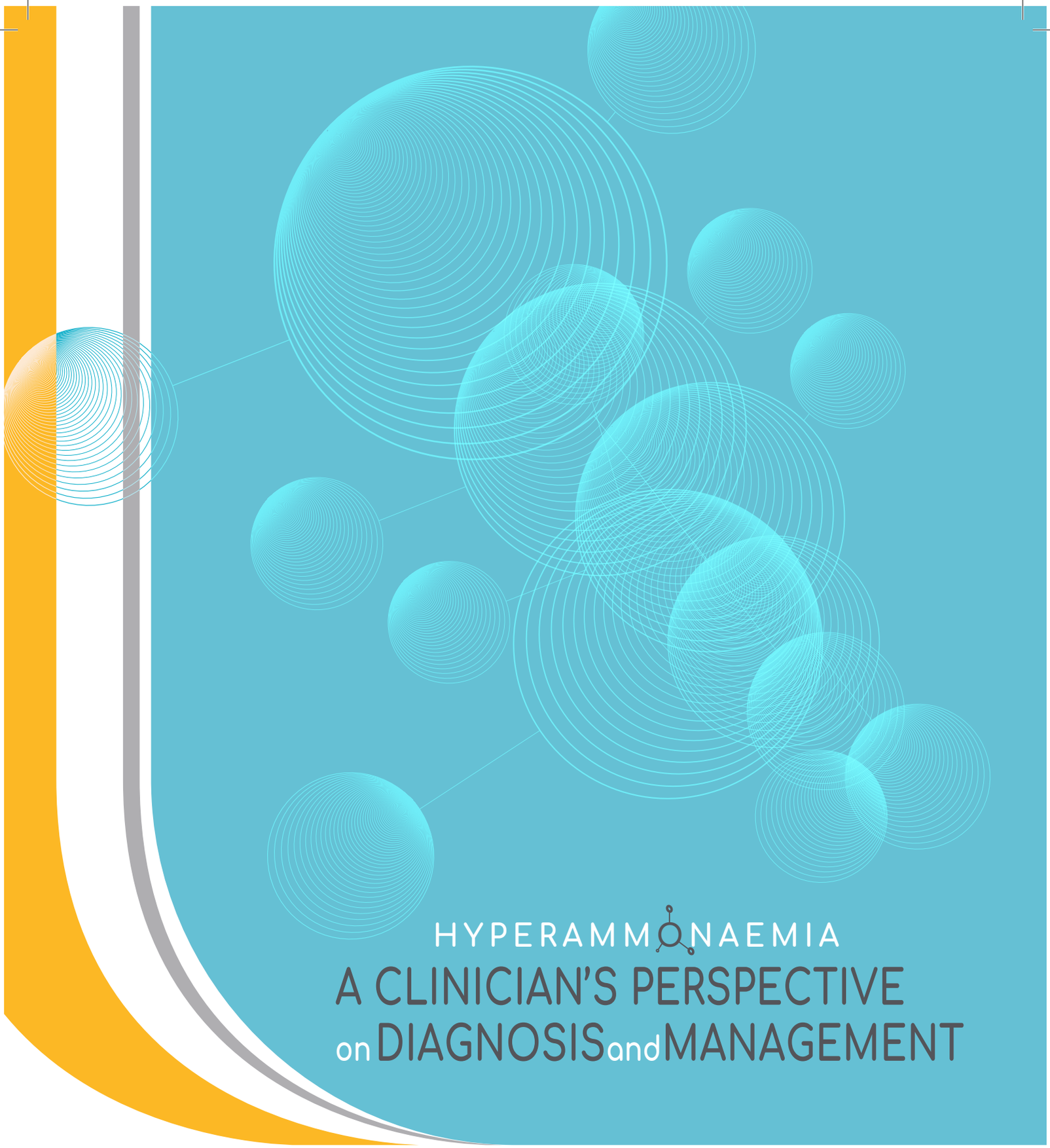

HYPERAMMONAEMIA
A CLINICIAN'S PERSPECTIVE on DIAGNOSIS and MANAGEMENT

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Springer Healthcare Education

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This booklet is provided by Springer Healthcare, supported by an educational grant from Recordati Rare Diseases – Recordati Group.

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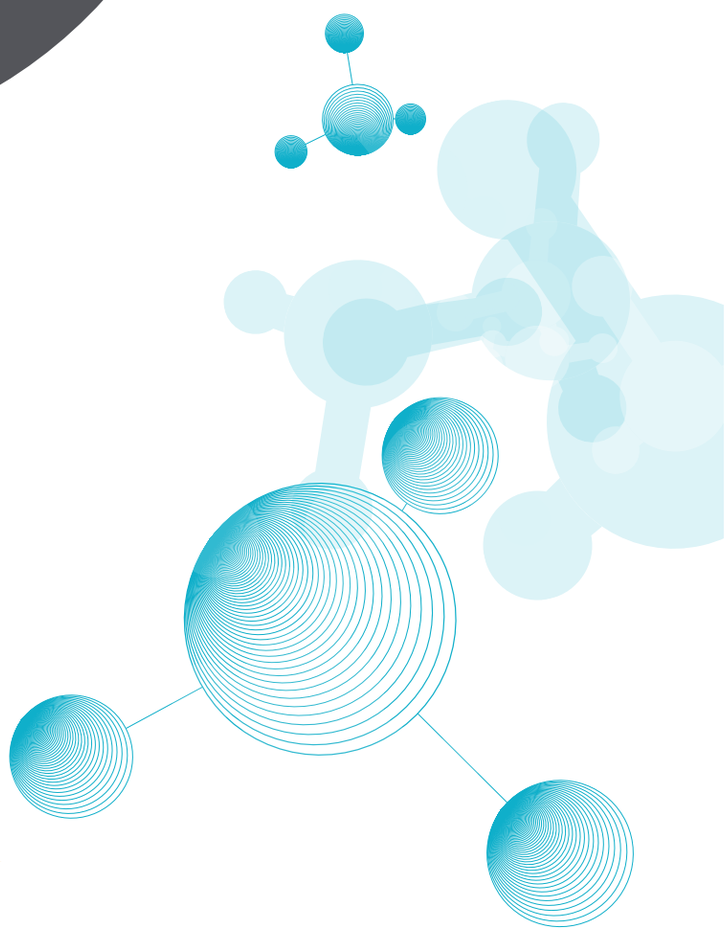


2-MCA	2-methylcitrate
ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
ARG(1)	Arginase (1)
ASL	Argininosuccinate lyase
ASA	Arginino Succinate
ASS	Argininosuccinate synthetase
ATP	Adenosine triphosphate
BMI	Body mass index
BW	Bodyweight
CAMP	Carglumic Acid in Methylmalonic Acidemia and Propionic Acidemia
CNS	Central nervous system
CoA	coenzyme A
CPS(1)	Carbamoyl phosphate synthetase [1]
DD	Differential diagnosis
DRI	Dietary reference intakes
EAA	Essential amino acids
ECG	Electrocardiogram
EEG	Electroencephalogram
FAO	Food and Agriculture Organisation
GLN	Glutamine
HCO3–	Bicarbonate
IMD(s)	Inherited metabolic disorder(s)
IQ	Intelligence quotient
IV	Intravenous
IVA	Isovaleric aciduria
IV-CoA	Isovaleryl-CoA
IVD	Isovaleryl-CoA dehydrogenase
LEU	Leucine
MMA	Methylmalonic aciduria
MM-CoA	Methylmalonyyl-CoA
MRI	Magnetic resonance imaging
MSUD	Maple syrup urine disease
MUT	Methylmalonyl-CoA mutase
NaB	Sodium benzoate
NAG	N-acetylglutamate
NAGS	N-acetylglutamate synthetase
NaP	Sodium phenylbutyrate
NCGA	N-carbamoyl-L-glutamic acid
NH3/NH4+	Ammonia/ammonium
OA(s)	Organic aciduria(s)
ORNT1	Ornithine translocase
OTC	Ornithine transcarbamylase
P5CS	Pyrroline-5-carboxylate synthase
PA	Propionic aciduria
PBA	Phenylbutyrate
PC	Pyruvate carboxylase
PCC	Propionyl-CoA carboxylase
P-CoA	Propionyl-CoA
PDH	Pyruvate dehydrogenase complex
P:E ratio	Protein: Energy ratio
PTH	Parathyroid hormone
RDA	Recommended daily allowances
TCA	Tricarboxylic acid
THAN	Transient hyperammonaemia of the newborn
UCD(s)	Urea cycle defect(s)
UNU	United Nations University
VAL	Valine
WHO	World Health Organization

HYPERAMMONAEMIA

A CLINICIAN'S PERSPECTIVE on DIAGNOSIS and MANAGEMENT

What is Hyperammonaemia?

Hyperammonaemia represents a medical emergency,[1] because high plasma levels of ammonia are neurotoxic, and the extent of intellectual disability is directly correlated with the duration of coma and severity of hyperammonaemia.[2,3]

Until recently, the only treatment for acute hyperammonaemia was ammonia-scavenging therapy or haemodialysis.[4] However, the recent development of new therapies such as carglumic acid offers an additional treatment modality for hyperammonaemia caused by a range of conditions[4]

The information provided here aims to equip physicians with the knowledge they need to quickly recognise the potential development of hyperammonaemia in their patients, and to accurately diagnose and treat it effectively.

Hyperammonaemia is defined as elevated plasma levels of ammonia. It commonly occurs in adults with advanced liver disease,[5] and hence the development of high levels of plasma ammonia in individuals without liver disease should raise suspicions for an inherited metabolic disorder (IMD) as a potential cause.[6] Age-appropriate reference levels of ammonia are shown in **Table 1**.

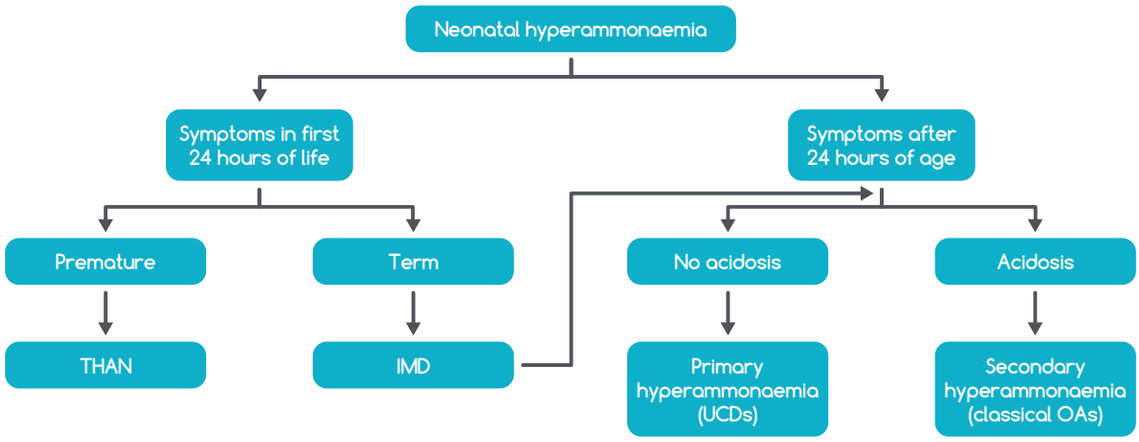
Table 1. Age appropriate plasma levels of ammonia.[7]

Age group	Plasma ammonia concentration, µmol/L
Neonates	
Healthy	<110
Sick	<180
Suspected IEM	>200
Post neonatal period	
Healthy	50–80
Suspected IEM	>100

IMD, inherited metabolic disorder.

Premature infants may (rarely) have elevated levels of ammonia because of immature hepatic function. This condition is called 'transient hyperammonaemia of the newborn' (THAN), and generally resolves without any symptoms or clinical consequences.[6] In neonates born at term or in older infants, hyperammonaemia is likely to be caused by an inherited metabolic disorder (IMD) **(Figure 1)**,[6] particularly if the ammonia level is >200 µmol/L in a neonate or >100 µmol/L in an older infant. Two major groups of IMDs which cause hyperammonaemia include urea cycle defects (UCDs) and organic acidurias (OAs). These are discussed in more detail in the section 'What causes hyperammonaemia?'.

Figure 1.
Differentiating conditions associated with hyperammonaemia in neonates (adapted with permission from Burton, 1998).[6]

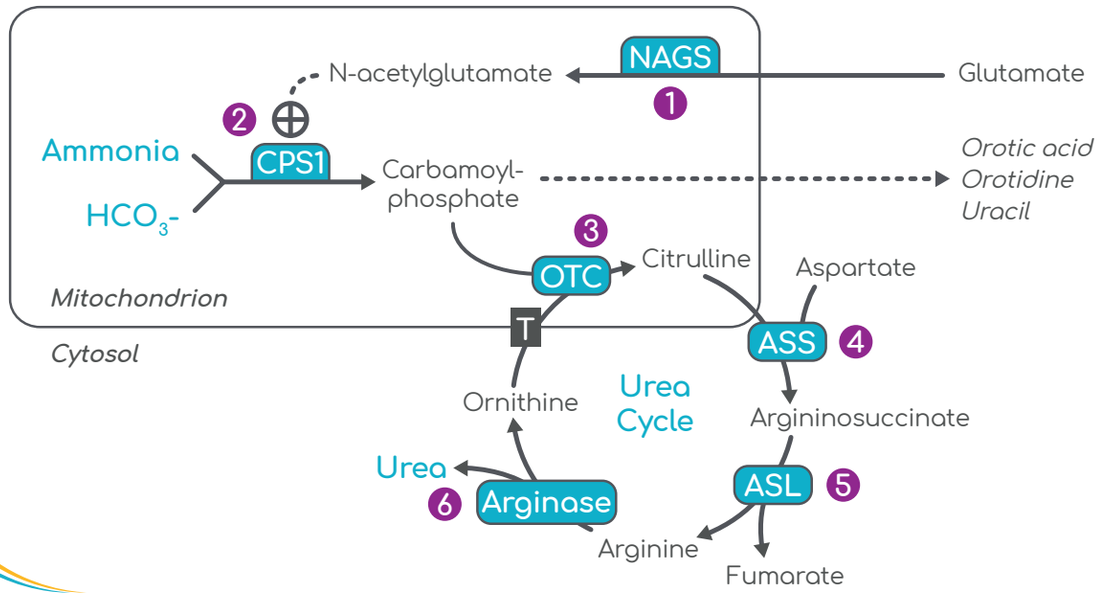


IMD, inherited metabolic disorder; OAs, organic acidurias; THAN, transient hyperammonaemia of the newborn; UCDs, urea cycle defects.

What causes hyperammonaemia?

The presence of hyperammonaemia is a hallmark sign of UCDs, which develop when there is an enzyme defect in the functioning of the urea cycle.[3,8] The urea cycle **(Figure 2)** is the main biochemical pathway by which nitrogen waste products are detoxified, and is the only means of producing endogenous arginine, ornithine and citrulline.[2] Some of the enzymes in the urea cycle overlap with enzymes in the nitric oxide production pathway, including argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL).[2]

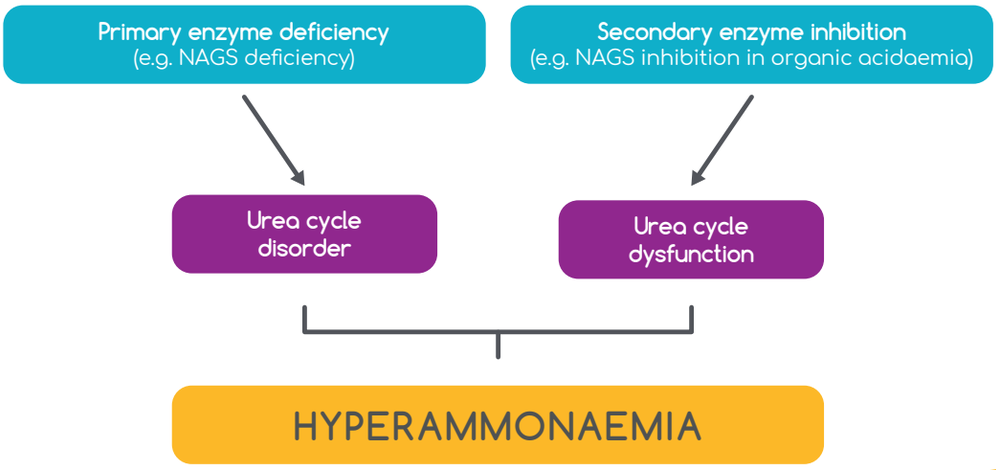
Figure 2. The urea cycle.[7]



1. N-acetylglutamate synthetase (NAGS); 2. Carbamoyl phosphate synthetase 1 (CPS1); 3. ornithine transcarbamylase (OTC); 4. Argininosuccinate synthetase (ASS); 5. Argininosuccinate lyase (ASL); 6. Arginase. T: Ornithine Transporter.

Hyperammonaemia can also develop in patients with a normally functioning urea cycle if there is another defect that affects the delivery of substrates to this cycle or that has an inhibitory effect on enzymes that feed into the urea cycle, such as occurs in some OAs **(Figure 3)**.[6]

Figure 3. Mechanisms involved in hyperammonaemia.[6]



Hyperammonaemia is the biochemical hallmark of urea cycle disorders or dysfunction

Therefore, hyperammonaemia can be divided into:

- Primary hyperammonaemia
  – resulting from an inherited enzyme deficiency in the urea cycle.

- Secondary hyperammonaemia
  – resulting from a defect in another pathway that affects the functioning of the urea cycle by inhibiting key enzymes or reducing the availability of certain substrates.

The age at onset and severity of hyperammonaemia depends upon the severity of the enzyme deficiency in the urea cycle.[2] It should be noted that hyperammonaemia may also be caused by other defects in the urea cycle (e.g. inherited disorders of nitrogen detoxification, transporter defects within the urea cycle, etc.), as well as defects in other biochemical pathways or secondary to valproic acid administration. Here, we exclusively focus on primary hyperammonaemia owing to urea cycle enzyme deficiency and secondary hyperammonaemia due to classical OAs.

What is primary hyperammonaemia?

