle-NH_3 uptake (6 μm/min/70 kg, Table 1) and C^0_{Art} is the normal NH_3 arterial concentration (30 μM). Substituting Equation 18 into Equation 15 and solving for C_{Art} as a function of f_{shunt}:

$$C_{Art} = \frac{C^0_{Art}(F_{Met} M_{GI} f_{shunt} - F_{Met} M_{GI} + M_{GI} + M_{Ren})}{C^0_{Art} F_{Met}(Q_{HA} + Q_{PV} - Q_{PV} f_{shunt}) - M^0_{Mus}} \quad (19)$$

The result of this calculation is plotted as the red line in Figure 5. It can be seen that this increase in muscle-NH_3 uptake reduces the increase in C_{Art}, which reaches a maximum of about 162 μM when $f_{shunt} = 1$.

As discussed in the "Muscle N balance" section, NH_3 taken up by muscle is converted to glutamine. To the extent that this new glutamine is actually metabolized, eg, in kidneys or intestines, to glutamate and NH_3, the assumption in Equation 19 that increased muscle-NH_3 uptake results in net NH_3 removal from the body is wrong. Holeček[30] suggested that this recycling of glutamine to NH_3 was a "vicious cycle", increasing the hyperammonemia in CLD. However, as discussed, the liver's conversion of glutamine to urea is the major pathway regulating plasma glutamine, suggesting that most of the additional glutamine production resulting from increased NH_3 uptake may be directly converted to urea by the liver.

The second factor that can blunt the increase in C_{Art} is a compensatory increase in Q_{HA} as Q_{PV} is reduced. There is a well-established "hepatic arterial buffer response" that tends to maintain total liver flow constant as Q_{PV} is decreased in the normal liver.[122,124] However, this response is less pronounced

in CLD,[125] and measurements in CLD are suggestive of only a small (if any) increase in Q_{HA}.[126,127] To illustrate the potential effect of a compensatory increase in Q_{HA}, it was assumed that Q_{HA} increases by 50% as f_{shunt} goes to 1:

$$Q_{HA} = Q^0_{HA}\,(1 + 0.5\,f_{shunt}) \tag{20}$$

where Q^0_{HA} is the normal hepatic artery flow (0.45 L/min, Table 1). Substituting Equation 20 into Equation 15 and solving for C_{Art} as a function of f_{shunt}:

$$C_{Art} = \frac{2(F_{Met}\,M_{GI}\,f_{shunt} - F_{Met}\,M_{GI} + M_{GI} + M_{Mus} + M_{Ren})}{F_{Met}\,(Q^0_{HA}\,f_{shunt} - 2Q_{PV}\,f_{shunt} + 2Q^0_{HA} + 2Q_{PV})} \tag{21}$$

This is plotted as the green line in Figure 6. It can be seen that this increase in Q_{HA} produces roughly the same blunting of the increase in C_{Art} as the increase in M_{Mus} (red line).

Creation of shunts between the portal system and the hepatic vein or vena cava has been commonly employed to reduce portal pressure in patients with intractable ascites or variceal bleeding. Walser et al[128] estimated that 3 months after establishing a transjugular intrahepatic portosystemic shunt (TIPSS), f_{shunt} ranged 0.84–1, with an average value of 0.93. Using Equation 17, one would predict that increasing f_{shunt} from the pre-TIPSS value of 0.65[120] to the post-TIPSS value of 0.93, with all other factors remaining unchanged, should increase C_{Art} from 107 to 204 μM. The most serious clinical complication of TIPSS is an increased HE,[4] presumably as a result of this increased NH_3. However, an increase in C_{Art} does not necessarily follow creation of the shunts. There is no significant increase in C_{Art} 2 weeks following end-to-side portacaval shunts[129] or 1 hour following a TIPSS,[102] suggesting that there must be compensating factors. An obvious candidate is the well-established increase in Q_{HA} (up to double) that results from decreased portal vein pressure following surgical creation of the shunt.[127,130,131] Recently, Damink et al[14,48,102,132] carried out the most detailed available measurements of the effect of TIPSS on N and NH_3 balance. They established that compensating changes in renal NH_3 release to the systemic circulation (M_{Ren}) can significantly alter C_{Art}. For example, they found that 1 hour following a TIPSS, M_{Ren} decreased by 22 μm/min/70 kg, presumably because of systemic hemodynamic changes produced by the stent.[102] Assuming that the overall effect of the stent is to increases f_{shunt} from 0.65 to 0.93, while Q_{HA} increases 50% and M_{Ren} decreases by 22 μm/min/70 kg, one finds using Equation 17 that pre- and poststent C_{Art} are 107 and 101 μM, respectively. Therefore, the calculated effect of these compensating factors can prevent a TIPSS-induced increase in C_{Art}, as is observed experimentally.

