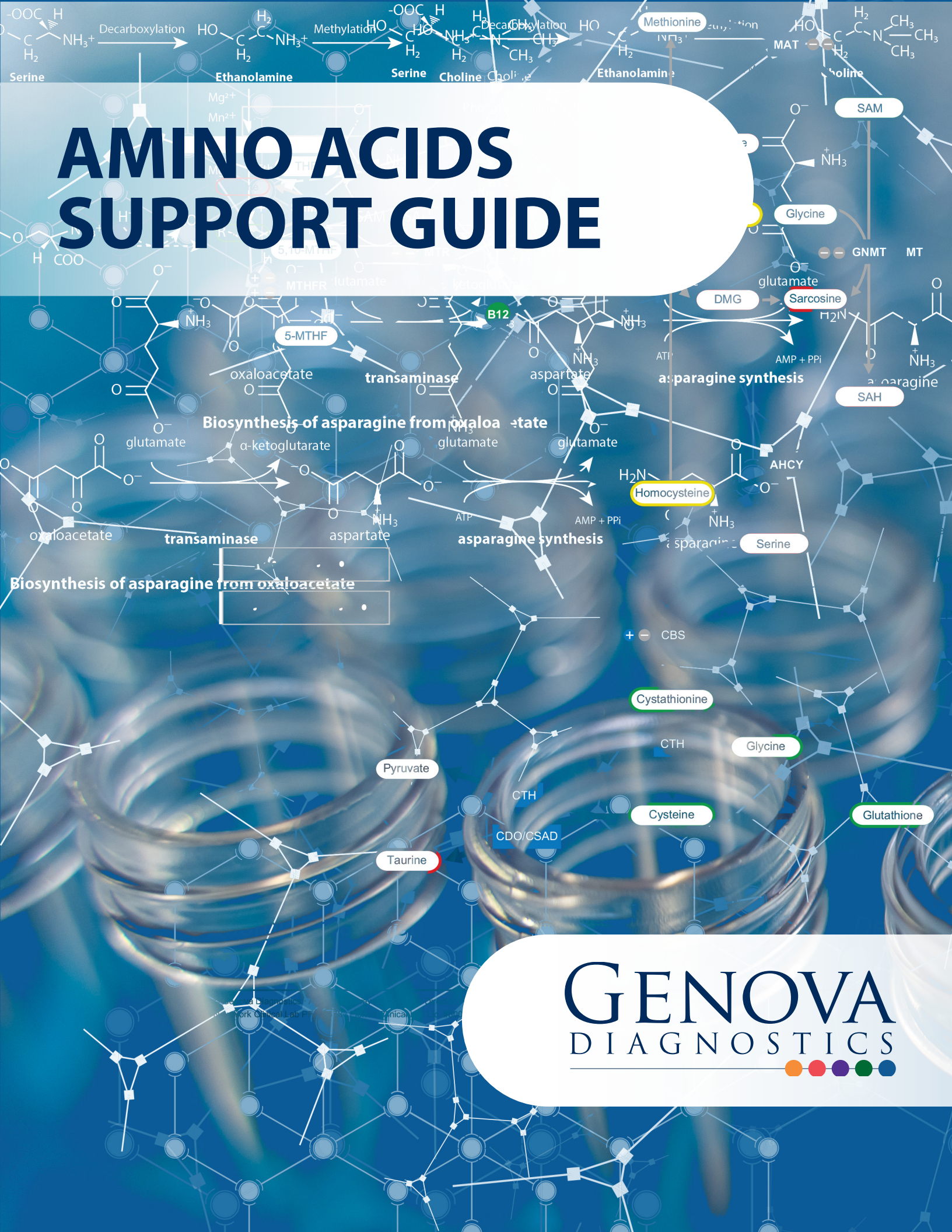


AMINO ACIDS SUPPORT GUIDE



GENOVA
DIAGNOSTICS

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Amino Acids Analysis

The **Amino Acids Analysis** measures essential and nonessential amino acids, intermediary metabolites involved in protein metabolism, and dietary peptide related markers. Amino acids are important building blocks for every cell and system in the body and require specific nutrients for metabolism and utilization. The report includes personalized amino acid recommendations based on amino acid levels, and functional vitamin and mineral cofactor recommendations based on amino acid metabolism. These nutrient need suggestions are synthesized depending on the patients' amino acid results, taking into account the age/gender of the patient and the severity of abnormal findings.

The Amino Acids Analysis Includes:

- **Essential Amino Acids** must be derived from dietary sources
- **Nonessential Amino Acids** are dietary or synthesized by the body
- **Intermediary Metabolites** are byproducts of amino acid metabolism
- **B-Vitamin Markers** are involved in biochemical reactions that specifically require B-vitamins
- **Urea Cycle Markers** are byproducts associated with nitrogen detoxification
- **Glycine/Serine Metabolites** are involved in the serine-to-choline pathway as well as methylation pathways
- **Dietary Peptide Related Markers** can indicate incomplete protein breakdown

Physiologic Importance and Patient Population:

Amino acids play many important roles in the body including energy generation, neurotransmitter and hormone synthesis, tissue growth and repair, immune function, blood cell formation, maintenance of muscle mass, and detoxification.

Testing is important in a variety of clinical scenarios including:

- Mood disorders¹
- Weight issues/Dietary guidance^{2,3}
- Malnutrition (often observed in the elderly or those with poor protein intake)^{4,5}
- Gut maldigestion/malabsorption
- Fatigue⁶⁻⁹
- Athletic optimization^{10,11}
- Increased nutrient demand in physical trauma/healing^{8,12}
- Kidney disease¹³
- Liver disease
- Obesity/Insulin resistance/Type 2 Diabetes^{14,15}
- Autism¹⁶⁻¹⁸

Diet and lifestyle factors, as well as certain clinical conditions, may predispose a person to having amino acid imbalances. There are multiple dynamic factors that influence amino acid

levels including dietary intake, liver and kidney function, protein metabolism, hormones, stress, exercise, and gastrointestinal health.¹⁹

There are amino acid abnormalities seen with various inborn errors of metabolism. Genova's amino acid reference ranges were not designed to be used for the diagnosis of inborn errors of metabolism; these are generally diagnosed in infancy. In fact, amino acid testing is not recommended for patients under 2 since Genova does not have reference ranges for this population.

Plasma Versus First Morning Void (FMV) Urine Amino Acids

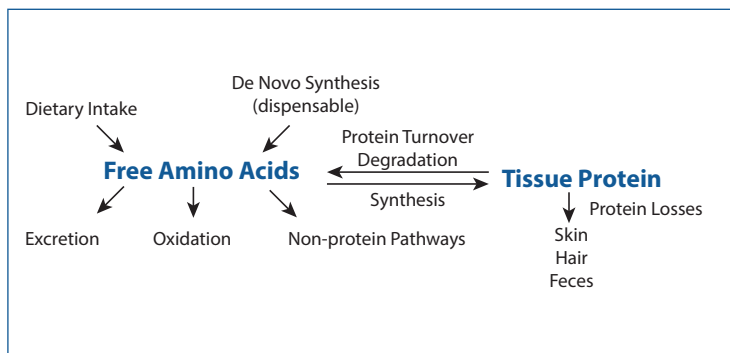
Different analytes are measurable in blood versus urine and selection of sample type depends on the clinical concern. Recent food intake briefly increases plasma amino acid levels, which is why a fasting sample is recommended. Short-term fasting does not result in depletion of plasma amino acids, but long-term malnutrition does. Many studies show a good correlation between plasma and urine amino acids. The key differences between plasma and urine amino acids are summarized below.^{13,19}

Plasma Amino Acids (Fasting)	Urine Amino Acids (First Morning Void)
Fasting sample represents "steady state" pool of amino acids; not affected by short-term diet fluctuations	Represents recent dietary intake and metabolism – more variable compared to plasma
36 analytes	40 analytes
Useful for mood disorders, or uncontrolled and fluctuating diets	Useful for controlled diets, or to assess the effects of a recent dietary change
Amino acid levels not influenced by abnormal kidney function; preferred if patient has proteinuria	Amino acid levels influenced by abnormal kidney function; urine testing dependent on healthy kidney function (biomarkers ratioed to urine creatinine)
Requires a blood draw	Ideal for children or adults averse to blood draws; urine conveniently collected at home

A urine creatinine concentration is part of every FMV analysis. All urinary biomarkers are ratioed to the creatinine concentration for standardization.

What Is an Amino Acid?

Amino acids are single unit building blocks that form protein. Amino acids contain a carboxyl group, an amino nitrogen group, and a side chain attached to a central alpha carbon. Functional differences between the amino acids lie in the structure of their side chains. Long chains of amino acids make up peptides and proteins which form the major structural and functional components of all cells in the body. Dietary protein must be digested into smaller peptides or individual amino acids to be absorbed, where they are then individually used by the body or synthesized into larger proteins. Essential amino acids must come from the diet, whereas nonessential amino acids can be synthesized by the body. The free amino acid pool is in constant flux and the diagram below illustrates the variables involved in protein metabolism.²⁰



Factors That Influence Amino Acid Levels:

- Dietary protein intake
- Amino acid composition
- Protein digestibility
- GI tract digestion and absorption
- Protein demand

Dietary Protein Intake

Adequate protein intake is essential for overall health. Protein and amino acid requirements change throughout the lifecycle. The recommended daily allowance (RDA) of protein is currently 0.8g/kg for the generally healthy adult population. Higher levels are required in cases of higher demand.²¹

Protein and amino acids consumed or supplemented in excess are degraded and excreted as urea. The keto acids left after removal of the amino groups are utilized as energy sources or converted to carbohydrate or fat.²²