Primary hyperammonaemia is caused by a deficiency in one of the six main enzymes involved in the urea cycle (**Table 2**, and see **Figure 2**).[2, 9-12] All such defects show autosomal recessive inheritance, with the exception of ornithine transcarbamylase (OTC) deficiency, which is X-linked.[9]

Table 2. Urea cycle disorders causing hyperammonaemia.[2, 9-12]

Condition and/or deficient enzyme	Gene	Inheritance (chromosome)	Incidence	Prevalence
Defect in the synthesis of the activator of the urea cycle				
1. NAGS deficiency	*NAGS*	Autosomal recessive (17)	<1:2,000,000	<1:1,000,000
Defects in the urea cycle enzymes				
2. CPS1 deficiency	*CPS1*	Autosomal recessive (2)	1:1,300,000	1:200,000 to 1:800,000
3. OTC deficiency	*OTC*	X-linked (X)	1:56,500	1:40,000 to 1:80,000
4. Citrullinaemia type I/ ASS deficiency	*ASS1*	Autosomal recessive (9)	1:250,000	1:100,000
5. Arginino Succinate or ASL deficiency	*ASL*	Autosomal recessive (7)	1:218,750	1:150,000
6. Argininaemia or arginase deficiency	*ARG1*	Autosomal recessive (6)	1:950,000	1:1,100,000

ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS1, carbamoyl phosphate synthetase 1; NAGS, N-acetylglutamate synthetase; OTC, ornithine transcarbamylase.

The specific enzyme deficiency in the urea cycle can be identified by plasma amino acids and urine for organic acid and orotic acid, as described in the section 'How is hyperammonaemia diagnosed?'

What is secondary hyperammonaemia?

There are many causes of secondary hyperammonaemia due to either urea cycle inhibition or substrate deficiency (Table 3).

Table 3. Secondary causes of hyperammonaemia.[1]

Urea cycle inhibition	Substrate deficiency
• PA: poor acetylglutamate production • MMA: propionyl Co A or methylcitrate accumulation • 3-Hydroxy-3-methylglutaryl-CoA-lyase deficiency • Secondary CPS1 deficiency stemming from low ATP levels • Hyperammonaemia due to valproic acid	• CoA deficiency due to: - Fatty acid oxidation defects - Carnitine cycle disorders - Pyruvate dehydrogenase defects • Cytosolic aspartate deficiency: pyruvate carboxylase deficiency • Fasting ornithine deficiency: Δ1 pyrroline-5-carboxylase deficiency • 3-hydroxyl-3-methlglutaryl-CoA lyase deficiency • Secondary CPS1 deficiency from low ATP levels • Hyperammonaemia due to valproic acid • Lysinuric protein intolerance: decreased ornithine/ citrulline/ arginine • Hyperinsulinism-hyperammonaemia syndromes: decreased glutamate and increased glutamate deamination

ATP, adenosine triphosphate; CoA, coenzyme A; CPS1, carbamoyl phosphate synthetase 1; MMA, methylmalonic aciduria; PA, propionic aciduria.

OAs are autosomal recessively inherited enzyme deficiencies affecting branched-chain amino-acid metabolism (isoleucine, valine, methionine and threonine in PA and MMA, and leucine in IVA) and additional substrates (odd chain fatty acids, cholesterol, nucleotides in PA and MMA).[8] These enzyme deficiencies lead to an accumulation of their respective precursors: propionyl-CoA (P-CoA) in PA, P-CoA and methylmalonyl-CoA (MM-CoA) in MMA, isovaleryl- CoA in IVA and their corresponding organic acids once released from CoA.[8] The 'classical OAs' can have an early onset in the neonatal period or infancy, and include the following:[13-16]

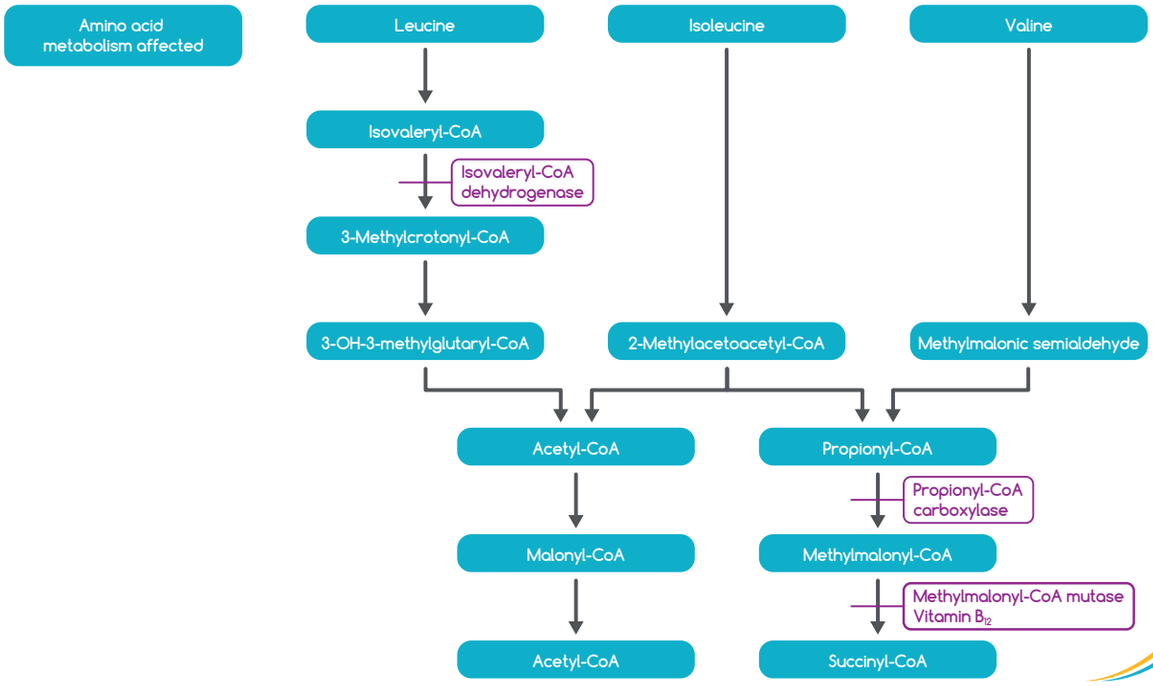
- Propionic aciduria (PA)
- Methylmalonic aciduria (MMA)
- Isovaleric aciduria (IVA)
- Maple Syrup Urine Disease (MSUD)

Only the first three of these classical OAs will be discussed here (MSUD will not be covered).

The characteristics of these conditions are summarised in **Figure 4**.[9] All three of these conditions affect the functioning of the urea cycle, as the accumulating intermediary metabolites cause inhibition of the N-acetylglutamate synthase (NAGS) enzyme and substrate deficiencies in the TCA cycle, ultimately leading to hyperammonaemia (see **Figure 3**).[14,16-18] **Figure 5** shows how these pathways are interrelated and the effects that the enzyme deficiencies have on metabolites.[19,20]

Figure 4. Characteristics of the OAs (adapted with permission from Testai et al. 2010).[9]

Characteristic	Isovaleric Aciduria	Propionic Aciduria	Methylmalonic Aciduria
Enzyme deficiency	Isovaleryl-CoA dehydrogenase	Propionyl-CoA carboxylase	Methylmalonyl-CoA mutase
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive
Chromosome	15	13	6
Metabolites in blood	Isovaleric acid, isovaleryl glycine, isovaleryl carnitine	Propionic acid, propionyl glycine, propionyl carnitine, methylcitrate, hydroxyvalerate, acetoacetate, β-hydroxypropionate, β-hydroxybutyrate	Methylmalonic acid, propionic acid, propionyl glycine, propionyl carnitine, methylcitrate, acetoacetate, β-hydroxybutyrate
Incidence	1:250,000	1:3000 to 1:35,000	1:50,000



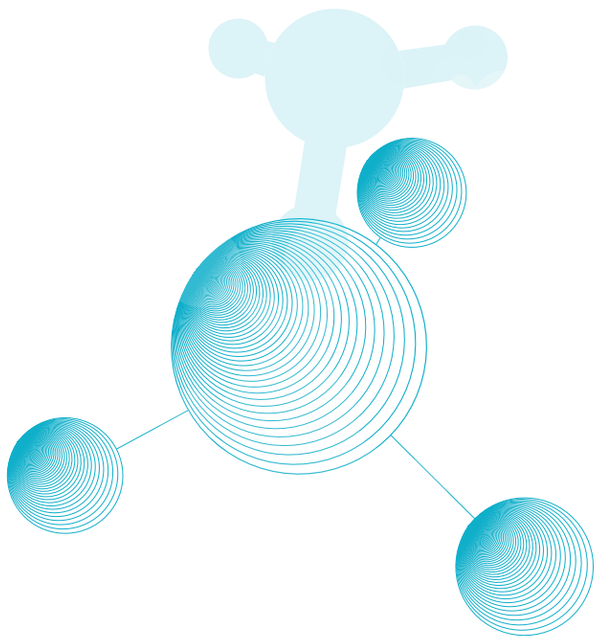
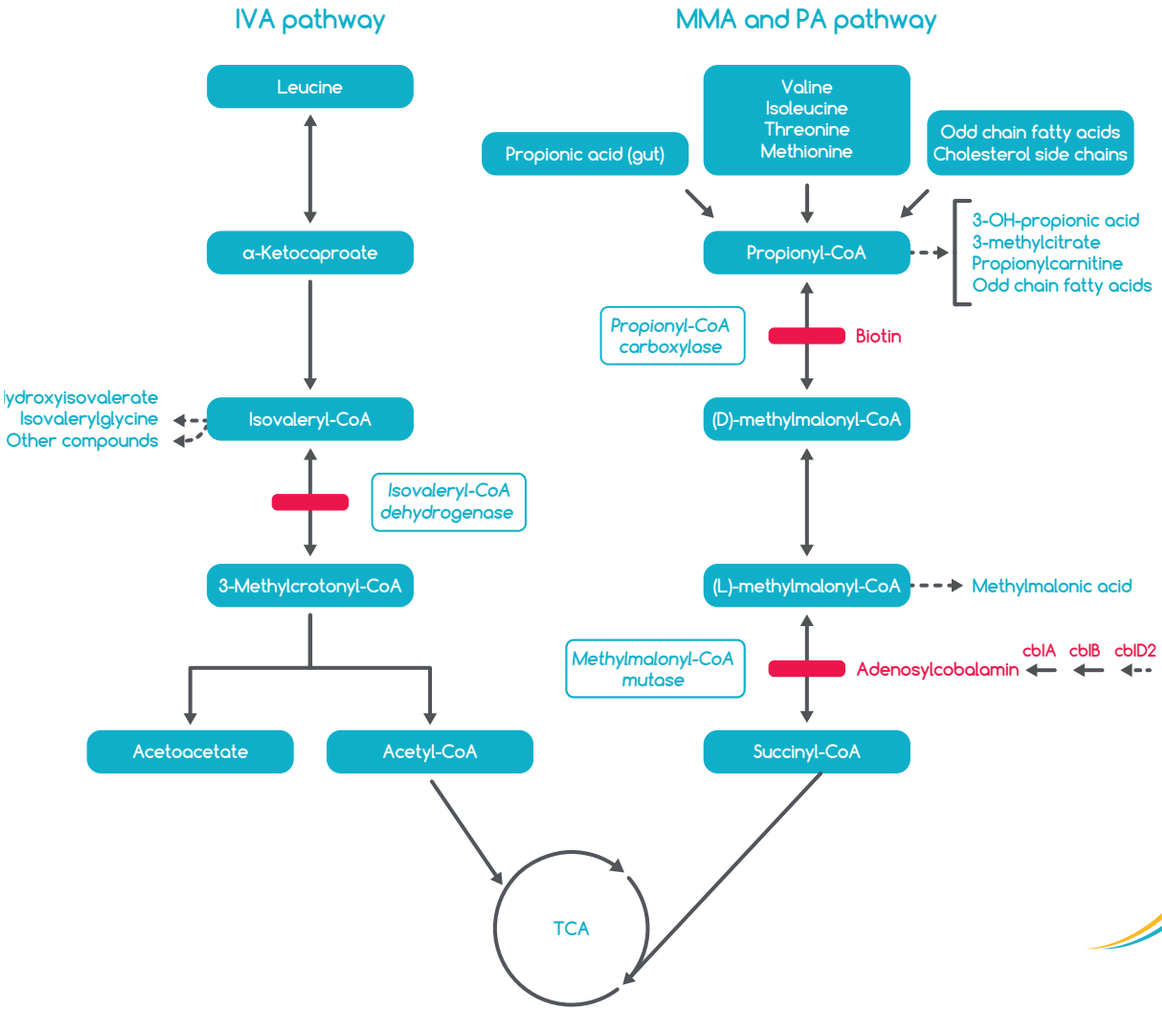


Figure 5. Metabolic pathway of IVA and the interrelationship between MMA and PA pathways, with the resultant metabolites from the respective enzyme deficiencies.[19,20]



Key points

- Hyperammonaemia is a medical emergency, which requires immediate treatment.
- Hyperammonaemia is the biochemical hallmark of a urea cycle defect or dysfunction caused by IMDs.
- UCDs cause primary hyperammonaemia, while classical OAs cause secondary hyperammonaemia.

HYPERAMMONAEMIA

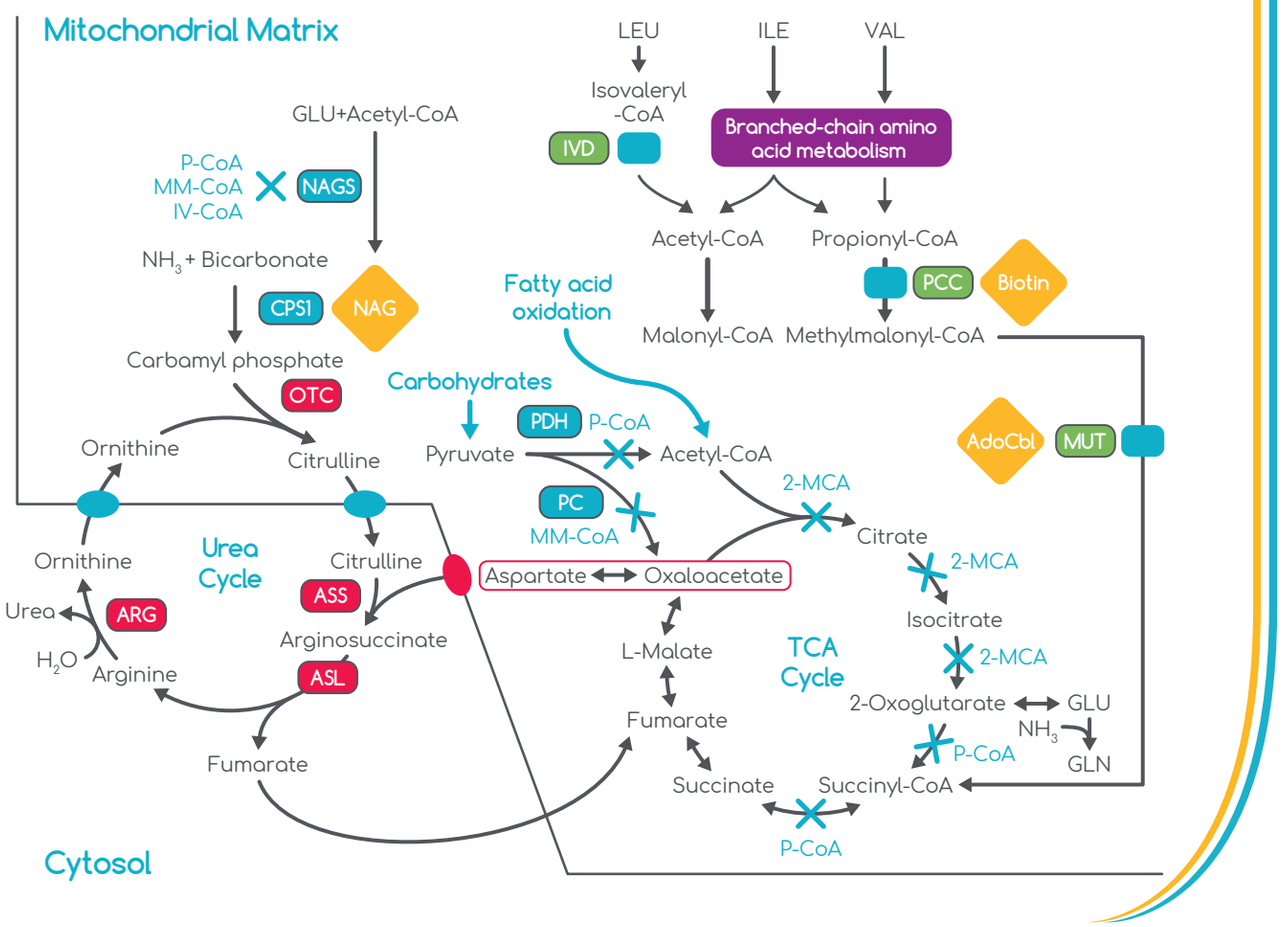
A CLINICIAN'S PERSPECTIVE on DIAGNOSIS and MANAGEMENT

What are the Biochemical Differences Between Primary and Secondary Hyperammonaemia?

What are the metabolic interactions between the urea and TCA cycles in the biochemical pathogenesis of OA?

Secondary hyperammonaemia in OA is due to the interaction of the resultant metabolites from the respective defective enzymes in the urea and TCA cycles **(Figure 6)**.

Figure 6. The biochemical pathways involved in pathogenesis of UCDs and OAs. Adapted with permission from Haberle, et al; 2008.[8]



Rectangles indicate key affected enzymes: red rectangles indicate the primary enzymes affected in UCDs (ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinate lyase, and arginase); green rectangles indicate the primary affected enzymes in OAs (propionyl-CoA carboxylase, methylmalonyl-CoA mutase, isovaleryl-CoA dehydrogenase); blue solid rectangles are positions of primary enzyme blocks. Blue crosses indicate secondary enzyme inhibition; blue texts are enzyme precursors; orange diamonds are key enzyme co-factors. Abbreviations: 2-MCA, 2-methylcitrate; CoA, coenzyme A; ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS1, Carbamoyl phosphate synthetase-1; GLN, glutamine; GLU, glutamate; H_2O, water; IV-CoA, isovaleryl-CoA; IVD, isovaleryl-CoA dehydrogenase; LEU, leucine; MM-CoA, methylmalonyl-CoA; MUT, methylmalonyl-CoA mutase; NAG, N-acetylglutamate; NAGS, N-acetylglutamate synthase; NH_3, ammonia; OTC, ornithine transcarbamylase; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; P-CoA, propionyl-CoA; PDH, pyruvate dehydrogenase complex; TCA, tricarboxylic acid; VAL valine.