A basic assumption of this discussion is that the F_{Met} (Equation 14) of the unshunted liver blood flow is normal in CLD, so the primary cause of the increased C_{Art} is portosystemic shunting. An alternative possibility is that liver-NH_3 clearance processes and F_{Met} are impaired in CLD. The definition of F_{Met} is problematic, because a fraction of the shunt may be intrahepatic and thus C_{HV} (Equation 14) represents both blood that has passed through functioning liver and shunted blood. One can correct for this by using ^{99m}Tc-macroaggreated albumin to quantitate the intrahepatic shunting. Nomura et al[110] found that liver hepatic extraction (Equation 13) decreased as the severity of the CLD increased: from 77% in controls (patients with idiopathic portal hypertension) to 50% in Child–Pugh class A and 40% in Child–Pugh class B or C cirrhosis. However, if these measurements are corrected for intrahepatic shunting, the F_{Met} of the unshunted blood remains relatively normal in these CLD patients. When the severity of CLD approaches the point where liver cells become nonfunctional, obviously significant impairment in F_{Met} would be expected. The classic human model of a severe defect in F_{Met} is ALF.

ALF is the sudden loss of a large fraction of liver function in a subject with no preexisting disease and presumably no portosystemic shunts. Among the liver functions reduced are NH_3-uptake processes along with metabolism of AAs (including glutamine and alanine) to urea. In 22 ALF patients, Clemmesen et al[103] made detailed measurements of urea, AA, and NH_3 balance across the splanchnic bed, ie, the GI tract plus liver determined from arterial–hepatic vein NH_3-concentration difference and hepatic blood flow. In addition, they measured arterial-venous differences across a lower extremity. The patients were in an approximate steady state, with arterial NH_3 increased to 182 μM (four times their normal of 46 μM) and glutamine increased to 2,393 μM (four times their normal of 568 μM). Most of the other AA concentrations are also increased three- to fourfold, with the major exception of BCAAs, whose concentrations were decreased from their normal values (valine 198–159 μM, leucine 110–85 μM, and isoleucine 57–28 μM). This is direct confirmation of the unique BCAA metabolism: as the normal liver cannot metabolize BCAAs because of the lack of the BCAA transaminase, liver failure should have no effect on their systemic plasma concentration. The decrease in plasma-BCAA concentration may be the result of increased muscle-BCAA conversion to glutamine stimulated by hyperammonemia[30] or more simply associated with general malnourishment in liver failure.

As discussed, normally the main site of NH_3 production is the GI tract, with all this NH_3 removed by the liver, so that overall there is a small net splanchnic NH_3 extraction. In marked contrast, in ALF there is a large net splanchnic production of 109 μm/min/70 kg compared to a splanchnic extraction of 9 μm/min/70 kg in normal controls (Clemmesen et al[103] could not determine liver extraction [F_{Met}], because they did not sample the portal vein). This decreased liver-NH_3 metabolism is balanced by increased lower-extremity (ie, muscle)-NH_3 uptake, which as discussed is roughly proportional to arterial NH_3. Some liver function remains because the liver urea-production rate is about 42% of normal, with arterial urea concentration increased threefold. Using this mathematical model with no shunt and assuming that muscle-NH_3 uptake is proportional to arterial NH_3 (C_{Art}, Equation 19), F_{Met} would have to be reduced to about 20% of normal in order to increase C_{Art} to 182 μM found in ALF patients. The ALF rate of muscle-NH_3 removal is linearly related to the rate of muscle-glutamine production, with about two glutamine molecules produced for each NH_3 consumed. The ALF rates of splanchnic alanine and NH_3 production are nearly equal, suggesting that the increased splanchnic NH_3 production arises primarily from GI-tract metabolism of glutamine to NH_3 and alanine (Equation 7), rather than from bacterial urea metabolism.