Amino Acid Composition

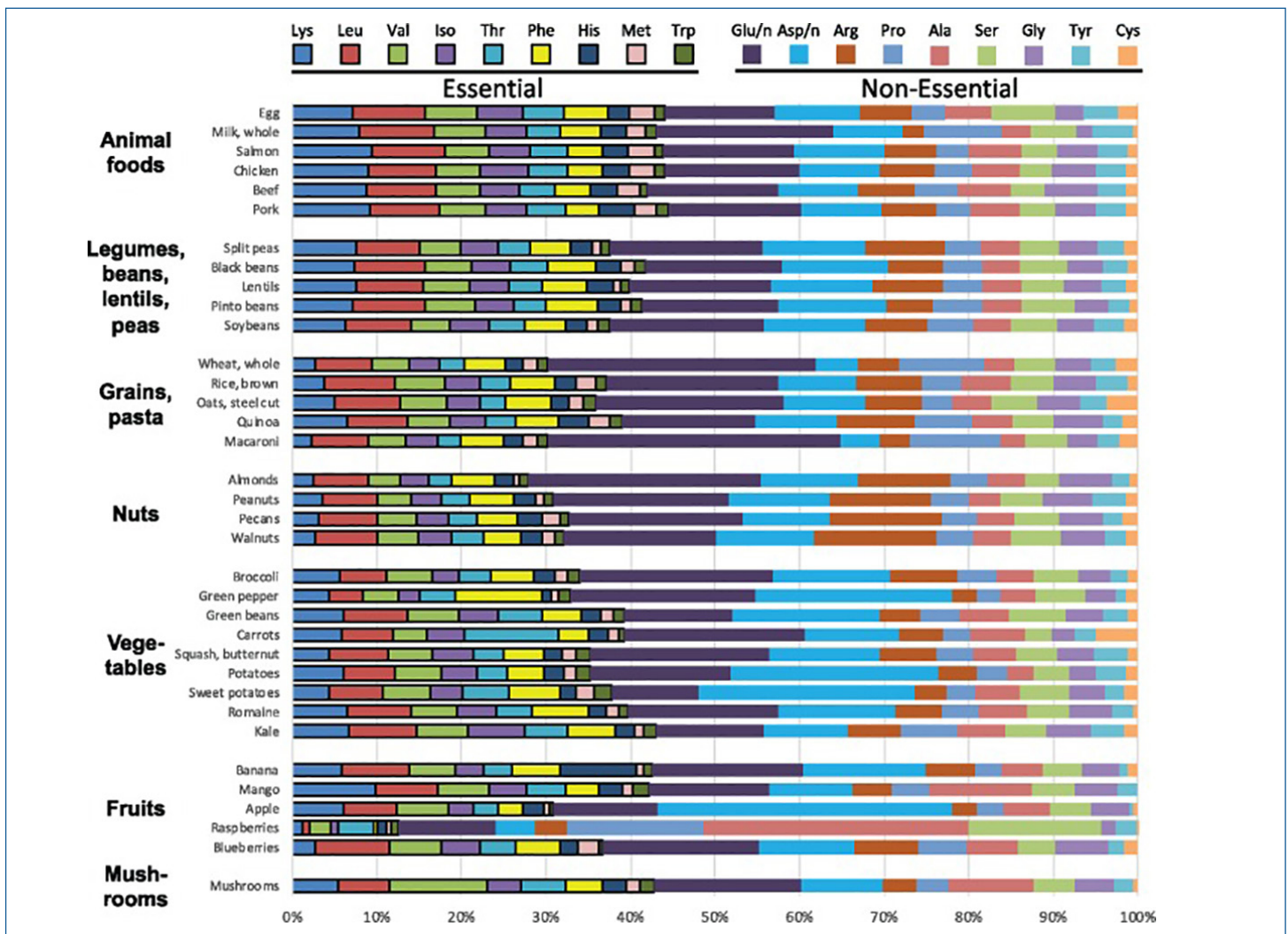
Protein-containing foods do not contain amino acids in equal proportions; however, all 20 dietary amino acids can be found in both plant and animal foods.²¹ If the diet is inadequate in any essential amino acid, protein synthesis cannot proceed

beyond the rate at which that amino acid is available. These essential amino acids that do not meet the minimal human requirement are called 'limiting' amino acids. This can be problematic in vegan or vegetarian diets. A diet based on a single plant food staple may not provide enough of certain amino acids and needs to be combined with other plants that provide the limiting amino acid(s). For example, most grains are good sources of methionine but contain very little lysine. Alternatively, legumes are high in lysine and low in methionine. Combining grains with legumes, grains with dairy, or legumes with seeds can provide all essential amino acids in adequate quantities. It is not necessary to eat all of the complementary amino acids in a single meal, though for optimal health they should be consumed within a day.²³

Animal-derived products generally provide the essential amino acids in ratios needed to sustain growth and metabolic processes.²⁴ Therefore, when food access is limited, animal foods provide better protein adequacy than plants. With that, a varied and diverse diet should adequately meet the daily protein requirement.²¹

Figure 1 provides a colorimetric representation of proportions of amino acids in 35 common plant and animal foods.²¹

FIGURE 1



Protein Digestibility

It is possible that a protein source has an excellent amino acid profile, but poor digestibility.²⁴ This may be due to the specific food source or how it is prepared. Modern cooking practices meant for convenience, safety, extended shelf life, and improved taste can in some cases decrease the digestibility of a food. Other processing techniques however, might increase digestibility, depending on the food.²⁵

Some examples include:

Animal protein is more easily digested than plants; plant cell walls are less susceptible to digestive enzymes.

- Antinutritional factors (ANF) in plants include phytates, enzyme inhibitors, polyphenols, tannins, lectins and non-starch polysaccharides. These can affect both the digestibility and bioavailability of protein and amino acids.²⁵
 - » In general, soaking, cooking, fermenting, and sprouting things like grains, legumes, and seeds has been shown to decrease ANF and lead to better digestibility of plant foods.^{24,25}

- Some plants contain enzymes which interfere with protein digestion and must be heat inactivated (i.e. soybeans contain trypsinase, which inactivates the protein-digesting enzyme, trypsin).²³
- Under severe heating conditions including smoking and broiling, all amino acids in food proteins become somewhat resistant to digestion.²³
- Mild heat treatment, in the presence of reducing sugars such as glucose and galactose, causes a loss of available lysine. This is referred to as the Maillard reaction. It can happen in foods such as skim milk, which can be heated to form milk powder. The Maillard reaction produces the characteristic browning for flavor in meats and other foods.^{23,25}
- Exposure to sulfur dioxide (a food preservative) and other oxidative conditions can result in loss of methionine.²³

The World Health Organization (WHO) and U.S. Food and Drug Administration (FDA) have adopted the 'protein digestibility corrected amino acid score' (PDCAAS) as the preferred method for assessing protein quality in human nutrition. The highest score a food can receive is 1- which indicates adequate levels and ratios of amino acids, as well as high protein digestibility. Some examples of foods receiving a score of 1 include milk and eggs. This indicates superior value, as compared to soy at 0.91, beef 0.92, wheat 0.42 and sorghum at 0.20. Wheat receives a low score because it is deficient in the essential amino acid lysine, while sorghum is even lower because it is poorly digestible.²³⁻²⁵

GI Tract Digestion and Absorption

Protein digestion and absorption are dependent on both the condition of the GI tract, as well as the digestibility of the protein-containing food.

In the stomach, hydrochloric acid denatures dietary protein, preparing it for enzymatic digestion.²⁶ The low stomach pH activates gastric pepsin. Pepsin then initiates protein digestion while stimulating cholecystokinin release, a step that is crucial to the secretion of pancreatic enzymes. Enterokinase, a brush border enzyme, then activates trypsin which then converts many pancreatic proteases to their active forms. Active pancreatic enzymes hydrolyze proteins into oligopeptides and amino acids, which are then absorbed by enterocytes.^{24,27}

Within the small intestine, amino acids, di-, and tripeptides are absorbed at different rates in different sections. Although the small intestine is the principal site of protein absorption, the colon does possess a capacity to absorb protein. Undigested or unabsorbed protein and amino acids can be fermented by the gut microbiota to form short chain fatty acids and amines which have biologic activity.^{24,28}

Low levels of amino acids with adequate dietary protein intake may prompt evaluation of the GI tract:

- Hydrochloric acid and pancreatic protease availability
 - » Assess use of acid-blocking medications
 - » Assess for pancreatic insufficiency (stool pancreatic elastase 1, chymotrypsin)²⁷
- Decreased absorptive surface area
 - » Assess for SIBO, celiac, IBD, surgery and other conditions that damage the GI tract or affect absorption²⁹

Protein Demand

Systemic demands for protein utilization might result in lower measurable amino acid levels, even with adequate protein intake. Protein can be used as an energy source at rate of 4kcal/g. Protein demands can be increased in wound healing, trauma, athletic performance, pregnancy, lactation, child and adolescent growth or development, and various conditions in the elderly.^{21,25}

Low carbohydrate diets can also increase protein demand and deplete amino acids. When the diet is low in carbohydrates or the individual is starving, the carbon skeletons of amino acids can be used to produce glucose in gluconeogenesis. These are called glucogenic amino acids. (Lysine and threonine are the only two amino acids that are not glucogenic.²³) Therefore, protein requirements may increase with low carbohydrate diets.²⁶

Essential Amino Acids

Essential amino acids must be derived from the diet and cannot be synthesized by the body. Some amino acids are semi-essential, or conditionally essential, meaning they can be synthesized in the human body in a certain developmental stage or in healthy states. Conditionally essential amino acids are needed more in times of illness and stress.³⁰

Of the 20 amino acids commonly found in proteins, 9 are considered essential for humans including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Two conditionally essential amino acids are also included: arginine and taurine.