The urea cycle and its role[8]

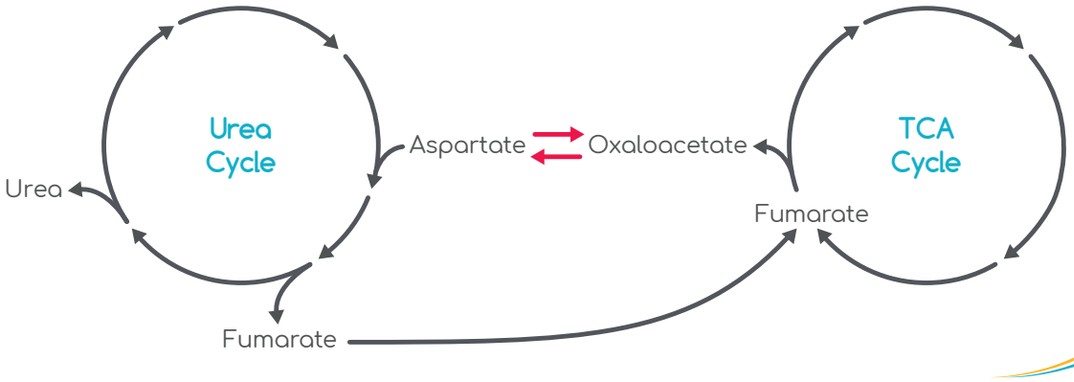
- A series of enzymes and transporters allows for the incorporation of ammonia into urea to be excreted in the urine.
- NAGS catalyses the formation of NAG from glutamate and acetyl-CoA.
- NAG is an essential activator of the rate-limiting enzyme of the urea cycle, CPS1.
- Ammonia that escapes the urea cycle in periportal hepatocytes conjugates with glutamate to form glutamine in pericentral hepatocytes, resulting in the increase in glutamine seen in all UCDs.

The urea cycle interacts with other mitochondrial pathways, including the TCA cycle, fatty acid oxidation pathway, and amino acid catabolism.[8,14,17,18]

The tricarboxylic acid (TCA) cycle and its role[8]

- The TCA cycle enables the extraction of reducing equivalents from acetyl-CoA that fuels the oxidative phosphorylation of the mitochondrial electron transport chain to generate adenosine triphosphate (ATP).
- The TCA and urea cycles are linked at oxaloacetate, which is the start and end point of the TCA cycle **(Figure 7)**. Oxaloacetate can be converted to aspartate via transamination in a reversible reaction.

Figure 7. Oxaloacetate–aspartate reaction is a reversible reaction between the TCA and the urea cycles. Fumarate links both the urea and TCA cycles (also known as the Kreb's cycle).



- 2-oxoglutarate is at the intersection between carbon and nitrogen metabolism, connecting the catabolic function of the TCA cycle with the anabolic function of the nitrogen assimilation reactions.[21]
- Glutamate can be also be generated from 2-oxoglutarate and ammonia during physiological cataplerosis.[22]
- Finally, the oxidation of fatty acids, carbohydrates and amino acids generates acetyl-CoA, which is one of the substrates necessary for the synthesis of NAG, in the urea cycle.

Proposed biochemical pathogenesis of OA

The mechanisms that contribute to hyperammonaemia in OAs remain complex, involving several interlinking metabolic cycles.

Ammonia levels increase in patients with PA when they become metabolically unstable. The severe hyperammonaemia may or may not be associated with metabolic acidosis.[23] Metabolic decompensations in PA are triggered by fever, fasting and infections, all of which are catabolic and prevent anabolism. Such conditions lead to a release of propionyl Co-A precursors, methionine and isoleucine from the muscle, which directly contribute to the production of propionic acid. As the propionyl Co-A increases, propionlycarnitine is formed and excreted in the urine. This forms the rationale for giving carnitine as part of the treatment.

How do OAs affect the urea cycle?

In OAs, the accumulated metabolites (propionyl Co-A, methylmalonyl Co-A and isovaleryl Co-A) compete with acetyl Co-A to inhibit the activity of NAGS, thereby diminishing the synthesis of Carbamoyl phosphate.[8] Excessive propionyl Co-A also favours the synthesis of N-propionylglutamate that cannot activate CPS1 in the urea cycle.[23] This secondary impairment of the urea cycle is only one of the mechanisms involved in the development of hyperammonaemia in OAs.

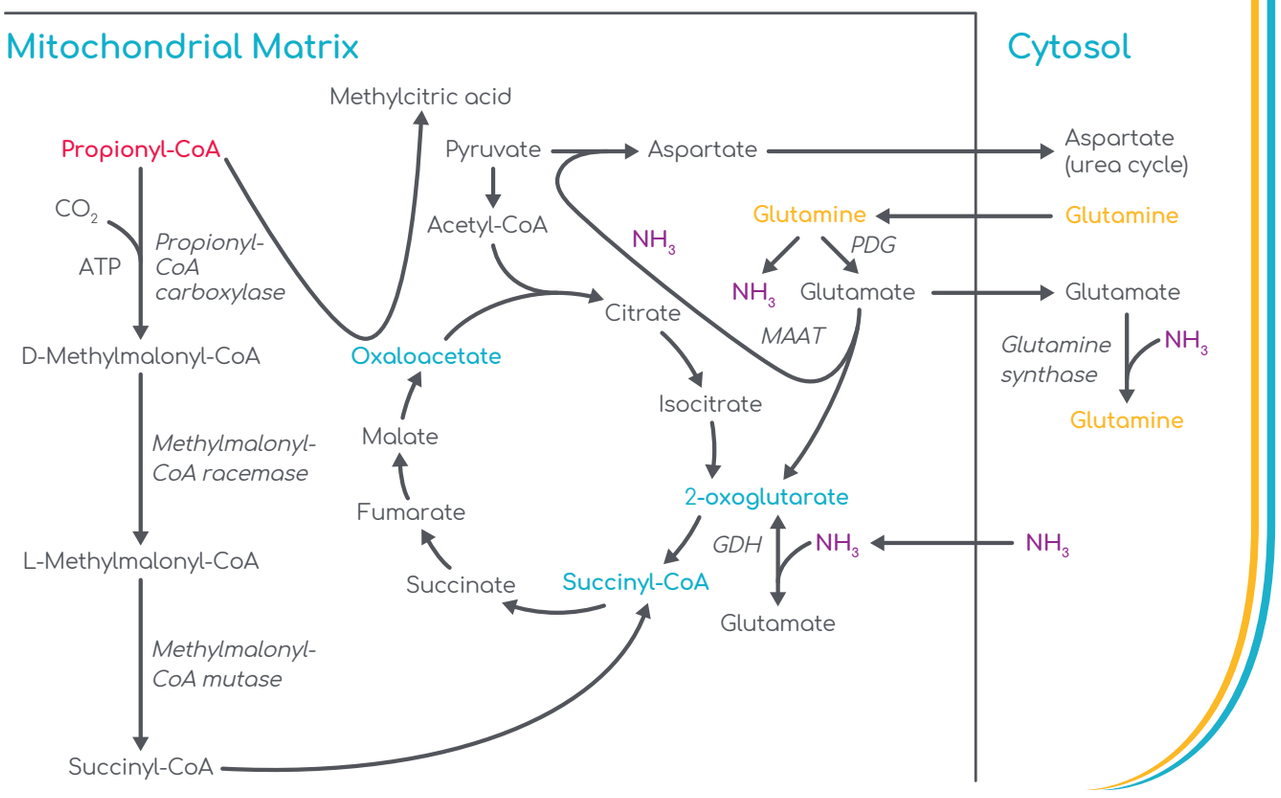
Glutamine levels in OA patients are often below normal ('glutamine paradox'), even when the patient is metabolically stable.[24]

There is also a negative correlation between ammonia levels and plasma glutamine, in contrast to the positive correlation seen in UCD patients.[23] The functional impairment of the urea cycle, such as that caused by a deficiency of NAG, with secondary deficit of CPS1 alone cannot account for such a discrepancy in elevated ammonia levels with lowered glutamine levels.

How do OAs affect the TCA cycle?

The lack of propionyl Co-A carboxylase in PA leads to an accumulation of propionic acid, which has a number of effects **(Figure 8)**.

Figure 8. Proposed pathway for propionyl CoA and glutamine metabolism in PA.[23]



Propionyl CoA is normally converted via several enzymatic reactions to succinyl CoA, which then enters the TCA cycle. In patients with PA, the accumulating propionyl CoA forms a complex with oxaloacetate to produce methylcitric acid. Because both oxaloacetate and succinyl CoA levels decrease, the TCA cycle is impaired, leading to reduced levels of 2-oxoglutarate. The deficit of 2-oxoglutarate can only be restored by using mitochondrial glutamine and glutamate, so levels of these compounds decrease. ATP, adenosine triphosphate; CO_2, carbon dioxide; CoA, coenzyme A; GDH glutamate dehydrogenase; MAAT, mitochondrial aspartate aminotransferase; NH_3, ammonia; PDG, phosphate-dependent glutaminase.

1. Propionic acid combines with oxaloacetate (from the TCA cycle) to form methylcitrate, causing a depletion of oxaloacetate.[23]
2. The lack of conversion of propionyl Co-A to methylmalonyl Co-A reduces the supply of succinyl CoA in the TCA cycle.[25-27]
3. Odd chain fatty acids eventually provide propionic acid that refills the TCA cycle with succinyl CoA.

The inability to utilise propionic acid in PA results in decreased availability of substrates in the TCA cycle and its secondary impairment with a functional lack of 2-oxoglutarate.[23]

Why are glutamine/glutamate levels reduced in PA?

The direct endpoint of PA and MMA metabolism is the production of succinyl Co-A, an important anaplerotic intermediate of the TCA cycle (see **Figure 8**). This degeneration (anaplerosis) pathway represents an important mechanism for replenishing the TCA cycle. Since anaplerosis is disrupted in PA and MMA, TCA intermediates must be derived from other pathways.[8] For example, the resultant lack of 2-oxoglutarate (TCA substrate) in PA shifts the equilibrium of the reactions catalysed by mitochondrial glutamate dehydrogenase and phosphate-dependent glutaminase, with a net production of ammonia and decreased levels of glutamine and glutamate in the muscle and liver (see **Figure 8**). In one reaction, glutamine is cleaved to form ammonia and glutamate:

$$\text{Glutamine} + \text{ADP} + P_i \leftrightarrow \text{Glutamate} + NH_4^+$$

In another, glutamate is split by glutamate dehydrogenase into ammonia and 2-oxoglurate to replenish the TCA cycle:

$$\text{Glutamate} + \text{NAD(P)}^+ + H_2O \leftrightarrow \text{2-oxoglutarate} + NH_4^+ + \text{NAD(P)H}$$

The above two reactions replenish the TCA cycle, resulting in increased ammonia production which may contribute to the chronic hyperammonaemia seen in OA.[8] In addition, the subsequent reduction of glutamate may further impair the synthesis of NAG by NAGS.[8]

Finally, the flow of glutamine to replenish 2-oxoglutarate in the TCA cycle leads to a decrease in glutamine.[8]

What are the biochemical differences between primary and secondary hyperammonaemia?

Although plasma ammonia levels remain elevated in both primary and secondary hyperammonaemia, the level of glutamine and urea cycle intermediates (citrulline and arginine) are helpful in distinguishing between primary and secondary hyperammonaemia **(Table 4)**.

Table 4. Differences in amino acid profiles between primary and secondary hyperammonaemia.

Parameters	Primary hyperammonemia	Secondary hyperammonemia
Ammonia	High	High
Glutamine	High	Normal-low*
Urea cycle intermediates Citrulline Arginine	 High/low Low (except in arginase deficiency when the level is high)	 No change No change

*Glutamine paradox is seen in secondary hyperammonaemia due to organic acidurias (OAs).

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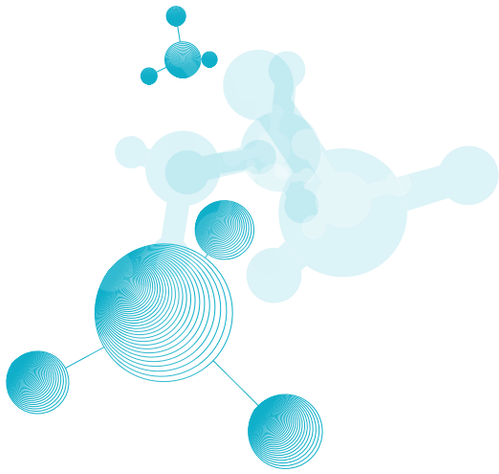
How Does Hyperammonaemia Present?



The IMDs that cause primary or secondary hyperammonaemia can present at any age as an acute or a chronic illness.

Early onset (neonatal presentation)

For most neonates with hyperammonaemia, whether from primary or secondary causes, pregnancy and the first few days of life are uneventful because excess urea in the foetus has been cleared by the maternal circulation.[1]

However, signs and symptoms of hyperammonaemia can develop within days, depending on the specific defect; some milder defects may have subtle, symptomatic episodes that spontaneously resolve or develop later in life.[1,3]

The symptoms of hyperammonaemia are nonspecific due to the very limited repertoire of symptoms in the neonate, frequently causing a delay in diagnosis.[3] Neonates with acute hyperammonaemia resemble those with sepsis, and show feeding difficulties, vomiting, temperature instability, and often respiratory distress or hyperventilation.[1,3] Respiratory alkalosis is present in about 50% of neonates with UCDs, and should prompt an immediate measurement of ammonia levels.[3] Progressive cerebral involvement may cause seizures and altered consciousness.[1,3] Neonates and infants with OAs that cause secondary hyperammonaemia all present with a high-anion-gap metabolic acidosis, in addition to hyperammonaemia.[15]

The initial symptoms of hyperammonaemia are nonspecific, frequently causing delay in diagnosis

Late onset (older infants and young children)

When a patient with an IMD presents later in life, acute hyperammonaemia may be triggered by an event such as an infection or vomiting **(Table 5)**.[3] In this case, the symptoms of an acute hyperammonaemia episode often mimic encephalitis or drug intoxication.[3] Patients may show an altered level of consciousness (from lethargy and somnolence, to coma), seizures, or psychiatric symptoms (such as hallucinations, paranoia, mania), and emotional or personality changes.[3] Like neonates, they may present with vomiting and loss of appetite. As other organs become affected, these children may develop signs of liver or multiple organ failure, as well as deteriorating peripheral circulation.[3]

Table 5. Potential triggers of hyperammonaemia episodes in patients with urea cycle defects (causing primary hyperammonaemia) or classical organic acidurias (causing secondary hyperammonaemia).[3]

Parameters
• Infection
• Fever
• Vomiting
• Gastrointestinal or internal bleeding
• Decreased energy or protein intake (e.g. fasting before surgery, major weight loss)
• Postpartum catabolism and involution of the uterus (mostly in females with OTC deficiency)
• Drugs: - Chemotherapy - High-dose corticosteroids - Valproate - L-asparaginase/pegasparagase - Others (less frequent causes include topiramate, carbamazepine, phenobarbitone, phenytoin, furosemide, hydrochlorothiazide, and salicylates)
• Prolonged or intense physical exercise
• Surgery under general anaesthesia
• Unusually high protein load (e.g. parenteral nutrition)

OTC, ornithine transcarbamylase.

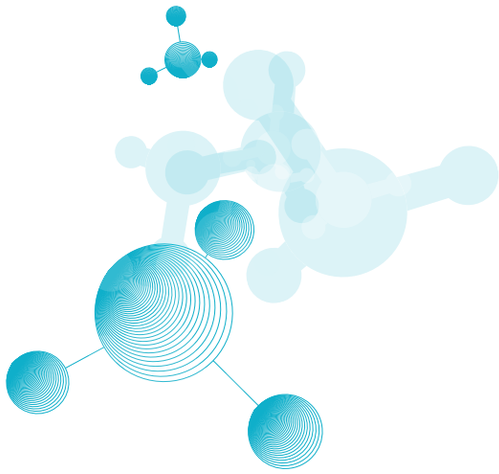
In older children and adults

When the genetic defects are not severe, hyperammonaemia can show a more chronic course, with signs and symptoms appearing episodically in response to triggers (see **Table 5**).[1,3] Many children with chronic hyperammonaemia choose to avoid protein intake by eating a vegetarian diet from a young age.[1,3] Psychiatric symptoms are very rare in patients with secondary hyperammonaemia, but are relatively common in primary hyperammonaemia associated with UCDs.[3,19] Certain conditions may be characteristic for individual UCDs or OAs; for example chronic renal failure is common in patients with MMA, whereas the heart is more often affected in PA.[19] Similarly, patients with ASL deficiency commonly develop fragility of the hair shaft (trichorrexis nodosa), and children with arginase 1 (ARG1) deficiency may develop a progressive spastic diplegia.[3]

Test ammonia levels whenever:

- A patient has unexplained encephalopathy
- A neonate has signs and symptoms of presumed septicaemia
- Respiratory alkalosis is present in a sick neonate
- A patient has signs and symptoms of chronic hyperammonaemia and they voluntarily choose to eat a vegetarian diet or limit their protein intake from a young age
- A patient has altered consciousness accompanied by loss of appetite or vomiting
- A patient has an unexplained change in consciousness

Differential Diagnosis of Hyperammonaemia



Once hyperammonaemia is suspected/confirmed, the following tests (many of which will already have been undertaken in a sick child) will assist in differentiating between causes of hyperammonaemia:

- Blood gases
- Electrolytes, including bicarbonate and calculation of anion gap
- Full blood count
- Urea
- Blood glucose
- Lactate
- Liver function test
- Plasma amino acids profile
- Acylcarnitines in plasma or blood spot
- Urinary amino acid and organic acid profile, including orotic acid
- Urine ketone bodies.