Enzyme deficiencies

As summarized in Figures 1 and 2, AA turnover produces about 1 M/70 kg/day of NH_3, most of which is converted to urea by the urea cycle and does not normally contribute to blood NH_3. In patients with genetic defects in the urea cycle, this conversion is slowed, with potential for massive increase in blood NH_3. There are eight enzymes required for normal urea-cycle function, and deficiencies in any of these can produce increased blood NH_3.[133] The severity of the defect depends on the enzyme involved and the degree of its loss of function. Unlike CLD, where defects in hepatic detoxification can produce myriad compounds that can contribute to HE, urea-cycle defects represent pure hyperammonemia. Although there are a variety of associated changes in the plasma concentration of urea-cycle intermediates (eg, ornithine, citrulline, arginine) the clinical symptoms are the result of the hyperammonemia, which is the defining feature.[134]

In the most severe forms, such as carbamoyl phosphate synthetase 1 (CPS1) deficiency, which affects the first step in the urea cycle, infants develop severe hyperammonemia (1,000 μM or greater) within 3 days of birth, producing catastrophic HE (eg, respiratory distress, convulsions) that is fatal unless treated acutely with mechanical ventilator and/or dialysis and chronically with protein restriction, IV NH_3 scavengers (eg, sodium benzoate), and urea-cycle intermediates.[135] About half of affected neonates die from HE coma.[134] Most of the longer-surviving subjects have moderate-severe long-term neurocognitive disorders.[135]

When there is only a partial enzyme deficiency, the onset of overt symptoms may be delayed for months or years, with acute episodes of symptomatic hyperammonemia (>100 μM) produced by some triggering event, such as an infection. These episodes are marked by a wide range of symptoms, including loss of appetite, cyclical vomiting, lethargy, hallucinations, and psychosis. Although these children have longer-term survival, they have high morbidity (eg, developmental disabilities, cerebral palsy, seizure disorders).[134] On occasion, a seemingly healthy adult with a minor defect may present with hyperammonemia and HE when some triggering event increases NH_3 production and/or reduces the borderline urea-cycle function.

Gastrointestinal therapeutic approaches to lowering blood ammonia

The association of increased arterial NH_3 with HE has led to therapeutic strategies for lowering NH_3.[3] Because the portal shunting of GI-produced NH_3 is the primary source of NH_3, the therapeutic focus is directed at reducing NH_3 production in the gut. As discussed, the quantitative estimates of the magnitude of the three GI-tract NH_3-producing processes are: bacterial urea hydrolysis = 122 μm/min/70 kg, bacterial protein deamination = 29 μm/min/70 kg, and intestinal mucosal glutamine metabolism = ~20 μm/min/70 kg.

In the past, low-protein diets were widely recommended for hyperammonemia and HE. The N-balance diagram in Figure 1 shows that the relationship between protein intake and blood NH_3 is indirect and ambiguous. As has been emphasized, since metabolism of dietary AAs by the liver to urea by the urea cycle does not alter blood NH_3, no direct relationship between protein intake and blood NH_3 would be expected. However, there is an indirect relation, because as discussed in detail in the "GI-tract N balance" section, 20% of the urea production is metabolized to urea in the GI tract by bacterial urease and 50% or more of portal vein NH_3 could be derived from this urea metabolism. Protein restriction should reduce blood urea, which in turn would reduce blood NH_3 if it resulted in less GI-tract bacterial production of urea-based NH_3 production. As discussed in the "GI-tract N balance" section, because the intestinal urease is nearly

saturated at normal blood-urea concentration, the relationship between blood-urea concentration and bacterial urea production is nonlinear. Picou and Phillips[136] found that children on a low-protein diet (30% of normal), had a fivefold decrease in the rate of urea production (and presumably blood urea), and as predicted a proportionally smaller twofold decrease in the absolute rate of bacterial urea hydrolysis. Young et al[137] compared rates of urea production and hydrolysis in adults on a normal-protein diet (11.6 g N/day) vs a high-protein diet (2.4 times normal) and low-protein diet (quarter of normal). On the low-protein diet, urea production was 52% of normal and urea hydrolysis 59% of normal. The high-protein diet increased the rate of urea production by a factor of 2.45 (identical to the 2.4-fold increase in protein intake). However, as predicted, there was no significant increase in the absolute rate of urea hydrolysis (relative to the normal-protein diet), since presumably bacterial urease was saturated at the urea levels achieved with the normal-protein diet. These results suggest that since urea hydrolysis represents the largest component of GI-tract NH_3 production, if severe enough, protein restriction should significantly reduce serum NH_3 in CLD patients.