Arginine

Arginine is found in all protein foods and is very abundant in seeds and nuts. It is considered a semi-essential amino acid during early development, infection/inflammation, or renal and/or intestinal impairment.³¹ It has many functions in the body including³¹⁻³⁶

- ammonia disposal in the urea cycle
- immune function
- stimulation of insulin release
- muscle metabolism (creatine/creatinine precursor)
- nitric oxide (NO) formation
- glutamic acid and proline formation
- glucose/glycogen conversion
- stimulation of the release of growth hormone, vasopressin, and prolactin
- wound healing

Because arginine is a precursor for nitric oxide synthesis, it is often used therapeutically in cardiovascular disease for its vasodilatory effects.³⁷

High Levels

A diet high in arginine, or exogenous supplementation with arginine or citrulline can elevate arginine levels.^{38,39} Levels might also be elevated in manganese (Mn) insufficiency since Mn is a necessary cofactor in the conversion of arginine to ornithine (and urea) in the urea cycle.^{40,41} Lastly, there is some literature to suggest that vitamin B₆ supplementation alters plasma amino acids resulting in increased arginine.⁴²

Low Levels

A low protein diet, gastrointestinal dysfunction, and increased amino acid utilization in acute phases of critical illness can all contribute to deficient arginine.⁴³ However, some chronic conditions, such as type 2 diabetes, are characterized by an

increase in the enzyme arginase, which can subsequently result in plasma arginine deficiency.⁴⁴

Clinically, arginine deficiency has been shown to contribute to increased susceptibility to infection, pulmonary hypertension, atherosclerosis, and impaired anti-tumor response.⁴⁵

Histidine

Histidine is a semi-essential amino acid which is formed in the breakdown of carnosine. Red meat is a common source of carnosine, and therefore histidine.⁴⁶ Other food sources include poultry, fish, nuts, seeds, and grains.

Histidine and histamine have a unique relationship. The amino acid histidine becomes histamine via a vitamin B₆-dependent enzyme called histidine decarboxylase.⁴⁷ With this, decreased amounts of histidine and insufficient vitamin B₆ can subsequently lead to a decrease in histamine concentration. This may impair digestion, since histamine binds to H₂ receptors located on the surface of parietal cells to stimulate gastric acid secretion, necessary for protein breakdown.⁴⁸

Histidine also inhibits the production of proinflammatory cytokines by monocytes and is therefore anti-inflammatory and antioxidant.⁴⁸⁻⁵⁰ With these beneficial effects, histidine supplementation has been shown to improve insulin resistance, reduce BMI, suppress inflammation, and lower oxidative stress in obese women with metabolic syndrome.⁵¹

Interestingly, histidine can also be broken down to form urocanic acid in the liver and skin. Urocanic acid absorbs UV light and is thought to act as a natural sunscreen.⁵²

High Levels

High levels of histidine are seen in high protein diets. And, as outlined above, vitamin B₆ is needed to convert histidine to histamine, therefore a functional need for vitamin B₆ may elevate levels of histidine.⁴⁷

There is also a relationship between histidine and folate metabolism. Histidine metabolizes to glutamic acid with FIGLU as an intermediary and tetrahydrofolate as a cofactor. Therefore, elevated histidine can be seen with vitamin B₁₂ and folate insufficiencies. Urinary levels have been shown to normalize with folate administration and plasma levels have been altered in supplementation with vitamin B₁₂.⁵³⁻⁵⁶

Lastly, there is a rare inborn error of metabolism involving impairment of histidase, which breaks down histamine and causes elevated histidine.

Low Levels

Low levels of essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.^{48,57}

Low histidine levels are clinically significant because as outlined above, histidine converts to histamine. Deficient histidine can contribute to gastric hypochlorhydria. This gastrointestinal dysfunction can, in turn, perpetuate histidine deficiency and therefore impair all protein digestion.⁴⁸ Low histidine has been reported in rheumatoid arthritis, chronic kidney disease, and cholecystitis.⁴⁹

Branched Chain Amino Acids - Isoleucine, Leucine, Valine

Branched Chain Amino Acids (Isoleucine, Leucine, Valine)

Isoleucine, leucine and valine are the three branched chain amino acids (BCAAs). Branched chain amino acids (BCAA) are essential amino acids and must be obtained from the diet (mainly meat, grains, and dairy).⁵⁸

Not only do the BCAAs account for almost 50% of muscle protein, but they have many metabolic functions.⁵⁹ BCAAs act as substrates for protein synthesis, energy production, neurotransmitter production, glucose metabolism, and the immune response. They are also involved in stimulation of albumin and glycogen synthesis, improvement of insulin resistance, inhibition of free radical production, and hepatocyte apoptosis with liver regeneration.^{60,61}

Unlike most amino acids, the initial step of BCAA metabolism does not take place in the liver. After dietary intake, BCAAs remain in circulation and are taken up by skeletal muscle, the heart, kidney, diaphragm, and adipose tissue for immediate metabolism. BCAAs are transaminated into α -keto acids and used within the tissues or released into circulation. The liver and other organs can then further catabolize these α -keto acids.⁶² The complete oxidation of valine yields succinyl CoA, and leucine and isoleucine produce acetyl CoA for use in the citric acid cycle. Isoleucine also produces propionyl CoA and succinyl CoA.⁶²

Skeletal muscle is a major site of BCAA utilization. During exercise, catabolism of the BCAAs is elevated; β -aminoisobutyric acid (β -AIB) is a metabolite of valine released during exercise which is evaluated in the B-Vitamin Marker section below.⁶³ There is much published literature on the use of BCAAs for muscle protein synthesis, however it's been shown that BCAA supplementation alone does not enhance muscle protein synthesis better than the consumption of a complete, high quality food protein containing the full spectrum of essential amino acids.

Of the three BCAA, leucine may have the most immediate impact. Leucine is one of the few amino acids that is completely oxidized in the muscle for energy, generating more ATP molecules than glucose. Additionally, leucine can be used to synthesize fatty acids in adipose tissue, and generates HMG CoA, an intermediate in the synthesis of cholesterol. Leucine also stimulates insulin secretion and promotes protein synthesis in the liver, muscle, and skin.^{62,64}

BCAAs, grouped in patterns and as single biomarkers, have been studied as predictors of obesity, insulin resistance, type 2 diabetes, metabolic syndrome, and cardiovascular disease outcomes.^{63,65,66}

High Levels

High protein intake may elevate BCAAs. In the catabolism of BCAAs, branched chain aminotransferase and branched chain alpha ketoacid dehydrogenase complex (BCKDC) require several cofactors such as vitamin B₆, vitamin B₁, and lipoic acid. Therefore, functional need for these cofactors may contribute to high levels of BCAAs.^{54,67-72}

Lastly, BCAAs can be elevated due to a rare inborn error of metabolism. Maple Syrup Urine Disease is an inherited disorder of branched chain amino acid metabolism due to deficiency of the BCKDC complex.⁷³

Low Levels

Low levels of essential BCAA may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.^{74,75}

Low levels of leucine can be seen after significant aerobic exercise or strength training.⁶⁴

Supplementation with zinc, vitamin B₃, and vitamin B₆, has improved outcomes in various conditions where low levels of BCAA's have been associated.^{60,76-78}

Lysine

Lysine is a nutritionally essential amino acid abundant in meat, fish, fowl, and legumes and is needed for formation of body proteins and enzymes.⁷⁹

Lysine can be methylated using S-adenosylmethionine (SAM) to synthesize carnitine, which is needed for fatty acid oxidation. Lysine also generates Acetyl CoA for use in the citric acid cycle. Lysine, proline, hydroxyproline, and vitamin C are important in the synthesis of collagen for skin, bones, tendons and cartilage.⁶²

L-lysine supplementation has also been studied for herpes simplex treatment and prophylaxis and may be beneficial.⁸⁰

High Levels

High dietary intake of protein can elevate lysine, as well as lack of cofactors needed in its utilization and catabolism, such as thiamine and niacin.⁷²

Hyperlysinemia is a rare inborn error of metabolism that causes a defect in the major catabolic pathway of lysine to acetyl CoA.⁸¹

Low Levels

Low levels of essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.⁴⁸

Lysine intolerance is a rare condition where intestinal absorption and renal tubular reabsorption of lysine, arginine and ornithine are impaired. This results in deficiencies of these amino acids and can lead to hyperammonemia.⁸²

Lastly, vitamin B₃ deficiency has been associated with low levels of lysine and other amino acids.⁷⁸

Methionine

Methionine is an essential amino acid that plays an important role in the methylation cycle. Methionine is obtained from dietary intake or through homocysteine remethylation. Methionine's dietary sources include eggs, fish, meats, Brazil nuts, and other plant seeds.⁸³

Methionine is converted to the body's main methyl donor, S-adenosylmethionine (SAM). This conversion requires the enzyme methionine adenosyltransferase (MAT).

High Levels

Methionine elevations are most commonly caused by

increased dietary intake.⁸³ However, increases can also be due to abnormalities within the methylation cycle itself producing a passive methionine elevation.