When seeking a patient history, ask about:[1,3,19]

- Drug/medication use
- Unexplained neonatal deaths in the family
- Neurological disorders in the family
- Patient's (or sibling's) voluntary avoidance of protein
- Consanguinity
- Risk factors for chronic liver disease
- Potential causes of increased ammonia production (e.g. asparaginase treatment, overgrowth of urease-positive bacteria, or genitourinary infection)
- Potential causes of increased protein catabolism (e.g. myeloma, chemotherapy, gastrointestinal haemorrhage)
- Potential causes of excessive nitrogen levels (e.g. total parenteral nutrition, glycine-containing irrigation during transurethral prostate resection).

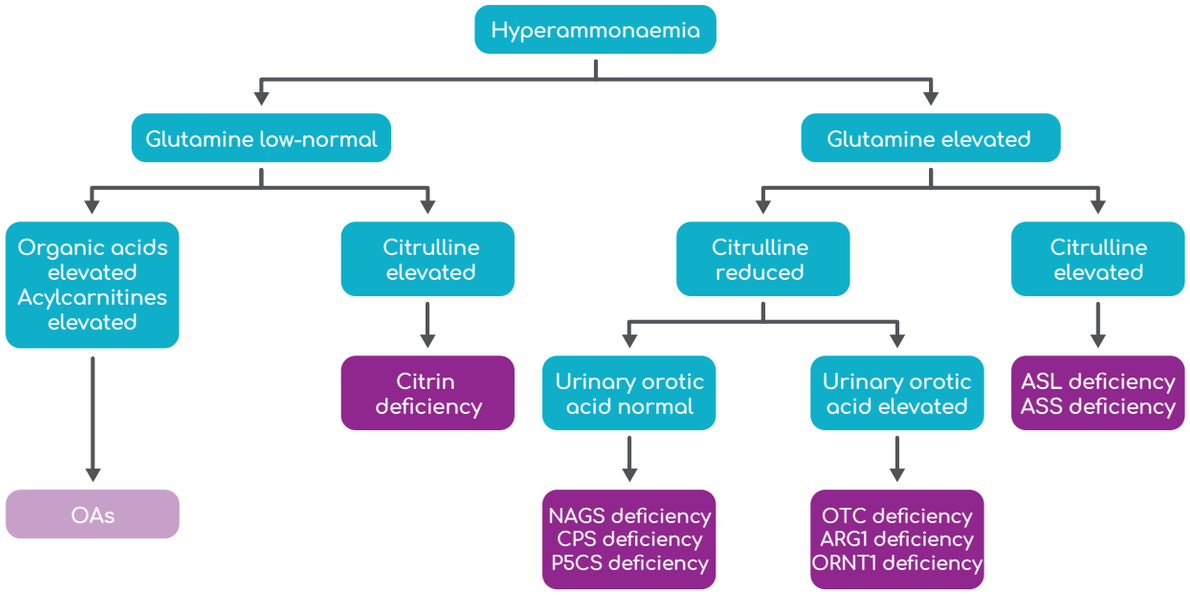
Once blood test results show hyperammonaemia, plasma amino acids and urine amino and organic acids must be measured to assist in the differential diagnosis of the specific UCD or OA. However, because of the neurotoxicity of elevated ammonia levels, treatment should be started immediately, without waiting for other definitive test results.

Once blood test results show hyperammonaemia, treatment should be started immediately, without waiting for other definitive test results

Differential diagnosis of primary versus secondary hyperammonaemia

The diagnostic algorithm for the presence of UCDs or OAs, based on blood and urine tests, is shown in **Figure 9**.[3] Both primary and secondary hyperammonaemia show elevated plasma ammonia levels. A key difference between primary and secondary hyperammonaemia is that glutamine levels are elevated in patients with UCDs, but normal or low in patients with OAs.[3] At times, the glutamine level will not allow this differentiation, especially when the ammonia levels are not particularly high, so another important distinguishing factor is that there are no changes in urea cycle intermediates in OAs.

Figure 9. Diagnosing urea cycle defects (primary hyperammonaemia) and organic acidurias (secondary hyperammonaemia) on the basis of key amino/organic acid tests.[3]



ARG1, arginase 1; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS, carbamoyl phosphate synthetase; NAGS, N-acetylglutamate synthetase; OA, organic aciduria; ORNT1, ornithine translocase; OTC, ornithine transcarbamylase; P5CS, pyrroline-5-carboxylate synthase.

Identifying the enzyme deficiency in primary hyperammonaemia

Almost all patients with primary hyperammonaemia show low plasma arginine levels, except those with arginase deficiency who have high levels of this amino acid **(Table 6)**. Similarly, citrulline levels can help in distinguishing between the enzymes causing primary hyperammonaemia: low citrulline levels indicate a deficiency in the first half of the cycle, namely NAGS, carbamoyl phosphate synthetase 1 (CPS1) or OTC (intra-mitochondrial enzymes), whereas higher/normal levels indicate either an ASS, ASL or arginase deficiency (extra-mitochondrial enzyme deficiency). Once the differential diagnosis has established that one of the first three (intra-mitochondrial) enzymes is involved, urine orotic acid levels can further help to determine if the cause is OTC deficiency.

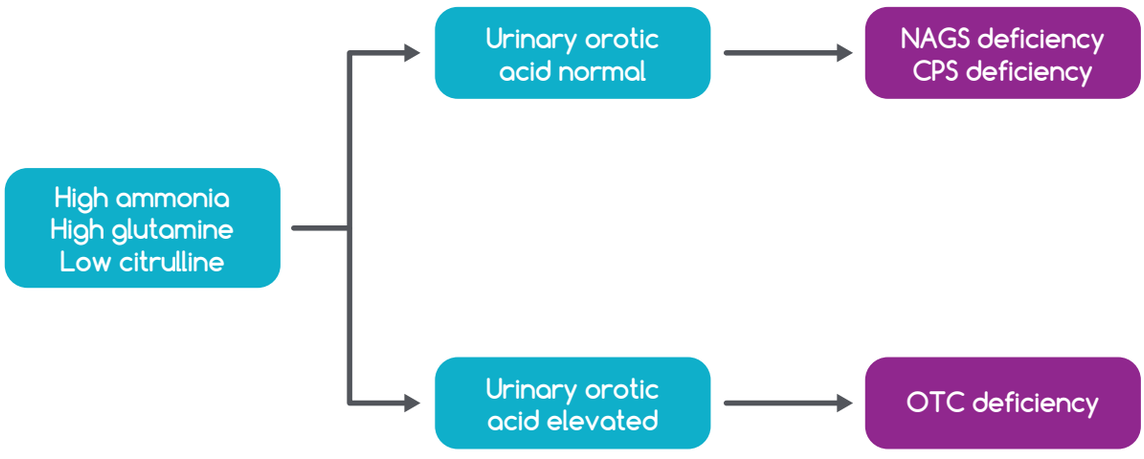
Table 6. Types of amino acid abnormalities seen in primary hyperammonaemia.[1,2,6]

Condition	Amino acid levels		
	Plasma citrulline	Plasma arginine	Other
1. NAGS deficiency	Absent/Normal/Low	Normal/Low	Low urine orotic acid*
2. CPS1 deficiency	Absent/Normal/Low	Normal/Low	Low urine orotic acid*
3. OTC deficiency	Normal/Low	Normal/Low	High urine orotic acid*
4. ASS deficiency (citrullinaemia type I)	High	Normal/Low	Absent plasma or urine ASA
5. ASL deficiency (argininosuccinate aciduria)	High	Normal/Low	High plasma or urine ASA
6. ARG1 deficiency (argininaemia)	Normal	High	–

ARG1, arginase 1; ASA, arginino Succinate; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS1, carbamoyl phosphate synthetase 1; NAGS, N-acetylglutamate synthase; OTC, ornithine transcarbamylase.*Not diagnostic when found in isolation – see below.

Urinary orotic acid is non-specific with many causes for low or high levels and is not diagnostic of OTC deficiency when found in isolation. In the presence of high ammonia and glutamine with low citrulline, the enzyme deficiency is narrowed down to the first three enzymes (NAGS, CPS1 and OTC). It is in this biochemical context that the presence of urinary orotic acid indicates OTC deficiency **(Figure 10)**.

Figure 10. Role of urinary orotic acid levels in the differential diagnosis of urea cycle defects.

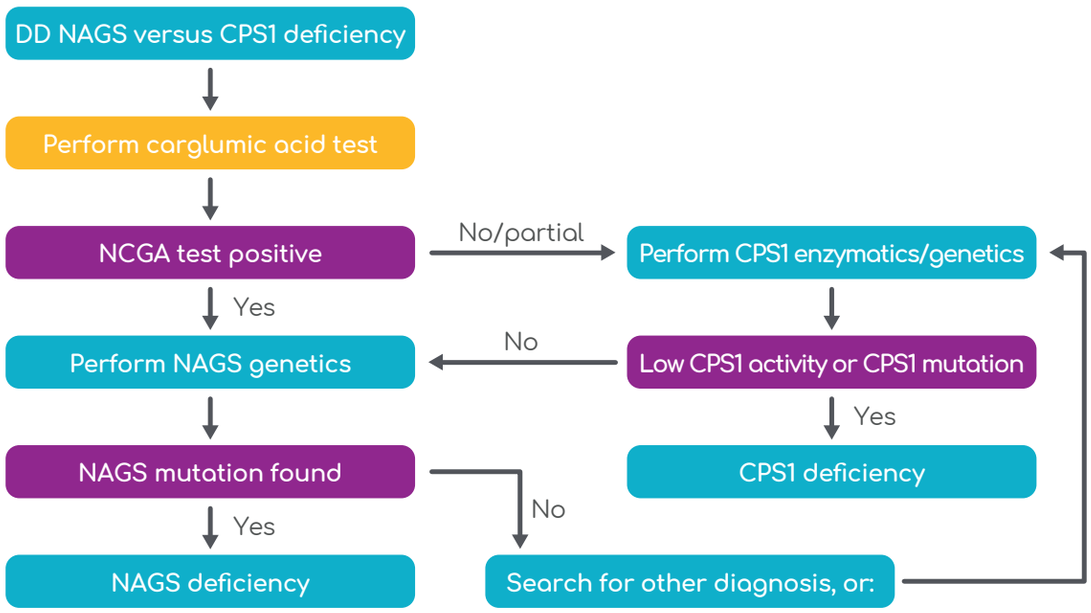


CPS, carbamoyl phosphate synthetase; NAGS, N-acetylglutamate synthetase; OTC, ornithine transcarbamylase.

Differential diagnosis of NAGS and CPS1 deficiencies by clinical ascertainment of responsiveness to carglumic acid.

Once the differential diagnosis has been narrowed down to either NAGS or CPS1 deficiency, it is important to perform the carglumic acid (N-carbamoyl-L-glutamic acid; NCGA) test, which involves administering carglumic acid at a dose of 100 to 250 mg/kg/day divided into two to four doses **(Figure 11)**.[28] In both NAGS deficiency and CPS1 deficiency, ammonia levels will normalise in response to nutritional intervention, including protein restriction, and treatment. When protein is reintroduced, patients with NAGS deficiency will be able to maintain normal ammonia levels during carglumic acid treatment without any protein restriction, whereas patients with CPS1 deficiency will not, and will need to continue with some moderate restriction of protein intake to maintain normal ammonia levels even with carglumic acid treatment.

Figure 11. Algorithm for the differential diagnosis of N-acetylglutamate synthetase and carbamoyl phosphate synthetase 1 deficiencies (adapted with permission from Häberle et al. 2012).[3]

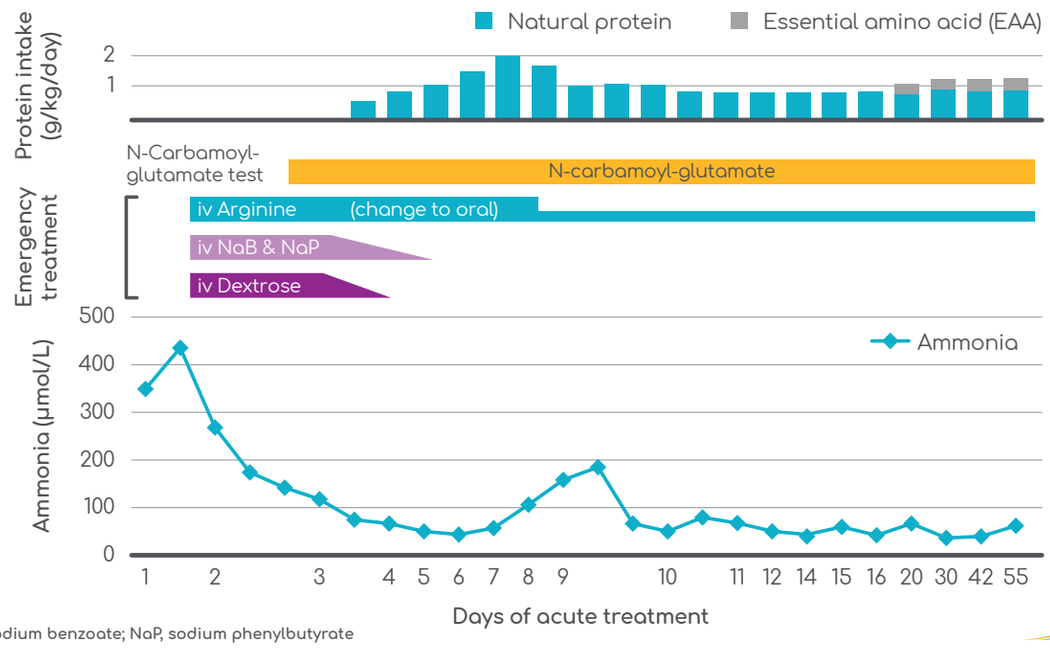


Genetic testing for NAGS and CPS1 are usually requested together, to ensure a correct genetics diagnosis. CPS1, carbamoyl phosphate synthetase 1; DD, differential diagnosis; NAGS, N-acetylglutamate synthetase.

Clinical point

Figure 12 illustrates the clinical management of a patient with acute hyperammonaemia of unknown cause, illustrating the use of pharmacotherapy and nutritional therapy, and the application of the carglumic acid test in practice.[29]

Figure 12. Clinical management of acute hyperammonaemia and metabolic stabilisation in a patient with an undiagnosed IMD.[29]



NaB, sodium benzoate; NaP, sodium phenylbutyrate

Key features of this case were:[29]

- Neonatal presentation of encephalopathy with high ammonia, 350 rising to 450 µmol/L, with no acidosis or urinary ketones.
- Diagnostic samples were taken and prioritised with the metabolic laboratory.
- Emergency treatment to control hyperammonaemia was implemented, comprising:
  - stopping protein
  - increasing calories through i.v. Dextrose
  - administering ammonia scavengers.
- The urgent amino acid profile showed normal glutamine and undetectable citrulline with low arginine – consistent with a differential diagnosis of UCD, either NAGS, CPS1 or OTC deficiency.
- Orotic acid was not detected in urine; this rules out OTC and the differential diagnosis is left with either NAGS or CPS1 deficiency.

Carglumic acid test in practice[29]

- Carglumic acid was given at 200 mg initially followed by 200 mg/day (63 mg/kg/day), divided into four doses.
- Ammonia scavengers were titrated down as ammonia levels decreased and were eventually discontinued.
- Dietary protein was gradually introduced and titrated against ammonia levels to reach a tolerance point for the patient, while maintaining treatment with carglumic acid.
- Final diagnosis was CPS1 deficiency
  - The patient was not able to tolerate full natural protein intake thus giving a clinical diagnosis of CPS1 deficiency, based on the findings of the carglumic acid test
  - The diagnosis was confirmed by molecular analysis of both the CPS1 and NAGS gene, which showed homozygous mutations in the CPS1 gene.

NAGS deficiency is the only urea cycle disorder that is fully responsive to carglumic acid while on a normal diet. Therefore, its diagnosis and treatment should not be missed

Identifying the enzyme defect in secondary hyperammonaemia

OAs are generally diagnosed by the presence of specific metabolites in urine and blood,[9] but usually measured in urine. **Table 7** shows the metabolites and amino acids that are present in urine in patients with OAs causing secondary hyperammonaemia.

Table 7. Metabolites present in urine of patients with classical organic acidurias.

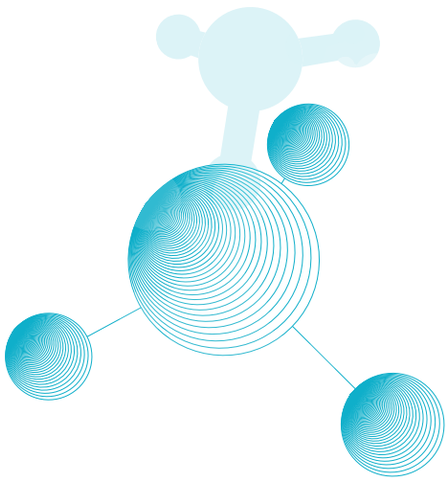
MMA	PA	IVA
• Methylmalonic acid	• Propionic acid	• Isovaleric acid
• Propionic acid	• Propionyl glycine	• Isovaleryl glycine
• Propionyl glycine	• Propionyl carnitine	• Isovaleryl carnitine
• Propionyl carnitine	• Methylcitrate	
• Methylcitrate	• Hydroxyvalerate	
• Acetoacetate	• Acetoacetate	
• β-hydroxybutyrate	• β-hydroxy-propionate	
	• β-hydroxybutyrate	

IVA, isovaleric aciduria; MMA, methylmalonic aciduria; PA, propionic aciduria.

Once a definitive diagnosis has been reached regarding the cause of the hyperammonaemia, confirmatory testing should include molecular analysis and/or enzymology in skin fibroblast cultures.[1,3,9,19]

Key points

- Urgently assess ammonia levels whenever a patient has unexplained neurological symptoms.
- Other blood and urine tests that can help to identify the cause and type of hyperammonaemia (primary vs secondary) should be done in parallel to assess metabolic acidosis, but should not delay treatment.
- Careful review of patient history should include family history indicative of IMDs and potential precipitating factors.
- Diagnosis should be confirmed by mutation analysis or assay of enzyme activity on liver biopsy sample.