Surprisingly, there have been no well-controlled studies on the effect of a low-protein diet on blood NH_3 in CLD.[138,139] In 2004, Cordoba et al[138] described the first randomized study of the effect of a low-protein diet plus neomycin (delivered by nasogastric tube) in patients with an episodic HE. The protein restriction had no significant effect on either the outcome of the HE or blood-NH_3 levels. Because of this result, coupled with the observation that low-protein diets have a deleterious effect on nutritional status in CLD patients, protein restriction is no longer recommended.[3,140] The apparent explanation for the failure of Cordoba et al to observe a decrease in blood NH_3 in their CLD patients may be attributable to the fact that both the control and low-protein arms of the study were receiving neomycin therapy, which would be expected to decrease bacterial urea metabolism.

Historically, the association between ingested protein and HE was supported by the original observation of Eck in 1877[115] that dogs with a portacaval shunt developed HE when they were fed meat. Similarly, the administration of bovine erythrocytes has been used as an experimental model to induce HE in rats with portacaval anastomosis.[141] In humans, this association of HE with dietary protein is supported by the common observation that HE is precipitated by GI bleeding in patients with hepatic cirrhosis[132,142] (in nonanemic subjects, blood contains about 20 g/dL protein). This effect of gastric bleeding was confirmed experimentally by Bessman and Mirick[142] in 1958, who showed that blood NH_3 roughly doubled 4 hours after intragastric administration of blood to patients with CLD, but had no effect in normal controls. This interesting result was recently studied in much more detail by Damink et al.[132] They directly measured the NH_3 balance across the GI tract, liver, leg (ie, muscle), and kidney in patients with cirrhosis and a TIPSS when administered an intragastric AA solution that simulated blood. The simulated blood increased arterial NH_3 by a factor of 1.62 after 4 hours. Surprisingly, this increase seemingly resulted from an increase in renal NH_3 production, with no significant change in either GI-tract or liver-NH_3 balance. Although the mechanism of the increased renal NH_3 production is not clear, these results indicate that a single oral protein dose does not increase GI-tract NH_3 production.

As discussed in the "Muscle N balance" section, muscle-glutamine production and NH_3 consumption (Equation 8) result from the conversion of muscle BCAAs to glutamine. In addition, it has been suggested that increased blood NH_3 in CLD leads to increased BCAA breakdown, contributing to the cachexia associated with CLD.[28] This has led to the idea that increased dietary BCAAs may be an effective way to lower blood NH_3 and treat the cachexia of CLD.[28,31] Unfortunately, clinical trials of BCAA diet supplements have shown conflicting results, with some studies actually finding an increase in blood NH_3.[31]

With the recognition in the 1950s of the large contribution of GI-tract bacterial urea metabolism to NH_3 production, focus was directed at reducing or eliminating this bacterial population. Antibiotic therapy became a standard in the treatment of HE after the report of Fisher and Falcon[117] in 1957 that venous blood NH_3 in CLD patients fell by half or more after 3 days of neomycin therapy. Because such antibiotics as neomycin, metronidazole, and vancomycin have some associated side effects, poorly absorbed rifaximin has now become the standard antibiotic therapy for HE.[3] A number of controlled randomized trials have shown that a few days of rifaximin therapy results in statistically significant reductions in blood NH_3, with posttreatment NH_3 33%–67% of pretreatment NH_3.[143–146]

In 1966, Bircher et al[147] showed that reductions in blood NH_3 could be obtained by chronic administration of the unabsorbable disaccharide lactulose. In a meta-analysis of four randomized comparisons of unabsorbable lactulose versus rifaximin, Wu et al[148] concluded that although the disaccharide lowered blood NH_3, the reduction was significantly less than that observed with rifaximin in three of the four studies. The exception was the study of Paik et al,[145] which found

that lactulose and rifaximin had a nearly identical effect on blood NH_3, with NH_3 being lowered from 192 μM to 128 μM by lactulose and from 204 μM to 138 μM by rifaximin. Because of their relative safety, unabsorbable disaccharides are regarded as the standard, first-line therapy for HE.[3]

Despite the relatively enormous use of lactulose as therapy for HE, the mechanistic basis of its NH_3-lowering action is uncertain.[149] Virtually all ingested lactulose reaches the colon, where it is fermented by colonic flora to short-chain organic acids with the production of acidic colonic contents, as well as diarrhea. Lactulose is more effective at reducing serum NH_3 than other cathartics, ie, polyethylene glycol; therefore, it has been assumed that the ability of lactulose to acidify the colon is responsible for its beneficial effect. Initially, it was postulated that this acidification increased the NH_4^+:NH_3 ratio, with a resultant luminal "trapping" of NH_3 via diminished nonionic diffusion. This excess NH_3 would then be fecally excreted, possibly facilitated by the cathartic effect of lactulose. However, the quantity of NH_3 excreted in feces has been shown to be minimal in both the basal and postlactulose situation,[150,151] ruling out the possibility that trapping of NH_3 accounts for the decline in serum NH_3 observed with lactulose.