Genetic SNPs for several methylation and transsulfuration enzymes, or the lack of necessary vitamin and mineral cofactors, can alter methionine's metabolism. For example, a nutritional cofactor deficiency (magnesium/potassium), ATP depletion, or a SNP in the MAT enzyme, can downregulate the conversion to SAM and may lead to elevated methionine.⁸⁴ Vitamin B₆ deficiency, a cofactor for the downstream enzyme responsible for homocysteine transsulfuration, can result in excess homocysteine re-methylation back to methionine, thus increasing methionine.⁸⁵

Additionally, molybdenum is a cofactor in methionine degradation and catabolism, therefore molybdenum insufficiency can contribute to high levels of methionine.⁸⁶

Mild elevations in methionine do not cause serious adverse clinical effects. There is literature regarding CNS abnormalities seen with excessive elevations, but this is rare and more commonly seen with inborn errors of metabolism (MATI/III deficiency also known as Mudd's disease).⁸⁷

Low Levels

Low levels of essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.^{48,57} A dietary methionine deficiency (low intake or malabsorption/maldigestion) can affect the methylation cycle, given its critical role. Increasing methionine dietary sources, methionine supplementation, or methylated product supplementation can mitigate the adverse impact.⁸⁸

Because vitamins B₁₂ and folate are needed to remethylate homocysteine into methionine, functional need for these cofactors may contribute to low methionine levels.⁸⁹

Lastly, vitamin B₃ deficiency has been associated with low levels of several amino acids.⁷⁸

Phenylalanine

Phenylalanine is an essential amino acid found in most foods which contain protein such as meat, fish, lentils, vegetables, and dairy.⁹⁰ Phenylalanine is the precursor to another amino acid, tyrosine. Because tyrosine is needed to form several neurotransmitters (dopamine, epinephrine, and norepinephrine), as well as thyroid hormone and melanin, phenylalanine intake is important.⁶²

High Levels

High dietary protein intake may elevate phenylalanine levels. Additionally, some artificial sweeteners contain phenylalanine (NutraSweet® and Equal®); use of these products can result in higher levels.⁹¹

Phenylketonuria (PKU) is a rare genetic mutation of the phenylalanine hydroxylase enzyme which results in high phenylalanine levels.⁹² The enzyme requires vitamin C, tetrahydrobiopterin, and iron as cofactors. The mainstay of treatment involves a low-protein diet, cofactor support, and the use of a phenylalanine-free formulas.^{62,93}

Low Levels

Low levels of essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.⁴⁸ Also, vitamin B₃ deficiency has been associated with low levels of several amino acids.⁷⁸

Taurine

Taurine differs from other amino acids because a sulfur group replaces the carboxyl group of what would be the non-essential amino acid, β -alanine. It takes part in biochemical reactions and is not fully incorporated into proteins. In most tissues, it remains a free amino acid. Taurine's highest concentration is in muscle, platelets, and the central nervous system. Taurine is mainly obtained via dietary sources (dairy, shellfish, turkey, energy drinks), but can also come from sulfur amino acid metabolism (methionine and cysteine).^{94,95} It has been proposed that taurine acts as an antioxidant, intracellular osmolyte, membrane stabilizer, and a neurotransmitter.⁹⁶

In the CNS, taurine is second only to glutamate in abundance. Taurine is extensively involved in neurological activities, (calming neural excitability, cerebellar functional maintenance, and motor behavior modulation), through interaction with dopaminergic, adrenergic, serotonergic, and cholinergic receptors, and through glutamate.^{96,97}

In cardiovascular disease, taurine's benefits are multifactorial. Because taurine's main physiologic role is in bile acid conjugation in the liver, it has been demonstrated that taurine is capable of reducing plasma LDL, total lipid concentration, and visceral fat in diabetic, obese patients.⁹⁷ Taurine has been shown to be a protector of endothelial structure and function after exposure to inflammatory cells, their mediators, or other chemicals.⁹⁷ Taurine is thought to be involved in cell volume regulation and intracellular free calcium concentration modulation. Because of these effects, experimental evidence shows promise for taurine therapy in preventing cardiac

damage during bypass surgery, heart transplantation and myocardial infarction. Moreover, severe taurine extravasation from cardiomyocytes during an ischemia–reperfusion insult may increase ventricular remodeling and heart failure risk.⁹⁸

Recent work has revealed taurine's action in the retina as a photoreceptor cell promoter.⁹⁹ The human fetus has no ability to synthesize taurine. Taurine is found in breast milk, but it is also routinely added to infant formulas.⁹⁹

Although taurine is very beneficial, it is often unnecessary to supplement. Dietary intake and sulfur amino metabolism are usually more than adequate to meet the body's needs. Newborns, patients with restricted diets, or patients with various diseases may be depleted in taurine and can benefit from supplementation.

High Levels

Excessive dietary intake of taurine-rich foods/beverages may result in elevated taurine levels (i.e. energy drinks, dairy, shellfish, and turkey).^{95,100}

Because taurine is part of the transsulfuration pathway, a single nucleotide polymorphism (SNP) in the cystathionine-beta-synthase (CBS) enzyme can elevate taurine, but only in the absence of oxidative stress and presence of adequate glutathione levels.¹⁰¹ However, because oxidative stress and inflammation can upregulate transsulfuration in general, taurine may also be elevated in response to those factors. Antioxidants, such as vitamins A and E, as well as plant-based antioxidants, can help to mitigate oxidative damage.¹⁰²

As with all sulfur-containing amino acids, the enzyme sulfite oxidase catabolizes the amino acid into sulfite for excretion. An important cofactor for this enzyme is molybdenum. With that, insufficient molybdenum can contribute to elevated taurine levels.¹⁰³

Because renal excretion of taurine depends on a sodium chloride transporter which is regulated by vitamin B₁, irregular renal excretion of taurine can be seen in functional vitamin B₁ insufficiencies.¹⁰²

Low Levels

Low levels of amino acids can be seen with poor dietary intake, GI tract malabsorption, or maldigestion.¹⁰⁰ Because of taurine's role in the transsulfuration pathway, as outlined above, low levels of taurine may also be due to excessive oxidative stress, lack of precursors, or deficient enzymatic cofactors.^{100,104-109}

Threonine

Threonine is a large neutral amino acid and a precursor for the amino acid glycine.¹¹⁰ Foods that contain relatively high amounts of threonine include cheeses (especially Swiss), meat, fish, poultry, seeds, walnuts, cashews, almonds and peanuts.

Threonine gets converted to glycine using a two-step biochemical pathway involving the enzymes threonine dehydrogenase and the vitamin B₆-dependent glycine C-acetyltransferase.¹¹¹ Threonine has been studied clinically as a supplement to increase cerebrospinal fluid levels of glycine in patients with spasticity related to neurological conditions such as multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS).¹¹²⁻¹¹⁴ Threonine may also play a role in tissue healing and liver health.¹¹⁵⁻¹¹⁷ It is used to synthesize body proteins and is found in high concentrations relative to other amino acids in mucus glycoproteins.⁶² Many amino acids, including threonine can be converted into citric acid cycle intermediates for mitochondrial ATP production or for gluconeogenesis, depending on the body's needs.^{118,119}

High Levels

High dietary intake of threonine-rich foods result in elevated levels, as well as lack of vitamin cofactors needed to utilize and metabolize threonine.⁷⁷

Low Levels

Low levels of essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.⁴⁸ Vitamin B₃ deficiency has been associated with lower levels of threonine, and other amino acids.⁷⁸

Tryptophan

Tryptophan is involved in serotonin production via vitamin B₆-dependent pathways resulting in the intermediate 5-hydroxytryptophan (5-HTP). 5-HTP is often used as a supplement for serotonin formation instead of tryptophan, which can be quickly metabolized in other pathways. Serotonin is further metabolized to melatonin via methylation. Because of these downstream conversions, therapeutic administration of 5-HTP has been shown to be effective for depression, fibromyalgia, binge eating associated with obesity, chronic headaches, and insomnia.¹²⁰

Tryptophan can be alternatively metabolized via the kynurenine pathway to produce various organic acids - kynurenic acid, quinolinic acid, and xanthurenic acid.¹²⁰ Two percent of dietary tryptophan is converted to niacin (vitamin B₃) in the liver and deficiencies of vitamin B₆, riboflavin,

iron, and heme as essential cofactors for enzymes can slow the reaction rate.¹²¹

- Hartnup disease is a rare genetic disorder involving an inborn error of amino acid metabolism with symptoms developing in childhood. The intestines cannot properly absorb neutral amino acids and the kidney cannot properly resorb them. This leads to increased clearance of neutral amino acids in the urine, and normal or low levels in the plasma. Tryptophan deficiency is thought to account for the symptoms, since tryptophan converts to vitamin B₃. This B₃ deficiency causes dermatitis, a characteristic feature of Hartnup disease.¹²²⁻¹²⁴

High Levels

Elevated tryptophan may be seen in high protein diets or supplementation. Stress, insulin resistance, magnesium or vitamin B₆ deficiency, and increasing age can all inhibit the conversion of tryptophan to 5-HTP and elevate tryptophan.¹²⁵ Lack of nutrient cofactors (vitamin B₆, riboflavin, iron, and heme) in several other tryptophan pathways can also contribute to elevations.^{120,121,126,127}

Lastly, glutaric aciduria is a rare inborn error of metabolism characterized by elevated tryptophan.

Low Levels

Low levels of essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.⁴⁸

Because some dietary tryptophan is converted to niacin, tryptophan-deficient diets have been associated with lower niacin production. Interestingly, niacin administration increased plasma tryptophan by 40%.¹²⁷⁻¹²⁹

Clinically, low serum tryptophan levels have been shown to correlate with depressive symptoms and cognitive impairment.^{130,131}

Nonessential Protein Amino Acids

Nonessential amino acids are synthesized by the body from amino acids and other intermediates. Although they can be obtained from the diet, it is not required (unlike essential amino acids). However, when dietary intake of protein is very limited, both essential and nonessential amino acids may trend low.

Alanine

Alanine is a nonessential amino acid. It is the second most abundant amino acid in circulation, after glutamine.¹³² It is found in many foods including eggs, meat, lentils, and fish. Alanine is involved in sugar metabolism for energy and is important in immune system function, specifically T lymphocyte activation.¹³² Interestingly, alanine is an agonist that binds to the glycine site of N-methyl-D-aspartate (NMDA) receptors in the brain and improves the positive and cognitive symptoms of patients with schizophrenia.¹³³

Alanine plays an important role in BCAA metabolism. As previously noted, BCAA are released from skeletal muscle during prolonged exercise. Their carbon backbones are used as fuel, while their nitrogen portion is used to form alanine. Alanine then gets converted to pyruvate and subsequently glucose in the liver using the glucose-alanine cycle (Cahill Cycle). This cycle is critical for regenerating glucose in prolonged fasting and is upregulated when glucagon, epinephrine, and cortisol are elevated.^{62,134} It ultimately helps clear ammonia and provides glucose to energy-deprived muscle tissue.