Acute Hyperammonaemia

Generic emergency treatment of hyperammonaemia

For acute hyperammonaemia, the goal of treatment is to quickly reduce ammonia levels in order to prevent or limit neuro-cognitive damage. Treatment approaches depend on the severity of the hyperammonaemia and whether or not the patient has a known IMD (**Figure 13**).[3,19] Patients with very high ammonia levels should also be assessed for extracorporeal detoxification, alongside initiation of pharmacological and nutritional therapy. The choice of detoxification method will depend on local facilities but would usually be haemodialysis.[3] However, haemofiltration is usually recommended in neonates or infants because of difficulties with dialysis in this age group.[3]

For acute hyperammonaemia, the goal of treatment is to quickly reduce or normalise ammonia levels in order to prevent or limit mortality and neurocognitive damage

Figure 13. Recommended treatment for acute hyperammonaemia.[3,19]

	Ammonia above ULN →	Ammonia >100 and <250 µmol/L (>150 and <250 µmol/L in neonates) →	Ammonia 250 to 500 µmol/L →	Ammonia 500 to 1000 µmol/L →	Ammonia >1000 µmol/L
Undiagnosed patient***	Stop protein intake Give IV glucose ± insulin[a] Monitor blood ammonia levels every 3 hours	+ Start treatment with i.v. L-arginine and nitrogen scavengers Start carbaglumic acid, carnitine, vitamin B12, and biotin	+ Prepare haemo(dia)-filtration if significant encephalopathy and/or early high blood ammonia or very early onset of disease (day 1 or 2) Begin haemo(dia)-filtration if no rapid drop of ammonia within 3–6 hours	+ Start haemo(dia)-filtration immediately	+ Evaluate whether to continue specific treatment or start palliative care
Patient with known UCD	Stop protein intake Give i.v. glucose ± insulin[a] Monitor blood ammonia levels every 3 hours	+ Continue treatment with i.v. L-arginine and sodium benzoate ± sodium phenylbutyrate/phenylacetate[b], increase dose or give IV Continue or add L-citrulline for NAGS, CPS1 or OTC deficiency Consider nasogastric carbohydrate and lipid emulsions unless child is vomiting	+ Give all drugs i.v. Prepare haemo(dia)-filtration if significant encephalopathy and/or early high blood ammonia or very early onset of disease (day 1 or 2) Begin haemo(dia)-filtration of no rapid drop of ammonia within 3-6 hours	+ Start haemo(dia)-filtration as fast as possible	+ Evaluate whether to aim at curative treatment or palliative care
Patient with known MMA/PA	Stop protein intake Give i.v. glucose ± insulin[a] Increase carnitine dosage to 200 mg/kg/day Monitor blood ammonia levels every 3 hours	+ Start treatment with sodium benzoate Consider carbaglumic acid	+ Consider extracorporeal detoxification dependent on patient's age and history	+ Consider extracorporeal detoxification dependent on patient's age and history	+ Consider extracorporeal detoxification dependent on patient's age and history

Nutritional treatment remains the cornerstone of management, in addition to pharmacotherapy and extracorporeal detoxification. Nutritional support aims to promote anabolism with the removal of ammonia.

As shown in **Figure 12**, principles of treatment in acute hyperammonaemia are:

- **Stop all protein**
  - Do NOT stop protein for more than 24–48 hours. Longer withdrawal of protein may induce catabolism leading to worsening of the hyperammonaemia.
- **Reduce catabolism** by providing alternative sources of energy (e.g. i.v. dextrose), and by maintaining protein-free nutrition until ammonia levels return to <100 µmol/L.
  - Provide 120% calories to promote anabolism.
  - Hyperglycaemia above 8 mmol/L can be managed by administering insulin under strict supervision without compromising the delivery of calories.
  - i.v. intralipid can be used to provide calories in a lesser volume, once fatty acid oxidation defects have been ruled out.
- **Remove toxic metabolites** by administering ammonia scavengers and carglumic acid, and implementing extracorporeal detoxification, as appropriate **(Table 8)**.
- **Adjust serum electrolytes by supplementation to maintain high normal levels.**
  - Beware that both ammonia scavengers (sodium benzoate and sodium phenylbutyrate) contain sodium. The sodium content for a maintenance dose of 250 mg/kg/day of sodium benzoate and sodium phenylbutyrate are 3.5 and 2.8 mmol/kg/day, respectively. Hypokalaemia can occur, particularly when insulin is used and must be appropriately supplemented.
  - Phosphate levels should be monitored and supplemented early if the patient is undergoing haemodialysis.
- **Prevent fluid overload**
  - Strict input/output chart.

Nutritional treatment is the cornerstone of acute hyperammonaemia management

a Administer glucose at an appropriate dose to prevent catabolism (e.g. 10 mg/kg/min in a neonate) ± insulin. Monitor blood glucose after 30 minutes and every hour thereafter as some neonates are highly sensitive to insulin.
b Where available, administer an equimolar solution of sodium benzoate and sodium phenylacetate/ phenylbutyrate (usually phenylbutyrate) as an i.v. bolus 250 mg/kg over 90 to 120 minutes then as 250 mg/kg continuous i.v. infusion over 24 hours.
CPS1, carbamoyl phosphate synthetase; i.v. intravenous; MMA, methylmalonic aciduria; NAGS, N-acetylglutamate synthase; OTC, ornithine transcarbamylase; PA, propionic aciduria; UCD, urea cycle defect; ULN, upper limit of normal.
**Diagnostic samples should be taken without delaying treatment

Table 8. Dosages of ammonia scavengers or carglumic acid to be administered in acute hyperammonaemia.[3,19]

		Dosage				
Patient	**Administration**	**Sodium benzoate**	**Sodium PBA/ sodium phenylacetate**	**L-arginine**	**Carglumic acid**	**L-carnitine**
Acute hyperammonaemia of unknown cause						
	Bolus[a]	250 mg/kg	250 mg/kg	250(–400) mg/kg	100 mg/kg	100 mg/kg
	Maintenance	250–500 mg/kg/day[b] (or 5.5 g/m²/day if BW >20 kg)	250–500 mg/kg/day[b]	250 mg/kg/day	25–62.5 mg/kg every 6 hours	100 mg/kg/day
Acute hyperammonaemia in diagnosed urea cycle defect						
NAGS deficiency	Bolus[a]	250 mg/kg	-	250 mg/kg	100 mg/kg	-
	Maintenance	250–500 mg/kg/day[b] (or 5.5 g/m²/day if BW >20 kg)	-	250 mg/kg/day	25–62.5 mg/kg every 6 hours	-
CPS1 or OTC deficiency	Bolus[a]	250 mg/kg	250 mg/kg	250 mg/kg	-	-
	Maintenance	250–500 mg/kg/day[b] (or 5.5 g/m²/day if BW >20 kg)	250(–500) mg/kg/day[b]	250 mg/kg/day	-	-
ASS deficiency	Bolus[a]	250 mg/kg	250 mg/kg	250 mg/kg	-	-
	Maintenance	250–500 mg/kg/day[b] (or 5.5 g/m²/day if BW >20 kg)	250(–500) mg/kg/day[b]	250 mg/kg/day	-	-
ASL deficiency	Bolus[a]	250 mg/kg	250 mg/kg	200–400 mg/kg	-	-
	Maintenance	250–500 mg/kg/day[b] (or 5.5 g/m²/day if BW >20 kg)	250(–500) mg/kg/day[b]	200–400 mg/kg/day	-	-
ARG1 deficiency	Bolus[a]	5.5 g/m²/day if BW >20 kg)	-	AVOID	-	-
	Maintenance	250 mg/kg 250–500 mg/kg/day[b] (or 5.5 g/m²/day if BW >20 kg)	-	AVOID	-	-
Acute hyperammonaemia in diagnosed organic aciduria						
MMA or PA	Bolus[a]	250 mg/kg	250 mg/kg	250 mg/kg	100 mg/kg	100 mg/kg
	Maintenance	250 mg/kg/day[c]	250 mg/kg/day[c]	250 mg/kg/day[c]	25–62 mg/kg	100 mg/kg/day

ARG1, arginase 1; ASLD, argininosuccinate lyase; ASS, argininosuccinate synthetase; BW, body weight; CPS1, carbamoyl phosphate synthetase; MMA, methylmalonic aciduria; NAGS, N-acetylglutamate synthase; ORNT1, ornithine translocase 1; OTC, ornithine transcarbamylase; PA, propionic aciduria; PBA, phenylbutyrate.
[a]For all i.v. agents (amino acids), this should be administered in 90 to 120 minutes. Carglumic acid is administered orally via nasogastric tube.
[b]If the patient is on haemodialysis, the maintenance dose should be increased to 350 mg/kg/day or increased proportionally based on body surface area calculations.
[c]Maximum daily dosage of 5.5 g/m² or 12 g/day for sodium benzoate or sodium PBA, and 12 g/day for L-arginine.

Principles of nutritional treatment in acute hyperammonaemia

The nutritional management of acute hyperammonaemia is the withdrawal of all protein for no more than 24–48 hours. A longer withdrawal period will cause catabolism leading to worsening of hyperammonaemia. To ensure anabolism, protein withdrawal must be accompanied by an intravenous hypercaloric protein-free solution, which may be given with insulin.[30,31] In parallel, the acute hyperammonaemia is controlled by extracorporeal detoxification and/or pharmacotherapy using ammonia scavengers and carglumic acid.

Once ammonia levels are sufficiently controlled, the next phase is to restart oral nutrition with high-quality protein. Dependent on the plasma ammonia and amino acid levels, and the activity of the gastrointestinal tract, the feeds may be started at 25–50% concentration of full protein requirements. It is important not to wait more than 48–72 hours without feeds.[32] At this point, it is important to supplement with essential amino acid (EAA) mixtures as well as L-arginine and/or L-citrulline, titrated against ammonia and amino acid levels in UCD. Protein tolerance should be determined for each patient individually.

Extracorporeal detoxification also affects nutritional status. Haemofiltration removes 20–25% of nutrients in the plasma, thus creating a catabolic state.[33] Hence, aggressive nutritional therapy must be instituted during dialysis to prevent a rebound of ammonia levels.

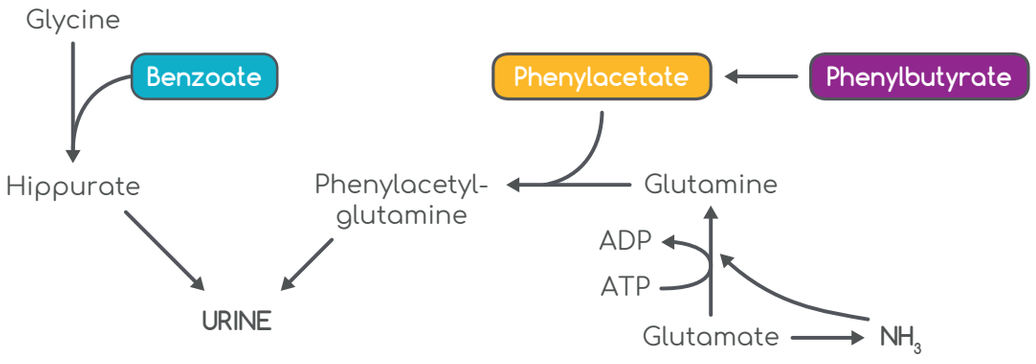
Pharmacotherapy: Modes of action

1. Ammonia scavengers

Ammonia scavenger agents include sodium benzoate, sodium phenylacetate and sodium phenylbutyrate. They are termed 'ammonia scavengers' because their hepatic metabolism involves conjugation with glycine (benzoate) or glutamine (phenylacetate or phenylbutyrate) in a process that uses ammonia ions to form an amine-containing metabolite **(Figure 14)**.[34]

Benzoate is conjugated to L-glycine to form hippurate, which is readily excreted in urine. Similarly, phenylacetate is conjugated to glutamine in a reaction that requires ammonia ions, to form the metabolite phenylacetylglutamine, which is also excreted in urine. Phenylbutyrate, which is converted to phenylacetate *in vivo*, acts in the same way.[34] By providing an alternative pathway for nitrogen excretion, these agents 'mop up' excess ammonia ions and reduce the extent of hyperammonaemia.[34]

Figure 14. Mechanisms of action of the ammonia scavengers-benzoate, phenylacetate and phenylbutyrate.



ADP, adenosine diphosphate; ATP, adenosine triphosphate; NH3, ammonia.

Vomiting can occur in patients receiving ammonia scavengers,[3,34] hence antiemetics may be given prophylactically with the initial bolus doses.[3] Maintenance doses may be associated with reduced appetite, taste disturbances, unpleasant body odour, and menstrual dysfunction (in postpubescent females).[34] There is also a potential for drug-to-drug interactions with agents that are metabolised by the cytochrome P450 enzyme system.[34]

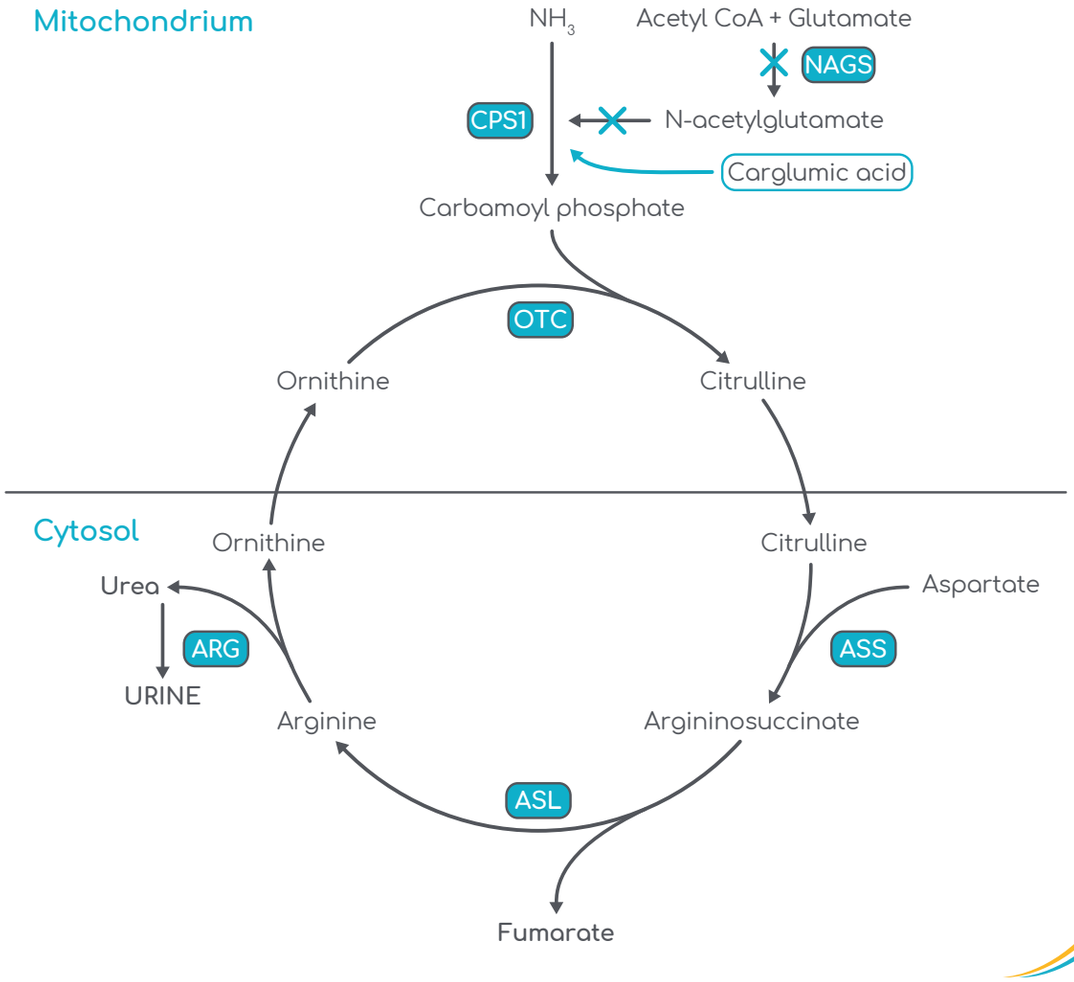
2. Arginine and citrulline

Arginine is commonly administered in patients with acute hyperammonaemia, unless the patient has ARG1 deficiency; citrulline may be given instead of arginine in severe OTC/CPS1 deficiency.[1,3] These amino acids ensure that the function of the urea cycle is maximised by supplementing the substrates in this cycle, thus avoiding any rate-limiting shortages in substrate.[3] L-arginine administration maximises nitrogen excretion via intermediary metabolites of the urea cycle (citrulline or argininosuccinate) and helps to avoid arginine deficiency.[1]

3. Carglumic acid

Carglumic acid is indicated for the acute- and long-term management of NAGS deficiency, IVA, MMA and PA.[35] Carglumic acid is a structural analogue of N-acetylglutamate (NAG) which increases the excretion of ammonia by directly activating the urea cycle at the site of the first and rate-limiting enzyme in this process – CPS1 **(Figure 15)**.[36]

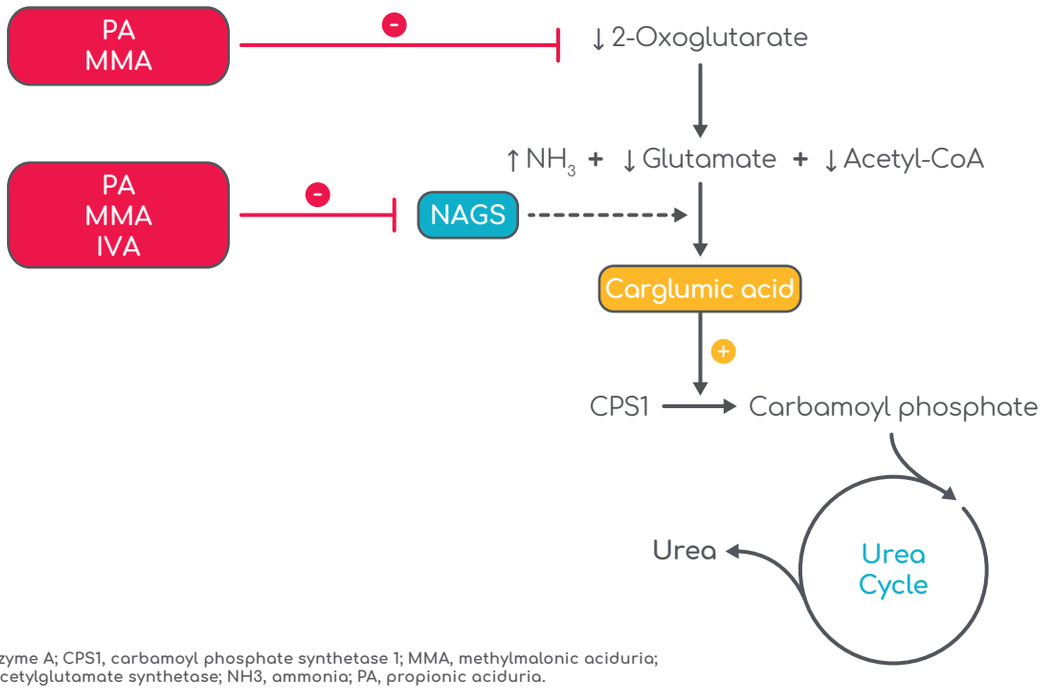
Figure 15. Mechanism of action of carglumic acid in patients with primary hyperammonaemia caused by a urea cycle defect in which the N-acetylglutamate synthetase enzyme is affected, and there is limited production of N-acetylglutamate.



ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS, carbamoyl phosphate synthetase; NAGS, N-acetylglutamate synthetase; NH4+, ammonium; OTC, ornithine transcarbamylase.

In patients with secondary hyperammonaemia caused by MMA, PA or IVA, the NAGS enzyme is not defective, but its action is inhibited by high levels of metabolites that accumulate as a result of other defective enzymes. These metabolites (propionyl-coenzyme A [CoA] in the case of PPA and MMA, methylmalonyl-CoA in the case of MMA, and derivatives of isolaveric-CoA in the case of IVA) inhibit the action of NAGS, so their accumulation limits the delivery of NAG to the urea cycle and CPS1, and causes an increase in ammonia levels. NAG levels may also be decreased in PA and MMA by a reduction in mitochondrial acetyl-CoA or free CoA levels that occurs when glutamate production is inhibited **(Figure 16)**.[36] Therefore, the lack of NAG in these conditions can be compensated for by the administration of the CPS1 activator, carglumic acid.[37]

Figure 16. Mechanism of action of carglumic acid in secondary hyperammonaemia caused by classical organic acidurias in which N-acetylglutamate synthetase is inhibited by accumulation of intermediate metabolites or by a reduction in acetyl coenzyme A supply.[36,38]



CoA, coenzyme A; CPS1, carbamoyl phosphate synthetase 1; MMA, methylmalonic aciduria; NAGS, N-acetylglutamate synthetase; NH3, ammonia; PA, propionic aciduria.

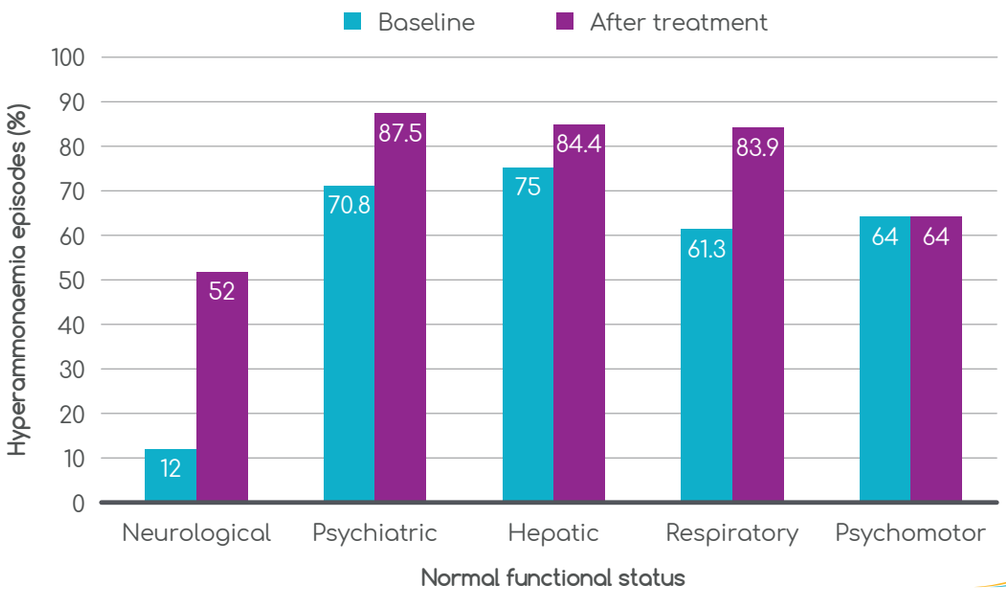
Carglumic acid is an oral medication, but can be administered by nasogastric tube in infants and young children who are unconscious or unable to swallow because of the effects of acute hyperammonaemia.[35]

In published reports, carglumic acid rapidly normalised ammonia levels in acutely ill neonatal patients with homozygous NAGS deficiency.[39-41] The unconscious patients rapidly regained consciousness,[39,40] and all patients were able to resume oral feeding within 48 hours.[39-41] Patients continued to receive treatment for NAGS deficiency after discharge and their ongoing neurological development was normal.[39-41]

Carglumic acid has also been used in the acute treatment of secondary hyperammonaemia associated with MMA,[25,37,38,42,43] PA,[37,38,42,44-47] and IVA.[38,48] Because of the rarity of IVA, published reports on the use of carglumic acid in these patients are limited to fewer than 10 patients.[38,48] The dosages of carglumic acid used for hyperammonaemia have varied between reports,[25,37,38,42-48] but guidelines recommend 100 mg/kg as the loading dose.[19] A loading dose of carglumic acid ≥100 mg/kg, rapidly normalizes ammonia levels and improves symptoms in patients with secondary hyperammonaemia.[25,37,42-48]

In the largest (retrospective, observational) study of carglumic acid use in patients with secondary hyperammonaemia (n=48 episodes), ammonia levels normalised to ≤60 µmol/L in a mean of 2.4 days after starting carglumic acid, and these changes were associated with normalization of neurological status in most patients **(Figure 17)**.[38] Patients in this group had received carglumic acid for between 1 and 15 days (median 4), with 86.7% of patients discontinuing carglumic acid because the target ammonia level had been reached. Additional ammonia scavengers made no significant difference to ammonia levels at endpoint (i.e. the last available ammonia measurement made within 18 hours of the last carglumic acid administration, or on day 15). However, the endpoint was reached much more rapidly when carglumic acid was given with ammonia scavengers (median 3.9 days) versus without ammonia scavengers (median 5.0 days) or with haemofiltration (median 4.8 days).[38]

Figure 17. Proportion of secondary hyperammonaemia patients with normal functional status in different organ systems before and after treatment with carglumic acid.[38]



A *post hoc* analysis of two retrospective studies that compared the efficacy of carglumic acid either alone or in combination with ammonia scavengers in patients with OAs during decompensation episodes reported a significant reduction in plasma ammonia levels from baseline at 72 hours with carglumic acid plus ammonia scavengers compared with ammonia scavengers alone ($p<0.01$; **Figure 18**).[49] It was also observed that the dose of carglumic acid, ≥100 mg/kg affected the median time required to reduce plasma ammonia concentrations by 50% in these patients **(Figure 19)**.

Figure 18. Median change from baseline in plasma ammonia levels in patients treated with carglumic acid alone, ammonia scavengers alone and a combination of carglumic acid plus ammonia scavengers.[49] Data were censored at extracorporeal detoxification. *n* represents the numbers of episodes evaluated at the time of the retrospective data collection.

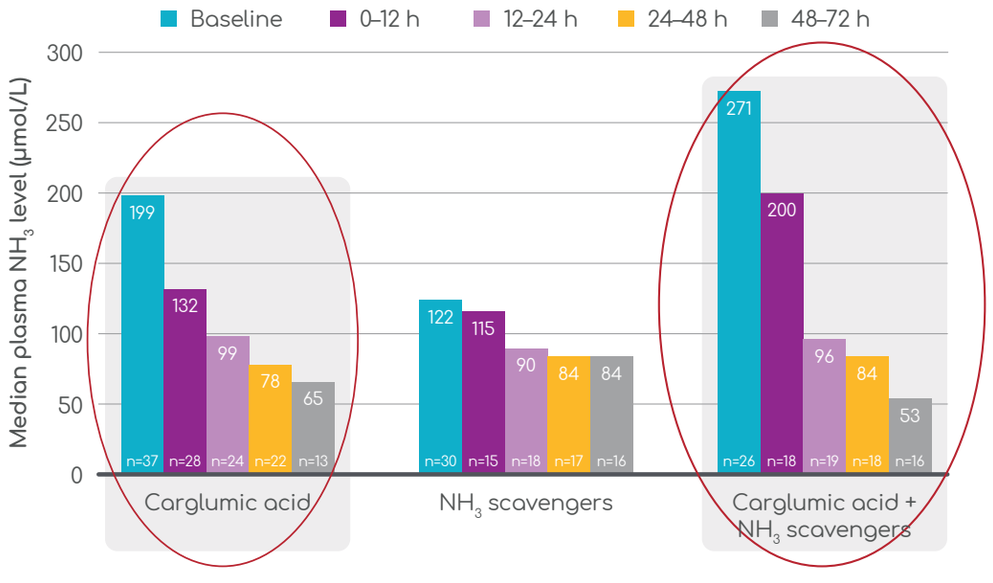
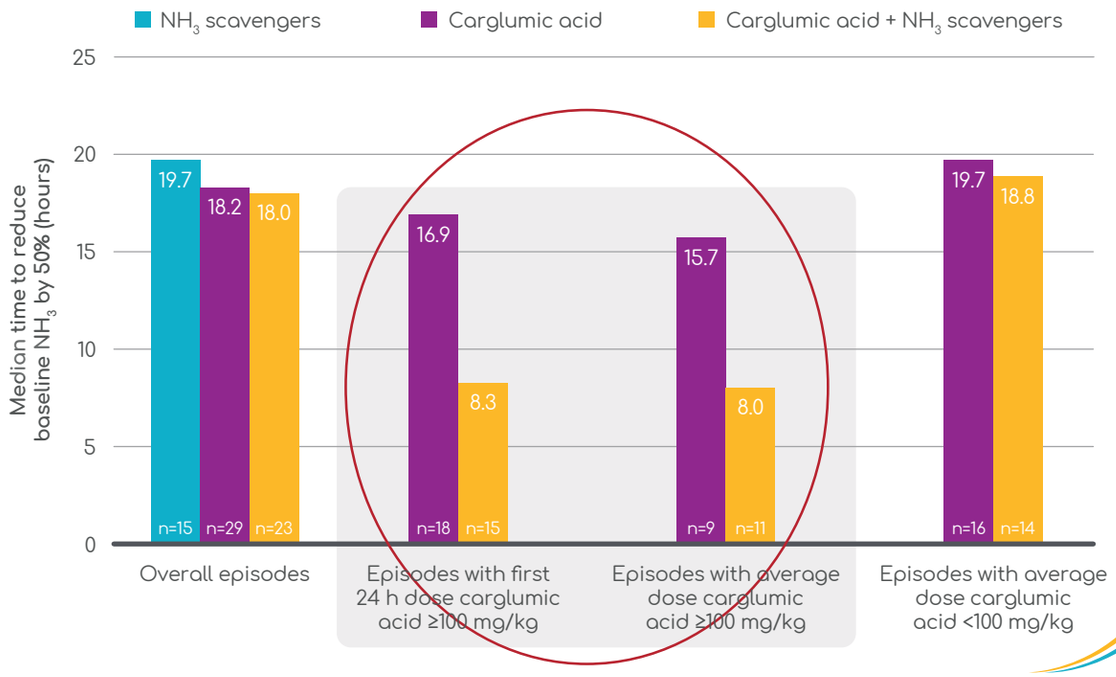
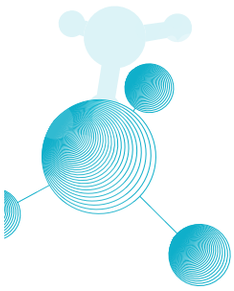


Figure 19. The median time required to reduce plasma ammonia concentrations by 50% of baseline values.[49] Data were censored at extracorporeal detoxification initiation. Numbers represent the number of episodes evaluated at the time of the retrospective data collection.





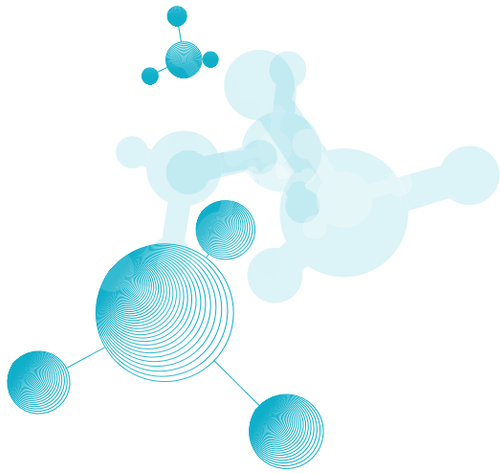
Key points

- The aim of acute treatment of hyperammonaemia is to rapidly reduce ammonia levels in order to prevent or limit neurological damage.
- Acute treatment of hyperammonaemia involves:
  - stopping protein (not more than 24-48 hours)
  - reducing catabolism by providing alternative sources of energy (e.g. i.v. dextrose; 120% calories), and by maintaining protein-free nutrition until ammonia levels return to <100 µmol/L
  - removing toxic metabolites by administering ammonia scavengers and/or carglumic acid and implementing extracorporeal detoxification, as appropriate
  - adjusting serum electrolyte levels at high normal range for sodium and potassium.
- In patients with hyperammonaemia of unknown cause, all pharmacotherapy including both ammonia scavengers and carglumic acid, with adequate nutritional treatment, should be instituted without delay.
- In patients who have a known cause for hyperammonaemia, the type of ammonia scavenger can be tailored to the underlying IMD.
- Carglumic acid rapidly normalises ammonia levels and improves symptoms in patients with primary hyperammonaemia due to NAGS deficiency or secondary hyperammonaemia due to MMA, PA or IVA.
- Haemodialysis is the preferred form of extracorporeal detoxification in most patients, but haemofiltration may be easier in neonates and young infants.

HYPERAMMONAEMIA

A CLINICIAN'S PERSPECTIVE on DIAGNOSIS and MANAGEMENT

Long-term Management of Hyperammonaemia due to UCD or OAs



Patients with UCD or OA require long-term management of their primary metabolic condition in order to prevent hyperammonaemia.[3,19]

Principles for the long-term management of hyperammonaemia are:

- Maintain metabolic stability to prevent episodes of acute toxicity and decompensation
- Achieve and maintain normal growth and development
  - By prevention of secondary nutritional deficiency
- Maintain quality of life
- Avoid/minimise the development of long term complications.

This is achieved primarily through dietary intervention, targeted treatment as needed with carglumic acid or ammonia scavengers, and preparation for potential triggers or emergencies.

Clinical point:

It has been reported that sodium benzoate can cause low potassium owing to its interference with renal tubular reabsorption of potassium. In contrast, the renal tubular reabsorption of sodium was not affected. Sodium phenylbutyrate did not affect plasma potassium levels.[50]

Nutritional Treatment

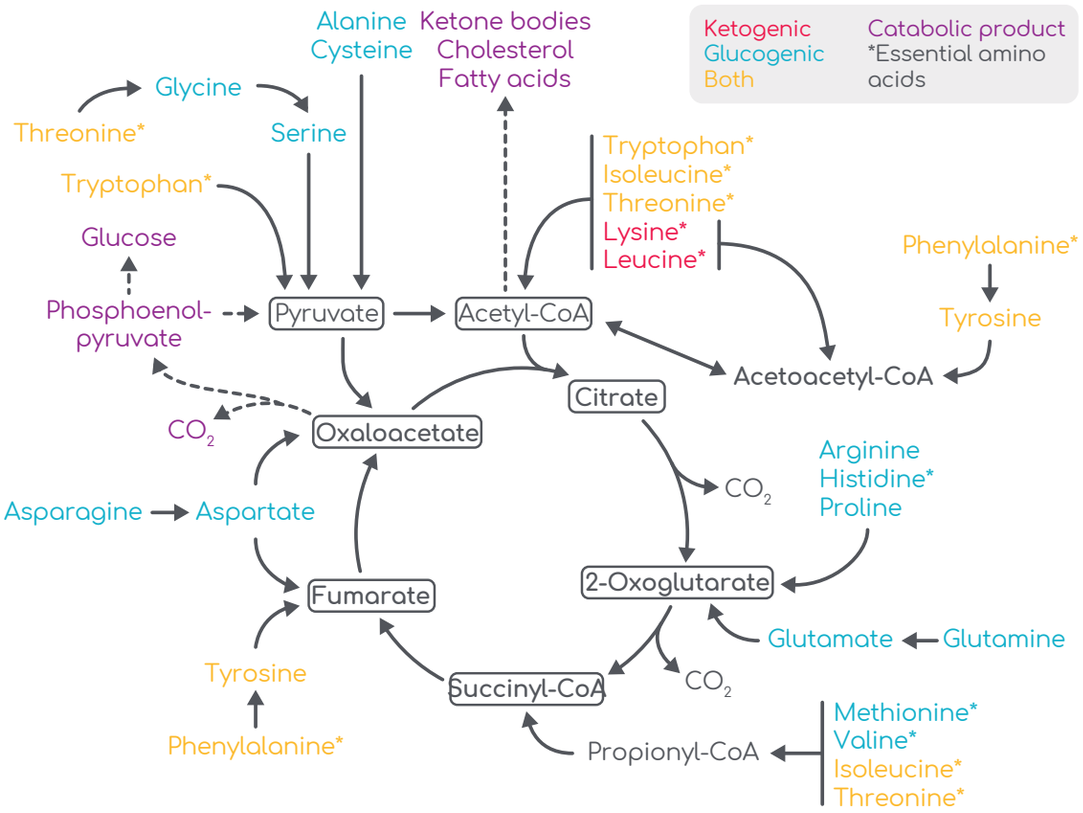
The prescribed diet in its entirety is the cornerstone of the long-term management of UCD (except for NAGS deficiency) and OAs, with additional pharmacotherapy as discussed above.