In vitro research has shown that colonic content acidity levels resulting from lactulose fermentation reduce the rate of fecal bacterial deamination of urea and protein.[152] However, if the quantitative estimates of NH_3 production discussed in the "GI-tract N balance" section are correct, protein deamination accounts for only about 16% of total GI-tract NH_3 production, and thus one would not expect this action of lactulose to have a large effect on plasma NH_3. Also as discussed in the "GI-tract N balance" section, the limitation in the rate that urea can diffuse to the large intestinal lumen suggests that any NH_3-lowering action of lactulose due to decreased bacterial urea breakdown may be an extracolonic action.

Lactulose has dramatic small-intestine effects. In patients with terminal ileostomies, adding 40 g lactulose to a meal reduces intestine-transit time by about 40% and increases total ileal effluent fivefold.[153,154] This produces a doubling in the amount of malabsorbed carbohydrate and a 55% increase in the malabsorbed protein. In normal subjects, high doses of lactulose (160 g/day) produce massive protein fecal losses of about 0.5 M/day, about 50% of the total protein intake,[155] presumably resulting from small-intestine protein malabsorption.

This lactulose-induced protein malabsorption is equivalent to a low-protein diet which as discussed earlier should decrease plasma urea and correspondingly the rate of NH_3 production from bacterial urea hydrolysis. In addition, the increased small- and large-intestine volume flows and decreased transit time should limit the time for bacterial urea hydrolysis and protein deamination. Both of these effects should decrease the rate of urea metabolism. This was directly confirmed by Weber, who looked at the effect of lactulose (40–80 g/day) on urea metabolism in six CLD subjects.[156] As predicted, if lactulose decreased protein absorption, lactulose increased stool-nitrogen output by a factor of 2.3 and decreased plasma urea by 17% and net urea production by 25%. Most importantly, it decreased bacterial urea metabolism by 38%, which could account for much of the decrease in plasma NH_3 associated with lactulose. Note that if colonic protein deamination was appreciable, the increased small-intestine protein malabsorption should have a deleterious effect, increasing colonic GI-NH_3 production. Since this is not observed, either protein deamination is not an important source of NH_3 (in the presence or absence of lactulose) or the increased colonic transit time produced by lactulose limits the time for deamination.

These studies show that reducing bacterial urea metabolism by antibiotics or unabsorbable disaccharides reduces blood NH_3 (C_{Art}) by about 50%. One can use this quantitative model to predict the magnitude of inhibition of GI-NH_3 production (M_{GI}) that would be required to produce this 50% fall in C_{Art}. The only change required is to make the substitution:

$$M_{GI} = (1 - f_{GI})M^0_{GI} \quad (22)$$

where f_{GI} is the fractional inhibition of GI-NH_3 production and M_{GI}^0 the uninhibited rate (66 μm/min/70 kg). Figure 7 shows a plot of C_{Art} as a function of f_{GI} for $f_{shunt} = 1$ (black line) and $f_{shunt} = 0.6$ (red line). It can be seen that in order to produce a 50% reduction in C_{Art}, it is necessary to reduce M_{GI} by about 60%. Antibiotics could account for such a reduction if these agents almost totally eliminated the bacterial metabolism of urea.

Clinical implications

Discussion of the vast array of clinical conditions causing hyperammonemia and the use of NH_3 measurements in the diagnosis and approach to therapy of these conditions is beyond the scope or goal of this paper. The following discussion provides a brief summary of selected aspects of the use of NH_3 determinations in clinical practice.

The laboratory assessment of blood-NH_3 concentration is complicated by the large number of blood proteins (plasma and red blood cell-derived) that may "spontaneously"