The Cahill Cycle uses the enzyme alanine aminotransferase (ALT). ALT catalyzes the transfer of the amino group from alanine to an alpha keto acid (typically alpha-ketoglutarate), forming pyruvate and glutamate as byproducts.⁶² ALT is commonly measured on standard laboratory chemistry profiles to assess liver health.⁶²

High Levels

High protein intake of alanine-rich foods can elevate levels. Because of the relationship between alanine and the clearance of ammonia and nitrogen, it may be elevated in urea cycle disorders to serve as a reservoir for waste nitrogen.¹³⁵ Biotin, thiamine, other nutrients are cofactors within the pathways of alanine metabolism. Functional need for these nutrients may elevate alanine levels.¹³⁶⁻¹³⁸

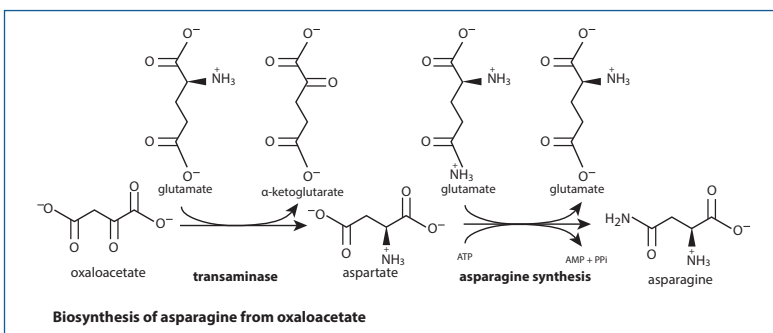
Low Levels

Low protein intake, low BCAA levels, gastrointestinal malabsorption and maldigestion, or increased demands in gluconeogenesis, may result in lower alanine levels. There is some literature to suggest that vitamin B₆ and vitamin B₃ normalized plasma alanine levels.^{77,78}

Because of its role in the CNS, lower plasma alanine levels have been seen in schizophrenic patients and an increase in plasma alanine correlated with symptom improvement.¹³³

Asparagine

Asparagine is a non-essential protein amino acid that is present in many fruits and vegetables including asparagus, from which it gets its name.¹³⁹ Other dietary sources include meat, potatoes, eggs, nuts, and dairy. It can also be formed from aspartic acid and glutamine using the enzyme asparagine synthetase.¹⁴⁰



In addition to being a structural component of many proteins, asparagine is also useful to the urea cycle. It acts as a nontoxic carrier of residual ammonia to be eliminated from the body.¹³⁵ Asparagine is rapidly converted to aspartic acid by the enzyme asparaginase.¹¹⁹ Interestingly, L-asparaginase has been successfully used as a chemotherapeutic agent for decades. It causes extracellular depletion of asparagine which seems to play a critical role in cellular adaptations to glutamine and apoptosis.¹⁴¹

High Levels

High dietary protein intake can elevate asparagine levels. Asparagine may also be elevated in hyperammonemia to serve as a reservoir for waste nitrogen.

Low Levels

Overall low amino acids from poor dietary intake or GI malabsorption/maldigestion may result in low levels of arginine. Low levels of its precursors (aspartic acid and glutamine), or enzymatic dysfunction in arginine synthetase can also result in low asparagine levels.

Upregulation of asparaginase may contribute to lower levels of asparagine and rarely can be associated with hyperammonemia.¹⁴²

Depleted levels of arginine due to genetic mutations in asparagine synthetase are associated with neurodevelopmental disorders.¹⁴³

Aspartic Acid (Aspartate)

Aspartic acid is a nonessential amino acid that plays roles in many important metabolic processes, such as energy production (citric acid cycle), hormone metabolism, CNS activation, and the urea cycle. It is found in many protein sources such as oysters, meats, seeds, avocado, asparagus, and beets. It is also an ingredient in artificial sweeteners.

Aspartic acid is a precursor to many amino acids and other molecules like asparagine, arginine, isoleucine, lysine, methionine, isoleucine, threonine, nucleotides, NAD, and pantothenate. Aspartate, like glutamine, can also be considered a neuroexcitatory neurotransmitter since it activates the N-methyl-D-aspartate receptor in the brain.¹⁴⁴⁻¹⁴⁶

Aspartate transaminase (AST) is an enzyme that catalyzes the transfer of an amino group from L-aspartate to alpha-keto glutarate. This reaction serves as a cellular energy source and takes place mainly in the liver, skeletal muscle, myocardium, and kidneys. Although AST is commonly measured on traditional laboratory profiles as a measure of liver dysfunction and muscle injury, it is not specific enough to be used alone as a diagnostic tool.

High Levels

Elevated aspartic acid may reflect high intake of aspartate-rich foods or use of artificial sweeteners containing aspartic acid ("NutraSweet" or "Equal").⁹¹ Elevations may also be due to impaired downstream metabolism from nutritional insufficiencies of enzymatic cofactors such as vitamin B₆, magnesium, and ATP.^{147,148}

Because aspartic acid is a major excitatory neurotransmitter, elevations have been noted in epileptic patients.¹⁴⁶

Cysteine

Cysteine is a nonessential sulfur-containing amino acid. It is obtained from the diet and is also endogenously made from the intermediate amino acid cystathionine. Dietary cysteine sources include poultry, eggs, beef, and whole grains.¹⁴⁹

This amino acid should not be confused with the oxidized derivative of cysteine called cystine. Cystine is formed by combining two cysteine molecules within a redox reaction. The urinary FMV amino acid test reports cysteine and cystine separately. The plasma amino acid test combines both cysteine and cystine as one biomarker -cyst(e)ine.

Cysteine is an important component of glutathione. Recent studies provide some data to support the view that cysteine may be a limiting amino acid for glutathione synthesis in

humans.¹⁵⁰ This synthesis requires the enzyme glutathione synthetase (GSS). Cysteine can alternatively be converted to taurine (another amino acid) and the organic acid pyruvate, which are used in the mitochondrial citric acid cycle and/or excreted in the urine.¹⁵¹ When cysteine levels are low, this favors their utilization in glutathione formation during oxidative stress, given the importance of glutathione. Conversely, high levels of cysteine in the absence of oxidative stress favor its metabolism towards pyruvate and taurine.¹⁵²

High Levels

A diet high in cysteine-rich proteins can elevate cysteine levels. As with all sulfur-containing amino acids, the enzyme sulfite oxidase catabolizes the amino acid into sulfite for excretion. An important cofactor for this enzyme is molybdenum. With that, insufficient molybdenum can contribute to elevated cysteine.^{86,153}

Homocysteine is pulled into the transsulfuration pathway via the enzyme cystathionine-beta-synthase (CBS) to become cysteine, with cystathionine formation as an intermediate step. Cysteine levels may be elevated due to a CBS SNP which results in an upregulation of the enzyme and more cystathionine and cysteine production.^{154,155} Zinc is an important cofactor downstream from cysteine in transsulfuration. Because of this, cysteine elevations can also be seen in zinc insufficiency.

Vitamin B₁₂ may also be a cofactor in the peripheral utilization of cysteine; therefore functional deficiencies of vitamin B₁₂ can contribute to higher levels.^{156,157}

Low Levels

Low dietary protein intake, GI malabsorption, and maldigestion may all contribute to lower amino acid levels.

Because vitamin B₆ is a cofactor in several steps within the transsulfuration pathways, deficiency may contribute to lower cysteine by inhibiting or slowing the enzyme that converts cystathionine to cysteine.^{105,158}

Cystine

Cystine is formed from the oxidation of cysteine, or from the degradation of glutathione oxidation products. It is two cysteines linked together with a disulfide bond.¹⁵⁹

As previously noted, the urine FMV amino acid test reports cysteine and cystine separately. The plasma amino acid test combines both cysteine and cystine as one biomarker.

High Levels

Anything that elevates cysteine, could potentially contribute to higher levels of cystine. (see above)

Elevations of cystine may be associated with increased oxidative stress; antioxidants such as vitamins A, C, E and plant-based antioxidants may be considered.¹⁶⁰⁻¹⁶³

In plasma, cystine is increased with age, obesity, cigarette smoking, alcohol abuse, HIV infection, carotid intima media thickness, endothelial cell function, type 2 diabetes, and age-related macular degeneration.¹⁵⁹

Cystinuria is an inherited renal transport disorder that features poor renal conservation and increased urinary excretion of cystine and other amino acids and metabolites. This condition is associated with renal calculi formation.¹⁶⁴⁻¹⁶⁸ Genova's profiles are not meant to diagnose inherited cystinuria. If suspected, due diligence with conventional medicine work up is recommended.

Low Levels

Anything that may lower cysteine, could potentially contribute to low cystine levels. (see above) Low cystine may be seen specifically in low animal protein diets.¹⁶⁹

γ -Aminobutyric Acid

Gamma-aminobutyric acid (GABA) is an amino acid that functions as an inhibitory neurotransmitter. It serves one-third of brain neurons and is involved in depression and mania.¹⁷⁰

Although there are some dietary supplement and food sources for GABA (cruciferous vegetables, spinach, tomatoes, beans, and rice), the primary source may be endogenous production.¹⁷¹ Nervous tissue, the gut microbiome, the liver, pancreas, and endothelial cells are important sources for production.¹⁷²

Endogenous GABA is produced by the decarboxylation of the excitatory neurotransmitter glutamic acid.¹⁷³ It can also be produced from the diamine putrescine using diamine oxidase (DAO).^{172,174,175}

Also, the gut microbiome is capable of synthesizing various hormones and neurotransmitters. For example, *Lactobacillus* and *Bifidobacterium* species can produce GABA.¹⁷⁶

In general, plasma GABA may reflect brain GABA activity, however urine GABA levels are felt not to correlate with CNS levels.¹⁷⁰

High Levels

High intake of protein and GABA-containing foods can contribute to elevated levels.

The metabolism and degradation of GABA requires a vitamin B₆- dependent enzyme; therefore vitamin B₆ deficiency can contribute to elevated GABA levels.¹⁷³

Elevated plasma GABA levels have been observed in autistic children.¹⁷⁷

Low Levels

Decreased protein intake, GI maldigestion, and malabsorption can contribute to lower levels. Also, since GABA can be made endogenously from glutamic acid and other pathways, low glutamic acid levels, issues with enzymes like DAO, or an altered microbiome should also be considered.