The foundation of nutritional treatment is a moderate-protein, high-energy diet that includes:

- intact natural protein, as a source of precursor amino acids,
- amino acid-based formula free of, or low in, the precursor amino acids, and
- additional energy sources, carbohydrate and fat to promote anabolism.

Fasting should be avoided to prevent catabolism of muscle protein and to reduce build-up of metabolites.[51] **(Figure 20)**

Figure 20. Catabolism of glucogenic and ketogenic amino acids in the TCA cycle. Adapted with permission from www.amboss.com/us/knowledge/Citric_acid_cycle.



Patients with UCD or OA are treated with a low-protein diet in order to minimise the nitrogen load on the urea cycle.[3,19] The level of protein should be individualised for each patient, and titrated to their serum ammonia level and their daily energy requirements. However, over-restriction should be avoided because too little protein can cause malnutrition, resulting in compromised growth and metabolic stability.[3,19] The amount of protein a patient needs will vary over time, dependent on their age, severity of the underlying UCD or OA, growth rate, physical activity, and any intercurrent illnesses.[3,19] Owing to the complexity of combining the diet with pharmacotherapy, it is essential that clinicians prescribing pharmacotherapy work closely with a metabolic dietician calculating the dietary prescription. This is to ensure adequate protein intake while maintaining good biochemical control. Titration of the drugs requires tandem monitoring of plasma ammonia and amino acid profiles with dietary protein and energy intake.[32] Other biochemical parameters for adequate growth should also be monitored throughout life. Some young infants with hyperammonaemia experience feeding difficulties, and older children may develop anorexia or dysphagia, which may require tube feeding.[3,19]

Vitamin, mineral and EAA supplements are often indicated.[3,19] Patients with MMA or PA will often require supplementation with L-carnitine and precursor-free amino acid and/or isoleucine/valine; those with MMA may benefit from vitamin B_{12} supplements.[19]

Dietary factors that may contribute to nutritional outcomes include:[52]

- quality and quantity of protein tolerated
- the frequency of metabolic decompensations treated with further protein restriction with high non-protein energy intake
- the abnormal feeding behaviours and food aversions observed in patients with UCD and OA
- lower-than-prescribed intake of overall protein and energy.

Therefore, protein-restricted dietary regimens may have short- and long-term nutritional risks.

General components of dietary treatment

1. Natural protein

- Natural protein intake needs to be restricted and titrated to achieve good biochemical control.
- Natural protein intake should be MAXIMISED in each patient individually according to their tolerance, before synthetic protein is added, if necessary.
- This minimises the need to supplement with additional single amino acids, e.g. L-isoleucine and/or L-valine, due to a deficiency in these EAA secondary to insufficient natural protein intake.

2. Synthetic protein (medical foods)

- Synthetic protein may be needed in order to achieve sufficient overall protein intake for growth and development.
- These medical formulas are devoid of the toxic precursors and include:
  - EAA for UCD
  - Amino acid formula, free of methionine, threonine and valine, and low in isoleucine for MMA/PA
  - A leucine-free, amino acid formula for IVA

3. 'Free' foods:

- These comprise non-protein containing foods to provide for caloric intake and variety in the diet.
- Often insufficient attention is given to this aspect of the diet thus creating a relative catabolic state, which could result in poor biochemical control.

General points regarding protein and energy requirements in UCD and OAs

Exact protein requirements in UCD or OA patients are not well established, partly due to the variability of individual tolerance, and compounded by the relative rarity of these disorders. As such, in practice, the adequacy of patients' diets is traditionally measured against recommendations for healthy populations. Dietary reference intakes (DRI), Recommended Daily Allowances (RDA) and Food and Agriculture Organisation (FAO/WHO/UNU) recommendations were not intended for children with chronic illness or with metabolic disorders.[32]

Nutritional treatment must be tailored and protein titrated to an amount that is tolerated, promotes anabolism and improves morbidity. The use of low biological value natural protein only may not support growth in these patients, particularly severely affected patients, and may result in increased morbidity.[32] Hence, for those patients who obtain the majority of protein from synthetic amino acid formulas, their total protein needs are often higher than the total protein recommendations in the DRI and FAO/WHO/UNU recommendations. The reasons for the increased requirements are as follows:[32]

- different nitrogen retention rates between natural protein and free amino acids
- differences in the absorption and catabolism rates of amino acids between natural protein sources and free amino acids.

The 1985 FAO/WHO/UNU consensus statement incorporated the concept of Protein: Energy ratio (P:E ratio).[53] The P:E ratio describes the proportion of dietary energy derived from protein and has been used to answer the following question:[52] *'If an individual or group consumes this diet, in amounts that will satisfy energy needs, will the concentration of protein also be high enough to meet their protein needs?'* Evans et al. have defined a P:E ratio of >1.5g to <2.9g of protein per 100 kcal per day for patients with IMDs because this ratio correlates with optimal growth outcomes in these patients.[52]

Condition-specific treatment

N-acetylglutamate synthetase deficiency

Patients with NAGS deficiency benefit from ongoing daily treatment with carglumic acid to maintain normal/safe ammonia levels.[39-41,54] The daily dosage may range from 10 to 100 mg/kg, and should be adjusted based on plasma ammonia levels.[35] Patients taking carglumic acid do not need to restrict their dietary protein intake except during acute episodes.[3]

NAGS deficiency is the only UCD that is effectively treatable with carglumic acid, without additional dietary protein restriction. Therefore, it is important to make the diagnosis early and start effective treatment as soon as possible.

Organic acidurias

Nutritional treatment is the primary treatment for OAs, alongside additional pharmacotherapy to control ammonia levels. Patients with OAs may also benefit from continuous or episodic treatment with antibiotics (usually metronidazole) to control the growth of anaerobic gut bacteria, which can cause fermentation of carbohydrates in the gut, producing high levels of propionyl CoA.[19] Carnitine supplementation may additionally help patients with OAs, although no clinical trials have been conducted to confirm this.[55]

Emerging studies have shown that patients with MMA and PA benefit from long-term maintenance treatment with carglumic acid. In a report of clinical experience in patients with PA and MMA (n=8; aged 2–20 years), treatment with carglumic acid (50 mg/kg/day) for 7–16 months resulted in a decreased number and severity of decompensation episodes (24 episodes at baseline *vs* 9 post-treatment), and none of the episodes required hospitalisation.[56] The treatment also led to a significant reduction in plasma ammonia levels in most patients (n=5), and patients had a 20–50% increase in natural protein intake during the treatment period.[56]

A case report of long-term use of carglumic acid (100 mg/kg followed by 50 mg/kg after 6 months) in a patient with decompensated PA and hyperammonaemia reported significantly decreased plasma ammonia levels with treatment (75.7 µmol/L *vs* 140.3 µmol/L before treatment; $p<0.005$), and fewer decompensation episodes (78 episodes during nine years before treatment *vs* 2 in six years of treatment).[57] A small increase in the patient's natural protein intake was also reported during the treatment period (0.7 ± 0.12 g/kg/day before treatment *vs* 0.85 ± 0.18 g/kg/day during treatment).[57]

Several studies to determine the effects long-term carglumic acid are currently underway. The Carglumic Acid in Methylmalonic Acidemia and Propionic Acidemia (CAMP) study is a randomised, multicentre study aims to determine the long-term effectiveness of carglumic acid in patients with PA and MMA.[58] Other ongoing studies include the PROTECT and the LOTUS studies.[59]

Liver transplantation can be considered in OA patients who are severely affected with recurrent metabolic decompensation.[19] The benefits of transplantation must be considered versus the risks associated with organ transplantation, and the need for lifelong immunosuppression.[3,19]

Emerging studies suggest that liver transplantation is not curative for MMA and that a protein-restricted diet should be continued post-transplant with close monitoring of renal function.[60] It is still possible that neurological and renal damage remains irreversible in these patients.[61]

Preparation and monitoring

Patients with IMD are at risk of acute episodes of hyperammonaemia in response to triggers (see **Table 5**); therefore, patients and their parents should have a written day-to-day treatment plan and instructions ('emergency regimens') for how to manage emergencies.[3,19] Even mild illness without vomiting or diarrhoea can affect the patient's nutritional status, so parents should have instructions for an emergency feeding regimen to implement, along with additional fluids **(Table 9)**.[3,19]

Table 9. Emergency nutritional regimen for children with inherited metabolic disorders during mild illness.[3,19]

Age	Protein intake	Glucose polymer concentration as % of carbohydrate	Energy as kcal per 100 mL	Suggested daily intake (mL/kg)	Feeding frequency
Patients with primary hyperammonaemia caused by urea cycle defects[3]					
≤6 months	Stop all protein	10	40	150 mL/kg	2 to 3 hourly oral bolus day and night or continuous tube feeds using enteral feeding pump
7–12 months		10–15	40-60	120 mL/kg	
1 year		15	60	1200 mL	
2–9 years		20	80	Calculate[a]	
≥10 years		25	100	Calculate[a]	
Patients with secondary hyperammonaemia caused by organic acidurias[19b]					
<12 month	Stop or reduce total protein intake by ≥50% depending on illness severity	10	72	120–150 mL/kg	Continuous tube feeds using enteral feeding pump
1–2 years		15	105	1200 mL	
2–9 years		20	125	Calculate[a]	
≥10 years		25	145	Calculate[a]	

[a] For children weighing 11–20 kg: 100 mL/kg for the first 10 kg, plus 50 mL/kg for each kg thereafter; for children weighing >20 kg: 100 mL/kg for the first 10 kg, plus 50 mL/kg for the next 10 kg, plus 25 mL/kg thereafter, up to a maximum of 2500 mL/day.
[b] Fat emulsion (50%) may be given at dose of 3.5% fat for children up to 12 months of age, and 5% fat for those ≥1 year, but may not be well tolerated and can be omitted.

This regimen should only be used for short periods (i.e. 24–48 hours) because prolonged protein exclusion can lead to protein malnutrition and also stimulate catabolism. More serious illness or any signs of acute decompensation should prompt a visit to hospital, preferably one with a specialist metabolic team.[3,19] Regular follow-up and monitoring is essential, based on the patient's metabolic stability and adherence to treatment and dietary intervention, in order to prevent toxicity and secondary nutritional deficiency **(Table 10)**.[3,19] Magnetic resonance imaging (MRI) of the central nervous system (CNS) is indicated early on in any episode that results in coma or stroke-like symptoms, and then every two years thereafter.[3] Surgery or treatment for concomitant conditions should be carefully planned in consultation with the specialist metabolic team.

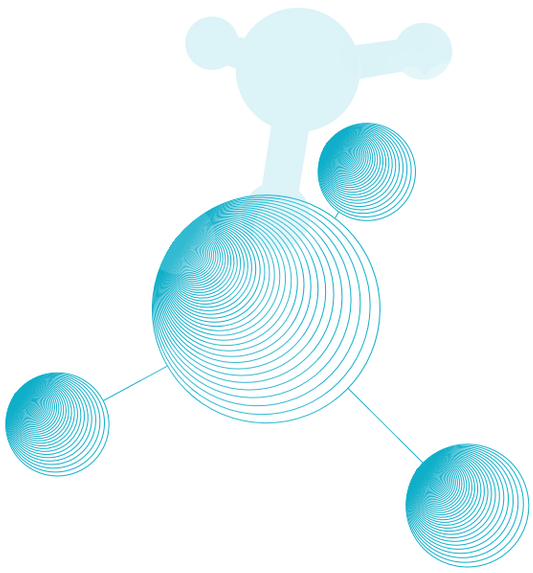


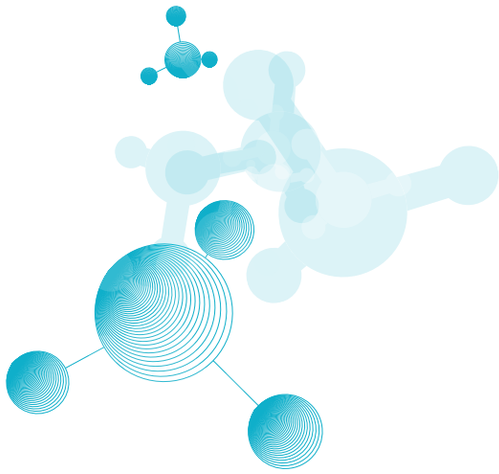
Table 10. Key aspects of monitoring and follow-up in patients with a urea cycle disorder or organic aciduria.[3,19]

UCD[3]	OA[19]
Metabolic status	
• Plasma ammonia • Plasma amino acid profile • Ketone bodies in urine	• Plasma ammonia • Blood gases • Lactate • Plasma amino acid profile (3–4 hours of fasting) • MMA in plasma and urine • Free carnitine in plasma or dried blood spot
Monitoring diet and nutritional status	
• Growth (head circumference, height, weight) • Diet history • Clinical examination: skin and hair, liver size • Vitamins (including vitamin B12), minerals, trace elements, ferritin, cholesterol, triglycerides and essential fatty acids	• Growth (length/height, weight, BMI) • Diet history • Clinical examination: skin and hair, liver size • Albumin, pre-albumin • Bone health (calcium, phosphorus, ALP, magnesium, PTH, vitamin D) • Full blood count, zinc, selenium, ferritin, folic acid, vitamin B12
Monitoring of long-term complications	
• Neurological status, cognitive function and IQ • Neuropsychological status (motor dexterity, nonverbal intelligence, visual memory, attention/ executive skills, verbal intelligence, memory, learning) • Health-related quality of life, anxiety, and stress (patient and family) • MRI for episodes involving coma or stroke-like symptoms • Liver size (clinical examination and ultrasound)	• Neurological examination, developmental milestones and IQ • Kidney function • Pancreatic function (amylase and lipase) • Cardiac function (ECG and echocardiography) • Ophthalmologic assessment • EEG, MRI and formal hearing test • Dentist/oral care

ALP, alkaline phosphatase; BMI, body mass index; ECG, electrocardiogram; EEG, electroencephalogram; IQ, intelligence quotient; MMA, methylmalonic acid; MRI, magnetic resonance imaging; OA, organic aciduria; PTH, parathyroid hormone; UCD, urea cycle defect.

Key points

- Nutritional management of protein intake is the key modality for the long-term management of patients with UCD or OA:
  - Patients should have coordinated specialist physician and dietician advice
  - Diet should be tailored to the patient's age, condition and ammonia level.
- Patients should be carefully monitored for adequate nutrition, and supplements of vitamins, minerals, and appropriate amino acids given as required.
- Patients with NAGS deficiency are treated with carglumic acid as maintenance therapy, which obviates the need for dietary protein restriction.
- Patients with MMA or PA may benefit from antibiotic therapy to reduce levels of propionyl CoA-producing bacteria in the gut.
- Patients should have a written 'emergency regimen' for how to manage acute episodes.
- Careful and regular follow-up is required to monitor metabolic status, nutritional status and for the presence of complications.
- Carefully selected patients with severe disease may benefit from liver transplantation.



ADDITIONAL RESOURCES

For more information about hyperammonaemia and IMDs, visit the following websites:

Orphanet: https://www.orpha.net/consor/cgi-bin/index.php

National Urea Cycle Disorders Foundation: http://www.nucdf.org/ucd.htm

Organic Acidemia Association: https://www.oaanews.org/

REFERENCES

1 • Haberle J. Clinical practice: the management of hyperammonemia. *Eur J Pediatr.* Jan 2011;170(1):21-34. doi:10.1007/s00431-010-1369-2

2 • Ah Mew N, Simpson KL, Gropman AL, Lanpher BC, Chapman KA, Summar ML. Urea cycle disorders. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, eds. *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2017.

3 • Haberle J, Boddaert N, Burlina A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis.* May 29 2012;7:32. doi:10.1186/1750-1172-7-32

4 • Daniotti M, la Marca G, Fiorini P, Filippi L. New developments in the treatment of hyperammonemia: emerging use of carglumic acid. *Int J Gen Med.* Jan 7 2011;4:21-28. doi:10.2147/IJGM.S10490

5 • Khan A, Ayub M, Khan WM. Hyperammonemia Is Associated with Increasing Severity of Both Liver Cirrhosis and Hepatic Encephalopathy. *Int J Hepatol.* 2016;2016:6741754. doi:10.1155/2016/6741754

6 • Burton BK. Inborn errors of metabolism in infancy: a guide to diagnosis. *Pediatrics.* Dec 1998;102(6):E69.

7 • Zschocke J, Hoffmann GF. Hyperammonemia. General clinical situations. 2011; http://evm.health2media.com/#/detail-view/generalsitu/0/2. Accessed 19 December, 2018.

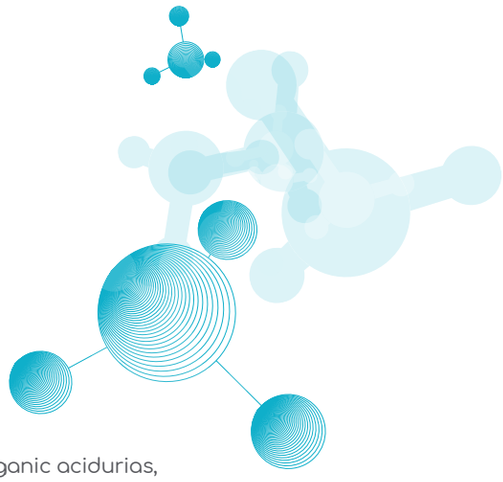
8 • Haberle J, Chakrapani A, Ah Mew N, Longo N. Hyperammonaemia in classic organic acidaemias: a review of the literature and two case histories. *Orphanet J Rare Dis.* Dec 6 2018;13(1):219. doi:10.1186/s13023-018-0963-7

9 • Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: homocystinuria, organic acidurias, and urea cycle disorders. *Arch Neurol.* Feb 2010;67(2):148-153. doi:10.1001/archneurol.2009.333

10 • Orphanet. Hyperammonemia due to N-acetylglutamate synthase deficiency. 2007; https://www.orpha.net/consor/www/cgi-bin/OC_Exp.php?lng=EN&Expert=927. Accessed October 23, 2018.

11 • Saheki T, Song Y-Z. Citrin deficiency. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, eds. *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2017.