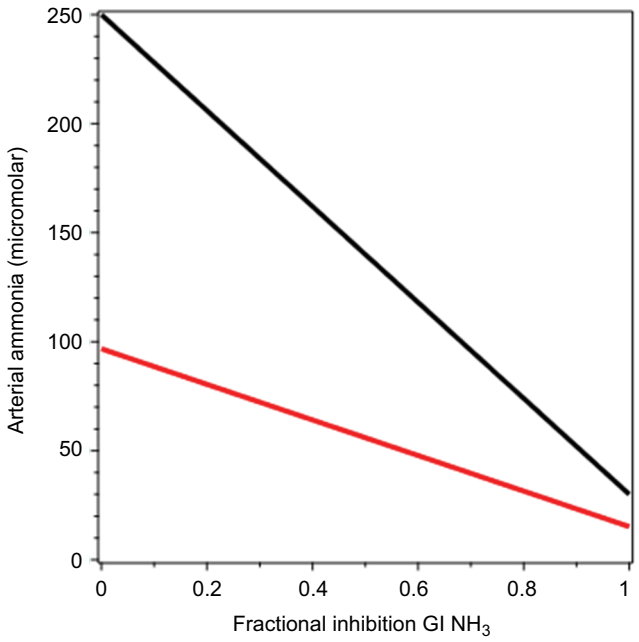

Figure 7 Arterial NH_3 concentration as a function of the fractional inhibition of GI-tract NH_3 production (eg, by oral antibiotics).
Notes: The black line represents 100% of portal blood shunted to the systemic veins. The red line represents 60% portosystemic shunt, which is the average value for chronic liver disease patients.
Abbreviation: GI, gastrointestinal.

deaminate, resulting in artifactual elevations of true blood-NH_3 concentration. For this reason, there was uncertainty that the blood of healthy subjects actually contained NH_3 until the 1960s, when analytical techniques requiring minimal blood manipulation became available. The most important factor in minimizing spurious elevations of blood NH_3 is maintenance of samples at ice-water temperature from collection to analysis, which should take place within 1 hour of sample collection. Care should also be taken when obtaining the venous blood sample, with minimal exercise of the arm and a short period of venous stasis. In addition, it has recently been shown that in patients with severe liver injury, deproteinization is required for accurate measurements using the glutamate dehydrogenase assay.[157] Despite apparent attention to these details, one laboratory found that of 86 pediatric patients with initially high blood NH_3 levels, subsequent values in about 50% normalized without treatment, suggesting that 50% of the initially elevated values were false positives.[158] It follows that the finding of elevated blood NH_3 requires confirmation with a repeat assay.

The clinical importance of hyperammonemia is its deleterious effect on the central nervous system with the induction of what is called HE. The most common manifestations of this problem are behavioral changes, confusion, somnolence to coma, and motor disturbances.[159] Since such symptoms are commonly observed in the absence of hyperammonemia, the obvious purpose of measuring blood NH_3 is to determine if NH_3 is playing a pathogenic role in the encephalopathic patient. Subjects with hyperammonemia tend to segregate into two groups: those with known liver dysfunction, either cirrhosis with portal collaterals, or acute hepatic necrosis, and patients with unexplained central nervous system dysfunction but normal liver function. Given the high prevalence of cirrhosis, the vast majority of NH_3 measurements at our hospital are obtained from patients with known CLD, usually with extensive collateral circulation. It is commonly accepted that the encephalopathy manifested by these patients is a response to a wide variety of factors in addition to blood NH_3, including hypokalemia, hyponatremia, infection, GI hemorrhage, altered neurotransmitter function, and drugs (that do not alter NH_3 homeostasis). While the degree of encephalopathy based on clinical assessment roughly correlates with the degree of hyperammonemia, the correlation coefficient (about 0.6) is poor.[113] Particularly problematic are patients who apparently demonstrate encephalopathy (usually mild) with a normal NH_3 level. While it is not clear to what extent the discrepancy between blood-NH_3 level and encephalopathy is a function of inaccurate clinical assessment of mental status, the present mantra is that the serum-NH_3 measurement adds little of value to simple bedside scoring systems for the severity of encephalopathy.

In contrast to the limited value of blood-NH_3 determinations in patients with CLD, this measurement is crucial to establishing hyperammonemia as the cause of encephalopathy in patients with normal liver-function tests. The vast majority of such patients have disorders of the urea cycle with severe defects manifesting in infancy, whereas minor defects may not become clinically apparent until some aggravating event occurs in adulthood, such as infection or a surgical procedure. A variety of drugs, including chemotherapeutic agents[160] and valproic acid,[161] have been observed to cause symptomatic elevations of blood NH_3, apparently via interference with the urea cycle. Other conditions associated with hyperammonemia include urinary-tract infections with NH_3-producing organisms[162] and bacterial overgrowth,[163] both presumably related to excessive production of NH_3. Since hyperammonemia can result in irreversible brain injury or death if not treated, it is important to identify this problem as soon as possible in the course of the disease. It follows that serum-NH_3 determination should be obtained early in the workup of any patient with unexplained encephalopathy.

Author contributions

Both authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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