Reduced GABA levels are known to exacerbate seizures.¹⁷⁸

Glutamic Acid (Glutamate)

Glutamic acid is a nonessential amino acid is derived from the diet and from the breakdown of gut proteins. Glutamate is a major excitatory neurotransmitter in the brain.¹⁷⁹ It plays a role in neuronal differentiation, migration, and survival in the developing brain. It is also involved in synaptic maintenance, neuroplasticity, learning, and memory.¹⁸⁰

Glutamate is present in many foods including cheese, seafood, meat, and spinach.¹⁷¹ In spite of intake, the total pool of glutamic acid in the blood is small, due to its rapid uptake and utilization by tissues including muscle and the liver (which uses it to form glucose and lactate).¹⁷⁹ Glutamic acid is also the precursor for arginine, glutamine, proline, GABA, and the polyamines (putrescine, spermine, spermidine).^{145,181}

As outlined in the previous BCAA section, the Cahill Cycle is used to generate pyruvate and glucose in the liver using branch chain amino acids. Glutamate is an end product of this reaction via the enzyme alanine aminotransferase (ALT).⁶² Glutamate is also an end product of the enzyme ornithine aminotransferase (OAT) in the urea cycle. This urea cycle reaction is a vitamin B₆-

dependent enzyme which catalyzes the reversible conversion of ornithine to alpha-ketoglutarate, yielding glutamate.¹⁸¹

High Levels

High dietary intake of glutamic acid-containing foods can elevate levels. The sodium salt of glutamic acid, monosodium glutamate (MSG), is common food additive. Intake of foods containing MSG can result in elevated glutamate levels.¹⁷⁹

Various cofactors are needed for glutamate metabolism including vitamin B₁, B₃, and B₆. Functional deficiencies in these cofactors can contribute to elevated levels. Administration of these nutrients can lower glutamate levels.^{42,72,78}

Low Levels

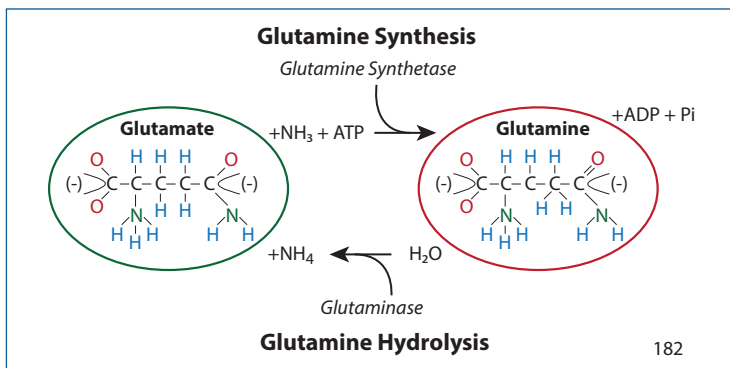
Low protein intake, GI malabsorption, and maldigestion can all contribute to low levels of amino acids. As above, there are many endogenous pathways which create glutamate, each with vitamin and mineral cofactors. Lack of those cofactors should also be considered.

No specific symptomatology has been attributed to low glutamic acid levels.

Glutamine

Glutamine is a nonessential amino acid and is the most abundant amino acid in the body. It is formed from glutamate using the enzyme glutamine synthetase.¹⁸²

Approximately 80% of glutamine is found in the skeletal muscle, and this concentration is 30 times higher than the amount of glutamine found in human plasma. Although glucose is used as fuel for many tissues in the body, glutamine is the main fuel source for a large number of cells including lymphocytes, neutrophils, macrophages, and enterocytes.^{62,183}



Glutamine is necessary for many physiologic processes including:

- growth of fibroblasts, lymphocytes, and enterocytes
- protein synthesis

- mitosis
- muscle growth
- immune function
- glutathione formation
- nucleotide synthesis
- apoptosis prevention
- regulation of acid-base homeostasis
- glutamate metabolism
- inter-organ nitrogen exchange via ammonia transport
- gluconeogenesis
- energy generation (ATP)^{180,183,184}

High Levels

High protein intake may contribute to higher levels. It should also be noted that glutamine is available as a nutraceutical supplement. Elevations can also be seen with supplementation. The metabolism of glutamine requires several cofactors, such as NADPH and vitamin B₁.¹⁸² Functional deficiencies of vitamin and mineral cofactors can also elevate levels. There is literature to suggest vitamin B₁ supplementation lowers elevated levels of glutamine, as well as other amino acids in thiamine deficiency.⁷²

Because of the relationship of glutamine and glutamate to both the Cahill Cycle and Urea Cycle, elevations of glutamine are associated with hyperammonemia due to increased production of glutamine from glutamate.¹³⁵

Low Levels

Decreased protein intake, GI malabsorption, and maldigestion can contribute to overall lower amino acid levels. However, given the extensive role of glutamine throughout the body, increased metabolic demand can also result in lower levels.

Glutamine is considered a conditionally essential amino acid in critically ill patients. Endogenous glutamine synthesis does not meet the body's demands in catabolic conditions including cancer, sepsis, infections, surgeries, traumas, and during intense and prolonged physical exercise.¹⁸⁰ Low plasma glutamine is associated with increased mortality and functional impairment in critically ill patients. Glutamine administration reduces infection-related morbidity, decreases mortality during the intensive care phase, and shortens the length of hospitalization.¹⁸⁴

Proline

Proline is a nonessential amino acid. It contains a secondary α -imino group and is sometimes called an α -imino acid. Proline, and its metabolite hydroxyproline, constitute a third of the total amino acids found in collagen. Lysine, proline, hydroxyproline, and vitamin C are all important in the synthesis of collagen for skin, bones, tendons, and cartilage.⁶²

Proline is abundant in meat, bone meal, poultry, salmon, wheat, barley, and corn.¹⁸⁵ In addition to dietary sources, proline can be synthesized from glutamate/glutamine, arginine, and ornithine. It can also be synthesized within enterocytes from degradation of small peptides.^{185,186}

In addition to collagen formation, proline has many other physiologic functions including regulation of gene expression, mTOR activation (integrating nutrient and growth factor signaling in cells), cellular redox reactions, protein synthesis, hydroxyproline generation, arginine synthesis, and it is a scavenging antioxidant.¹⁸⁵

High Levels

High dietary intake of proline-rich foods can elevate levels. There are vitamin and mineral cofactors needed for downstream metabolism of proline in its many physiologic processes. Functional deficiency of nutrient cofactors, such as vitamin B₁, can result in elevated levels. Furthermore, administration of vitamin B₁ has been shown to lower proline levels, as well as other amino acids in severe thiamine deficiency.⁷²

Low Levels

Low levels may be reflective of poor dietary intake, GI malabsorption, maldigestion, or low levels of its precursors.

Tyrosine

Tyrosine is a conditionally essential amino acid which can come directly from the digestion of dietary protein. Common food sources include dairy, beans, whole grains, meat, and nuts.¹⁸⁷

If intake is insufficient, tyrosine can be formed from the essential amino acid phenylalanine using a tetrahydrobiopterin reaction. Tyrosine itself is a precursor to several neurotransmitters including dopamine, epinephrine and norepinephrine. It is also needed to create thyroid hormone and melanin skin pigments.¹¹⁹

Within the metabolism of tyrosine to form neurotransmitters and other hormones, there are several important nutrient cofactors involved including vitamin B₁, vitamin B₆, tetrahydrobiopterin, copper, vitamin C, among others.¹²⁵

High Levels

High dietary intake of tyrosine-rich foods can elevate levels. Additionally, functional need for vitamin and nutrient cofactors for tyrosine metabolism can contribute to elevations.^{72,125}

Low Levels

Low levels of essential and conditionally essential amino acids may indicate a poor-quality diet, or maldigestion due to deficient digestive peptidase activity or pancreatic dysfunction.⁴⁸

Phenylketonuria (PKU) is an inborn error of metabolism involving a deficiency of the hepatic enzyme phenylalanine hydroxylase, and results in elevated phenylalanine and low tyrosine levels.

Vitamin B₃ deficiency has been associated with altered levels of amino acids.⁷⁸

B-Vitamin Markers

Some intermediary amino acid metabolites specifically require B-vitamins as cofactors for enzymatic reactions. Elevations may signify a functional need for vitamin cofactors.

α -Aminoadipic Acid

Alpha-aminoadipic acid (also known as 2-aminoadipic acid) is an intermediary biomarker of lysine and tryptophan metabolism. The further metabolism of alpha-aminoadipic acid to alpha-ketoadipic acid requires vitamin B₆.¹⁸⁸

Plasma alpha-aminoadipic acid is strongly associated with the risk of developing diabetes as seen in an assessment of the Framingham Heart Study data. Circulating levels were found to be elevated for many years prior to the onset of diabetes.¹⁸⁹ Preclinical data shows it may also play a role in oxidation and atherosclerotic plaque formation.¹⁹⁰

High Levels

The excretion of alpha-aminoadipic acid correlates well with lysine intake.¹⁹¹ Elevations of alpha-aminoadipic acid may be due to rate limitations of downstream enzymes that require nutrient cofactors including vitamin B₂, B₆, B₁₂, and choline. Lastly, alpha-aminoadipic aciduria is an extremely rare inborn error of metabolism.^{192,193}

Low Levels (urine)

Low levels of this metabolite can be seen when its precursors, lysine and tryptophan, are also low. There is no known clinical significance of low levels of alpha-aminoadipic acid.

α -Amino-N-butyric Acid (α -ANB)

Alpha-Amino-N-butyric acid (α -ANB), also known as alpha-aminobutyric acid, is a nonessential amino acid derived from the catabolism of methionine, threonine, and serine.⁷⁵ α -ANB is both formed and metabolized by reactions which require vitamin B₆ as a cofactor.¹⁹⁴

High Levels

Levels of this metabolite may be elevated if its precursors are also elevated.