12 • Orphanet. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. 2015; https://www.orpha.net/consor/cgi-bin/Disease_Search.php?lng=EN&data_id=770&Disease_Disease_Search_diseaseGroup=415&Disease_Disease_Search_diseaseType=ORPHA&Disease(s)/group%20of%20diseases=Hyperornithinemia-hyperammonemia-homocitrullinuria-syndrome&title=Hyperornithinemia-hyperammonemia-homocitrullinuria%20syndrome&search=Disease_Search_Simple. Accessed September 20th, 2019.



REFERENCES

13 • Dionisi-Vici C, Deodato F, Roschinger W, Rhead W, Wilcken B. 'Classical' organic acidurias, propionic aciduria, methylmalonic aciduria and isovaleric aciduria: long-term outcome and effects of expanded newborn screening using tandem mass spectrometry. J Inherit Metab Dis. Apr-Jun 2006;29(2-3):383-389. doi:10.1007/s10545-006-0278-z

14 • Kolker S, Burgard P, Sauer SW, Okun JG. Current concepts in organic acidurias: understanding intra- and extracerebral disease manifestation. *J Inherit Metab Dis.* Jul 2013;36(4):635-644. doi:10.1007/s10545-013-9600-8

15 • Vaidyanathan K, Narayanan MP, Vasudevan DM. Organic acidurias: an updated review. *Indian J Clin Biochem.* Oct 2011;26(4):319-325. doi:10.1007/s12291-011-0134-2

16 • Organic Acidemia Association. Current Research on Organic Acidemias: Organic Acidemia Association; 2007. Accessed September 20th, 2019.

17 • Schiff M, Ogier de Baulny H, Dionisi-Vici C. Branched-chain organic acidurias/acidaemias. In: Saudubray J-M, Baumgartner M, Water J, eds. *Inborn Metabolic Diseases: Diagnosis and Treatment.* 6th edition ed. Berlin: Springer 2016:277-294.

18 • Vianey-Saban C, Acquaviva-Bourdain C, Levrat V, Boyer S, Forest I, Fouilhoux A. Role of N-carbamylglutamate in undiagnosed neonatal hyperammonaemia. In: Bachmann C, Haberle J, Leonard J, eds. *Pathophysiology and management of hyperammonaemia.* Heilbron: SPS Publications; 2006:114-128.

19 • Baumgartner MR, Horster F, Dionisi-Vici C, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. *Orphanet J Rare Dis.* Sep 2 2014;9:130. doi:10.1186/s13023-014-0130-8

20 • Vockley J, Ensenauer R. Isovaleric acidemia: new aspects of genetic and phenotypic heterogeneity. *Am J Med Genet C Semin Med Genet.* May 15 2006;142C(2):95-103. doi:10.1002/ajmg.c.30089

21 • Huergo LF, Dixon R. The Emergence of 2-Oxoglutarate as a Master Regulator Metabolite. *Microbiol Mol Biol Rev.* Dec 2015;79(4):419-435. doi:10.1128/MMBR.00038-15

22 • Cooper AJ. The role of glutamine synthetase and glutamate dehydrogenase in cerebral ammonia homeostasis. *Neurochem Res.* Nov 2012;37(11):2439-2455. doi:10.1007/s11064-012-0803-4

23 • Filipowicz HR, Ernst SL, Ashurst CL, Pasquali M, Longo N. Metabolic changes associated with hyperammonemia in patients with propionic acidemia. *Mol Genet Metab.* Jun 2006;88(2):123-130. doi:10.1016/j.ymgme.2005.11.016

24 • Ierardi-Curto L, Kaplan P, Saitta S, Mazur A, Berry GT. The glutamine paradox in a neonate with propionic acidaemia and severe hyperammonaemia. *J Inherit Metab Dis.* Feb 2000;23(1):85-86.

25 • Gebhardt B, Vlaho S, Fischer D, Sewell A, Bohles H. N-carbamylglutamate enhances ammonia detoxification in a patient with decompensated methylmalonic aciduria. *Mol Genet Metab.* Aug 2003;79(4):303-304.

26 • Mochel F, DeLonlay P, Touati G, et al. Pyruvate carboxylase deficiency: clinical and biochemical response to anaplerotic diet therapy. *Mol Genet Metab.* Apr 2005;84(4):305-312. doi:10.1016/j.ymgme.2004.09.007

27 • Roe CR, Sweetman L, Roe DS, David F, Brunengraber H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. *J Clin Invest.* Jul 2002;110(2):259-269. doi:10.1172/JCI15311

28 • Guffon N, Schiff M, Cheillan D, Wermuth B, Haberle J, Vianey-Saban C. Neonatal hyperammonemia: the N-carbamoyl-L-glutamic acid test. *J Pediatr.* Aug 2005;147(2):260-262. doi:10.1016/j.jpeds.2005.04.059

29 • Yap S, Gougeard N, Hart AR, Barcelona B, Rubio V. N-carbamoylglutamate-responsive carbamoyl phosphate synthetase 1 (CPS1) deficiency: A patient with a novel CPS1 mutation and an experimental study on the mutation's effects. *JMID Reports.* 2019;48:36-44. doi:10.1002/jmd2.12034

30 • Fryburg DA, Jahn LA, Hill SA, Oliveras DM, Barrett EJ. Insulin and insulin-like growth factor-I enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. *J Clin Invest.* Oct 1995;96(4):1722-1729. doi:10.1172/JCI118217

31 • Wolfe RR. Regulation of skeletal muscle protein metabolism in catabolic states. Curr *Opin Clin Nutr Metab Care.* Jan 2005;8(1):61-65.

32 • Singh RH. Nutritional management of patients with urea cycle disorders. *J Inherit Metab Dis.* Nov 2007;30(6):880-887. doi:10.1007/s10545-007-0718-4

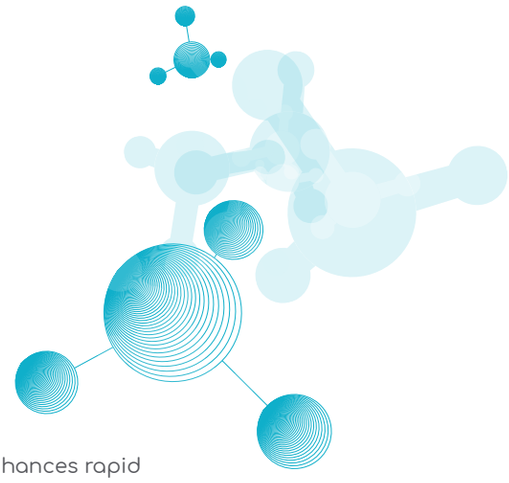
33 • Ikizler TA, Pupim LB, Brouillette JR, et al. Hemodialysis stimulates muscle and whole body protein loss and alters substrate oxidation. *Am J Physiol Endocrinol Metab.* Jan 2002;282(1):E107-116. doi:10.1152/ajpendo.2002.282.1.E107

34 • De Las Heras J, Aldamiz-Echevarria L, Martinez-Chantar ML, Delgado TC. An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease. *Expert Opin Drug Metab Toxicol.* Apr 2017;13(4):439-448. doi:10.1080/17425255.2017.1262843

35 • European Medicines Agency. Carbaglu (carglumic acid). Summary of product characteristics. 2011; https://www.ema.europa.eu/documents/product-information/carbaglu-epar-product-information_en.pdf. Accessed September 20th, 2019.

36 • Ah Mew N, McCarter R, Daikhin Y, Nissim I, Yudkoff M, Tuchman M. N-carbamylglutamate augments ureagenesis and reduces ammonia and glutamine in propionic acidemia. *Pediatrics.* Jul 2010;126(1):e208-214. doi:10.1542/peds.2010-0008

37 • Filippi L, Gozzini E, Fiorini P, Malvagia S, la Marca G, Donati MA. N-carbamylglutamate in emergency management of hyperammonemia in neonatal acute onset propionic and methylmalonic aciduria. *Neonatology.* 2010;97(3):286-290. doi:10.1159/000255168



REFERENCES

38 • Valayannopoulos V, Baruteau J, Delgado MB, et al. Carglumic acid enhances rapid ammonia detoxification in classical organic acidurias with a favourable risk-benefit profile: a retrospective observational study. *Orphanet J Rare Dis.* Mar 31 2016;11:32. doi:10.1186/s13023-016-0406-2

39 • Al Kaabi EH, El-Hattab AW. N-acetylglutamate synthase deficiency: Novel mutation associated with neonatal presentation and literature review of molecular and phenotypic spectra. *Mol Genet Metab Rep.* Sep 2016;8:94-98. doi:10.1016/j.ymgmr.2016.08.004

40 • Reigstad H, Woldseth B, Haberle J. Normal Neurological Development During Infancy Despite Massive Hyperammonemia in Early Treated NAGS Deficiency. *JIMD Rep.* 2017;37:45-47. doi:10.1007/8904_2017_13

41 • Van Leynseele A, Jansen A, Goyens P, et al. Early treatment of a child with NAGS deficiency using N-carbamyl glutamate results in a normal neurological outcome. *Eur J Pediatr.* Dec 2014;173(12):1635-1638. doi:10.1007/s00431-013-2205-2

42 • Levrat V, Forest I, Fouilhoux A, Acquaviva C, Vianey-Saban C, Guffon N. Carglumic acid: an additional therapy in the treatment of organic acidurias with hyperammonemia? *Orphanet J Rare Dis.* Jan 30 2008;3:2. doi:10.1186/1750-1172-3-2

43 • Yap S, Leong HY, Abdul Aziz F, et al. N-Carbamylglutamate Is an Effective Treatment for Acute Neonatal Hyperammonaemia in a Patient with Methylmalonic Aciduria. *Neonatology.* 2016;109(4):303-307. doi:10.1159/000443630

44 • Fernandez de Miguel S, Gimeno Diaz de Atauri A, Torres Peral R, Fernandez Carrion F, Serrano Ayestaran O. [N-carbamylglutamate treatment in hyperammoniemia decompensated propionic acidaemia]. *An Pediatr (Barc).* Dec 2009;71(6):579-580. doi:10.1016/j.anpedi.2009.08.007

45 • Jones S, Reed CA, Vijay S, Walter JH, Morris AA. N-carbamylglutamate for neonatal hyperammonaemia in propionic acidaemia. *J Inherit Metab Dis.* Dec 2008;31 Suppl 2:S219-222. doi:10.1007/s10545-008-0777-1

46 • Schwahn BC, Pieterse L, Bisset WM, Galloway PG, Robinson PH. Biochemical efficacy of N-carbamylglutamate in neonatal severe hyperammonaemia due to propionic acidaemia. *Eur J Pediatr.* Jan 2010;169(1):133-134. doi:10.1007/s00431-009-1036-7

47 • Soyucen E, Demirci E, Aydin A. Outpatient treatment of propionic acidemia-associated hyperammonemia with N-carbamoyl-L-glutamate in an infant. *Clin Ther.* Apr 2010;32(4):710-713. doi:10.1016/j.clinthera.2010.04.004

48 • Kasapkara CS, Ezgu FS, Okur I, Tumer L, Biberoglu G, Hasanoglu A. N-carbamylglutamate treatment for acute neonatal hyperammonemia in isovaleric acidemia. *Eur J Pediatr.* Jun 2011;170(6):799-801. doi:10.1007/s00431-010-1362-9

49 • Chakrapani A, Valayannopoulos V, Segarra NG, et al. Effect of carglumic acid with or without ammonia scavengers on hyperammonaemia in acute decompensation episodes of organic acidurias. *Orphanet J Rare Dis.* 2018;13(1):97.

50 • Singh RH, Acosta PB, Kennedy MJ, Longo N, Elsas LJ. Potassium retention in patients treated for argininosuccinate lyase deficiency. *J Inherit Metab Dis.* 2006;29(Suppl. 1):48.

51 • Thompson GN, Chalmers RA. Increased urinary metabolite excretion during fasting in disorders of propionate metabolism. *Pediatr Res.* Apr 1990;27(4 Pt 1):413-416. doi:10.1203/00006450-199004000-00021

52 • Evans M, Truby H, Boneh A. The Relationship between Dietary Intake, Growth, and Body Composition in Inborn Errors of Intermediary Protein Metabolism. *J Pediatr.* Sep 2017;188:163-172. doi:10.1016/j.jpeds.2017.05.048

53 • Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements (1981: Rome I, Food and Agriculture Organization of the United Nations, World Health Organization & United Nations University, *Energy and protein requirements : report of a Joint FAO/WHO/UNU Expert Consultation [held in Rome from 5 to 17 October 1981].* Geneva: World Health Organization;1985.

54 • Gessler P, Buchal P, Schwenk HU, Wermuth B. Favourable long-term outcome after immediate treatment of neonatal hyperammonemia due to N-acetylglutamate synthase deficiency. *Eur J Pediatr.* Feb 2010;169(2):197-199. doi:10.1007/s00431-009-1006-0

55 • Nasser M, Javaheri H, Fedorowicz Z, Noorani Z. Carnitine supplementation for inborn errors of metabolism. *Cochrane Database Syst Rev.* Apr 15 2009(2):CD006659. doi:10.1002/14651858.CD006659.pub2

56 • Burlina A, Cazzorla C, Zanonato E, Viggiano E, Fasan I, Polo G. Clinical experience with N-carbamylglutamate in a single-centre cohort of patients with propionic and methylmalonic aciduria. *Mol Genet Metab Rep.* Sep 2016;8:34-40. doi:10.1016/j.ymgmr.2016.06.007

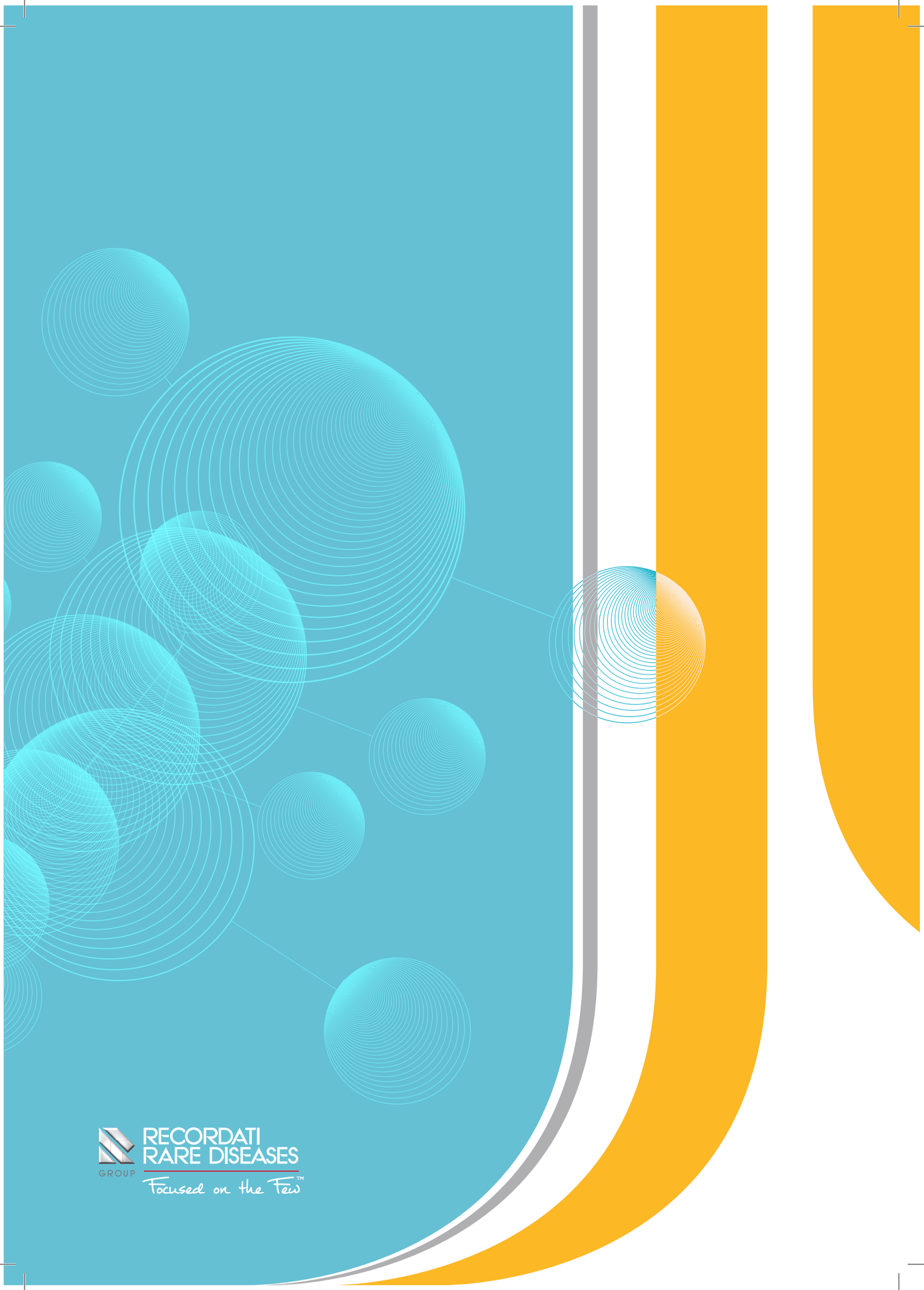
57 • Tummolo A, Melpignano L, Carella A, et al. Long-term continuous N-carbamylglutamate treatment in frequently decompensated propionic acidemia: a case report. *J Med Case Rep.* Apr 22 2018;12(1):103. doi:10.1186/s13256-018-1631-1

58 • National Institute of Health. Carglumic Acid in Methylmalonic Acidemia and Propionic Acidemia (CAMP). 2018; https://clinicaltrials.gov/ct2/show/NCT02426775 Accessed 21 December, 2018.

59 • Blair H. Carglumic acid in hyperammonaemia due to organic acidurias: a profle of its use in the EU. *Drugs & Therapy Perspectives.* 2019;35(3):101-108. doi:10.1007/s40267-018-00595-4

60 • Sakamoto R, Nakamura K, Kido J, et al. Improvement in the prognosis and development of patients with methylmalonic acidemia after living donor liver transplant. *Pediatr Transplant.* Dec 2016;20(8):1081-1086. doi:10.1111/petr.12804

61 • Jiang YZ, Sun LY. The Value of Liver Transplantation for Methylmalonic Acidemia. *Front Pediatr.* 2019;7:87. doi:10.3389/fped.2019.00087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