A functional need for vitamin B₆ can limit the further metabolism of α -ANB and contribute to elevated levels. Elevations of this metabolite have been studied in several conditions which contribute to a functional vitamin B deficiency, such as alcoholism, sepsis, hypocaloric weight loss, and excessive exercise.^{75,195-199}

Low Levels

Low levels of α -ANB may be seen when overall amino acids are low, especially its precursors. Additionally, a functional deficiency in vitamin B₆ has been associated with lower levels of α -ANB and levels can increase with B₆ supplementation.⁴² Clinically, low levels of plasma α -ANB have been associated with depressive symptoms.^{200,201}

β -Aminoisobutyric Acid (β -AIB)

Beta-aminoisobutyric acid (also known as 3-aminoisobutyric acid) is a non-protein amino acid formed by the catabolism of valine and the nucleotide thymine. It is further catabolized to methylmalonic acid semialdehyde and propionyl-CoA.²⁰² Levels are controlled by a vitamin B₆-dependent reaction in the liver and kidneys.²⁰³ β -aminoisobutyric acid can also be produced by skeletal muscle during physical activity.

High Levels

Elevated levels may be associated with increased intake of the precursor amino acid valine. Levels are higher with exercise.⁶³ A functional need for vitamin B₆ can also contribute to elevations.²⁰³

Clinically, transient high levels have been observed under a variety of pathological conditions including lead poisoning, starvation, total body irradiation, and malignancy.²⁰²

Low Levels

Low levels of β -AIB may be seen with decreased precursors, such as valine.

Dihydropyrimidine dehydrogenase deficiency is a rare inborn error of metabolism that results in lower levels of urinary β -AIB.²⁰²

Cystathionine

Cystathionine is an intermediate dipeptide within the process of transsulfuration. Transsulfuration is the main route for irreversible homocysteine disposal, glutathione production, and energy. The initial step involves the enzyme cystathionine β -synthase enzyme (CBS). This reaction requires nutrient cofactors such as vitamin B₆ and iron.

Cystathionine is then converted to cysteine, and eventually goes on to either make glutathione or feed the Krebs cycle. Currently, there is no known source or physiologic function for cystathionine other than serving as a transsulfuration intermediate. Some literature suggests that cystathionine may exert protection against endoplasmic reticulum stress-induced tissue damage and cell death, but studies are sparse.²⁰⁴

High Levels

Because cystathionine is an intermediate of the transsulfuration pathway, elevation of this biomarker may indicate a downstream backup of the transsulfuration pathway. Conversion of cystathionine to glutathione, or other transsulfuration metabolites, requires necessary cofactors, such as vitamin B₆, zinc, glycine, and magnesium. Therefore, transient elevations of this metabolite may indicate increased need for these cofactors.^{105,205}

Elevated cystathionine may be seen in individuals who have a CBS SNP which upregulates this enzyme and therefore upregulates the conversion of homocysteine to cystathionine.^{154,155}

Elevated S-adenosylmethionine (SAM) directly upregulates the CBS enzyme leading to higher cystathionine levels.²⁰⁶ Dimethylglycine (DMG) or trimethylglycine (betaine) supplementation contribute to maintaining methylation. If the methylation cycle is adequate, transsulfuration is then upregulated. With this, supplementation of DMG or betaine have been associated with elevated cystathionine.²⁰⁶

Elevated homocysteine may increase its metabolism into transsulfuration. Therefore, in vitamin B₁₂ and folate deficiencies which result in high homocysteine, cystathionine might also be elevated.²⁰⁷

Low Levels (urine)

Abnormalities within the methylation cycle can result in lower levels of cystathionine. Low levels of SAM, or methylation imbalances, result in the body preferentially deferring transsulfuration to maintain methylation.

Because the CBS enzyme requires vitamin B₆ as a cofactor, deficiencies in vitamin B₆ may result in lower cystathionine.¹⁵⁸

3-Methylhistidine

Both 1-methylhistidine and **3-methylhistidine** are histidine metabolites which have been proposed as markers of meat intake.^{208,209} Note that some confusion exists in the literature regarding the numbering of atoms in the imidazole ring of histidine – 1 versus 3 – and thus, there is caution with interpretation and clinical significance of these two markers.^{46,208} 3-methylhistidine is a constituent of actin and myosin, the contractile proteins of skeletal muscles. Urinary excretion of 3-methylhistidine may be a result of muscle breakdown or consumption of meat fibers. Unlike 1-methylhistidine, 3-methylhistidine has been shown to increase in fasting states indicating catabolism of muscle tissue. Therefore, this marker is more variable with regards to animal protein consumption.⁴⁶

High Levels

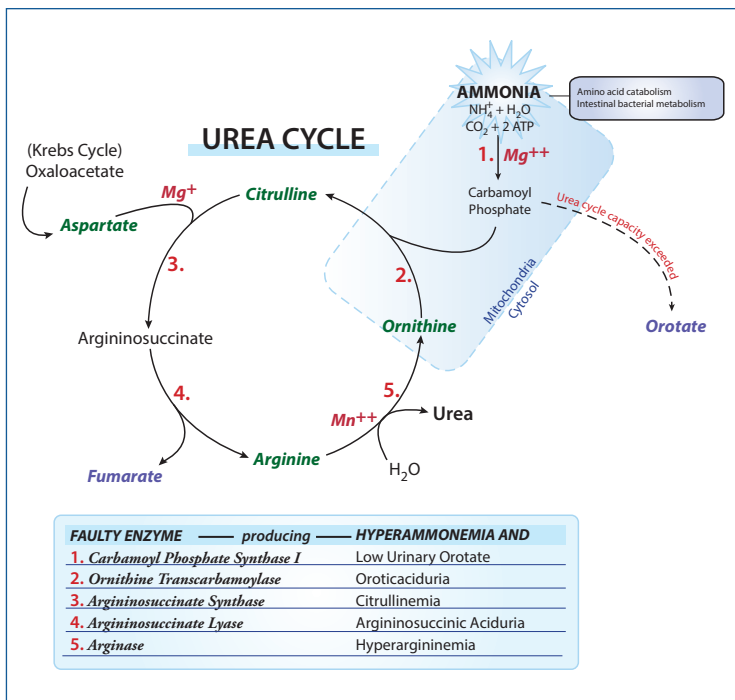
Urine and plasma levels of 3-methylhistidine can be higher with meat consumption.^{46,208} And, as noted above, elevations have been seen in catabolism or fasting states.

Low Levels (urine)

3-methylhistidine is lower with low protein diets, or in vegetarian and vegan diets.

Urea Cycle Markers

The urea cycle takes place in the liver and is important for detoxifying nitrogen (ammonia) into non-toxic urea. The main sources of ammonia in the body are the catabolism of protein and production by bacteria in the gut. The urea cycle involves the amino acids arginine, ornithine, and citrulline - with one intermediate, argininosuccinate. Impairments in the urea cycle can lead to hyperammonemia, a serious condition involving the buildup of ammonia in the blood. Symptoms can range from mild (irritability, headache and vomiting), to severe (encephalopathy, seizures, ataxia and coma). A serum ammonia level should be obtained if hyperammonemia is suspected.²¹⁰



Citrulline

Citrulline is an intermediate, nonprotein-forming amino acid in the urea cycle serving as a precursor to arginine. It derives its name from the watermelon (*Citrullus vulgaris*), where it was first isolated and identified. It is easily absorbed by the gut and bypasses the liver, making it an effective method for replenishing arginine.^{211,212} Other food sources of citrulline include muskmelons, bitter melons, squashes, gourds, cucumbers and pumpkins.²¹³

Citrulline can also be synthesized from arginine and glutamine in enterocytes, which can then be metabolized by the kidneys back into arginine.²¹⁴ Because citrulline is produced in enterocytes, it has been proposed as a marker of enterocyte mass in conditions of villous atrophy.^{215,216}

Given the importance of arginine in nitric oxide production

for vasodilation and muscle protein synthesis, citrulline is sometimes administered therapeutically to deliver arginine to endothelial and immune cells. It is also supplemented in sarcopenia to stimulate protein synthesis in skeletal muscle through the rapamycin (mTOR) pathway.^{211,217} Citrulline supplementation has been studied in conditions like erectile dysfunction, sickle cell anemia, short bowel syndrome, hyperlipidemia, cancer chemotherapy, urea cycle disorders, Alzheimer's disease, multi-infarct dementia, and as an immunomodulator.²¹³

High Levels

Elevated citrulline can occur with urea cycle defects. Lack of nutrient cofactors or enzymatic SNPs within the urea cycle can contribute to elevated citrulline levels.

Citrullinemia is an inherited autosomal recessive disease that affects the enzyme argininosuccinate synthase and is diagnosed in infancy. In most cases, a serious problem related to citrulline is unlikely and may be a limitation in the cofactors associated with citrulline metabolism: aspartic acid and magnesium. Elevated plasma levels may result from citrulline supplementation.³⁹ Orally administered citrulline is highly bioavailable since plasma levels rise dramatically, whereas urinary citrulline loss is minimal.³⁸

Elevated citrulline in urine can be a consequence of a urinary tract infection where bacterial action reduces arginine and produces citrulline.

Administration of thiamine (vitamin B₁) has been found to lower elevated citrulline, as well as other amino acids, in thiamine deficiency.⁷²

Low Levels

Low citrulline may be secondary to a relatively low protein diet and/or intestinal malabsorption. Because citrulline can be formed from glutamine, glutamine depletion has been associated with low citrulline levels in plasma.³⁸

Ornithine

Ornithine is an intermediate nonprotein-forming amino acid of the urea cycle. Arginine is converted to ornithine via the arginase enzyme, with urea as a byproduct. Ornithine combined with carbamoyl phosphate is then converted into citrulline via the ornithine transcarbamylase (OTC) enzyme. The contribution of carbamoyl phosphate results from the metabolism of ammonia by the enzyme carbamoyl phosphate synthase, and if this magnesium-dependent process is impaired, ammonia buildup, or hyperammonemia can occur. Ornithine can also form polyamines including putrescine via the ornithine decarboxylase (ODC) enzyme, which requires pyridoxal-5-phosphate (vitamin B₆) as a cofactor.^{218,219} Putrescine and other polyamines are crucial to the growth and proliferation of cells.²²⁰

Ornithine forms glutamate via ornithine aminotransferase (OAT), requiring pyridoxine (vitamin B₆) as a cofactor. OAT deficiency is a rare congenital disorder characterized by gyrate atrophy of the choroid and retina, and is treated with vitamin B₆ to prevent vision loss.^{221,222}

High Levels

Elevations of ornithine may be due to a limitation in the cofactors associated with metabolism including vitamin B₆ and magnesium.^{181,223-226}

Elevations may also result from supplementation of citrulline or ornithine.^{39,227}

Administration of thiamine (vitamin B₁) lowered elevated ornithine, as well as other amino acids in thiamine deficiency.⁷² OTC deficiency resulting in hyperammonemia is an inborn error of metabolism and is the most common of the inborn errors of the urea cycle. While most inborn errors present during the neonatal period or early childhood, some can have a later onset in adulthood, including OTC deficiency. It is characterized by elevated ammonia and orotic acid (an organic acid) due to the metabolic block.^{210,228}

Low Levels

Low protein intake can result in low levels of urea cycle intermediates.

Low ornithine may be of no clinical consequence; evaluate other urea cycle intermediates and metabolites.

A nonspecific finding of decreased plasma ornithine and arginine may be seen with OTC deficiency; this would be accompanied by hyperammonemia and elevated orotic acid, plasma glutamine and alanine.²²⁸

Urea

Urea is a nontoxic byproduct of nitrogen (ammonia) detoxification. It is formed in the liver via the urea cycle and is the end product of protein metabolism. It is essentially a waste product with no physiological function.

High Levels

Elevated urea may reflect high dietary protein intake. It can also be seen in underlying renal issues, abnormal urea transporters, or abnormal urinary concentration capabilities.²²⁹

Low Levels

Low levels may be secondary to low protein diets or protein malabsorption, or renal and liver issues. There are some urea cycle disorders that are due to enzymatic cofactor need, such as manganese and magnesium, which may in turn lead to lower urea levels.^{62,230}

Glycine/Serine Metabolites

Glycine and serine are nonessential amino acids that have multiple functions. The metabolites measured are involved in the choline synthesis pathway. Choline is important for the production of the neurotransmitter acetylcholine. The reactions in these pathways are reversible, depending on the body's need for certain compounds.

Glycine

Glycine is a nonessential amino acid that is synthesized from choline, serine, hydroxyproline, and threonine.^{110,231} It has many important physiologic functions. It is one of three amino acids that make up glutathione. Glycine's dietary sources include meat, fish, legumes, and gelatins.

Glycine is a major collagen and elastin component, which are the most abundant proteins in the body. Like taurine, it is an amino acid necessary for bile acid conjugation; therefore, it plays a key role in lipid digestion and absorption.²³² Glycine is the precursor to various important metabolites such as porphyrins, purines, heme, and creatine. It acts both as an inhibitory neurotransmitter in the CNS and as an excitatory neurotransmitter on N-methyl-D-aspartate (NMDA) receptors.²³³ Glycine has anti-oxidant, anti-inflammatory, immunomodulatory, and cytoprotective roles in all tissues.²³² In the folate cycle, glycine and serine are interconverted. These methyltransferase reactions and interconversions are readily reversible depending on the needs of the folate cycle to synthesize purines.²³⁴

Glycine can also be generated from choline, betaine, dimethylglycine, and sarcosine within the methylation cycle itself.²³⁵ Glycine accepts a methyl group from S-adenosylmethionine (SAM) to form sarcosine. This conversion functions to control SAM excess.²³⁶

Supplementation with glycine has been used to ameliorate metabolic disorders in patients with obesity, diabetes, cardiovascular disease, ischemia-reperfusion injuries, inflammatory diseases, and cancers.²³² Because of glycine's excitatory effects on CNS NMDA receptors, research regarding the treatment of psychiatric disorders, such as schizophrenia, using glycine transport antagonists have shown great promise.²³³

Oral glycine can boost tissue levels of glutathione, especially with concurrent NAC and/or lipoic acid. Because glutathione levels decline during the aging process, supplementing with glycine can impact elderly patients with low protein intake.²³⁷

High Levels

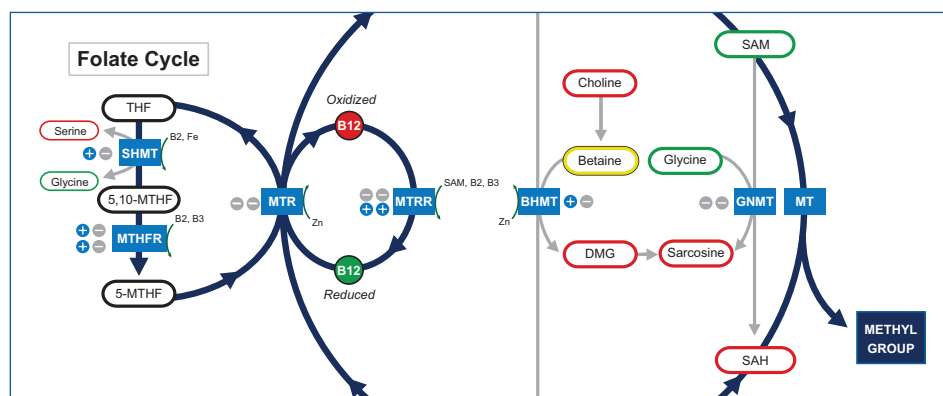
Elevated glycine may be due to dietary intake (i.e. meat, fish, legumes, and gelatin) or supplementation.

Enzymatic SNPs or cofactor deficiencies in glycine production and metabolism (vitamin B₆, B₁₂, and folate) may result in abnormal levels of glycine.^{62,77,205,238,239}

Low Levels

Low glycine may be due to decreased intake, or GI malabsorption and maldigestion.

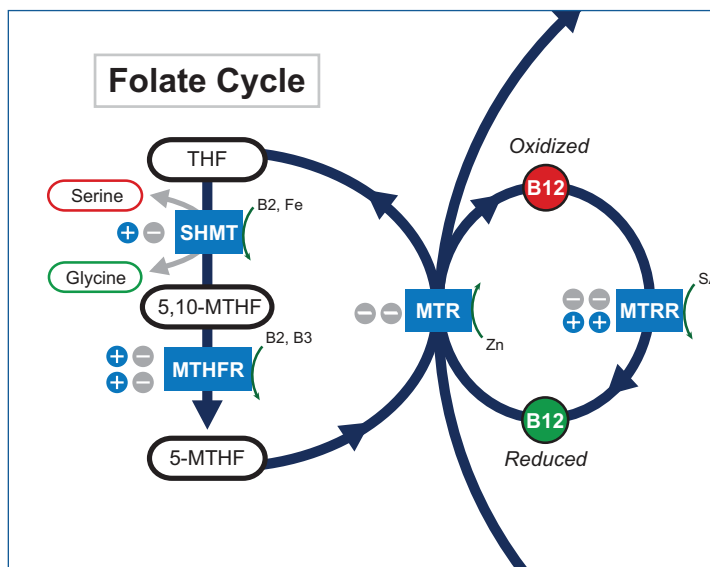
Glycine's function as an antioxidant plays an important role in disease processes and is incorporated into glutathione, an important antioxidant. Therefore, low levels have significant clinical impact. Antioxidants such as vitamins A and E can help mitigate damage from oxidative stress.²⁴⁰



Serine

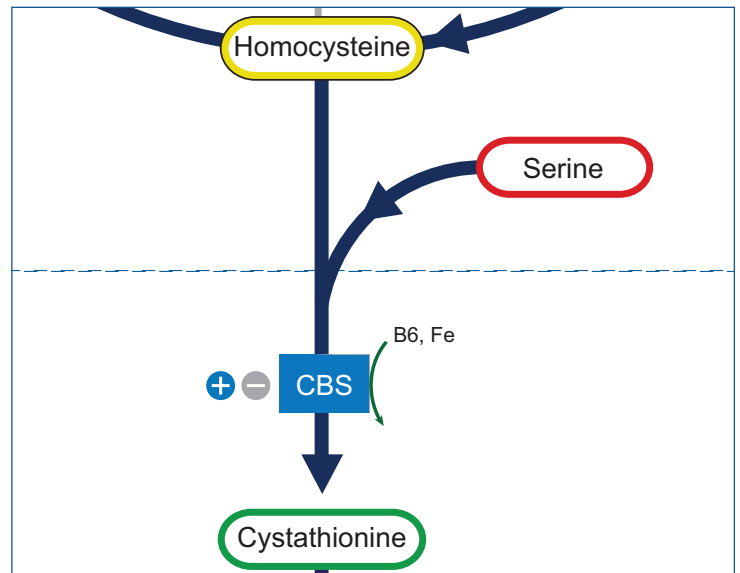
Serine is a nonessential amino acid used in protein biosynthesis and can be derived from four possible sources: dietary intake, degradation of protein and phospholipids, biosynthesis from glycolysis intermediate 3-phosphoglycerate, or from glycine.²⁴¹ Serine is found in soybeans, nuts, eggs, lentils, shellfish, and meats.

Serine is used to synthesize ethanolamine and choline for phospholipids.⁶² Serine is essential for the synthesis of sphingolipids and phosphatidylserine in CNS neurons.²⁴² In the folate cycle, glycine and serine are interconverted.²⁴³ These methyltransferase reactions and interconversions are readily reversible depending on the needs of the folate cycle.^{62,234} Dietary serine is not fully converted to glycine; therefore, serine supplementation has little value, though is not harmful.²⁴⁴



Glycine and serine's interconversion are important in mitochondrial glycolysis. Glycolysis provides ATP and energy in most cell types. Serine-glycine biosynthesis is a component in glycolysis-diverting pathways and nucleotide biosynthesis. This is clinically important, and specifically evident, in cancer. Cancer cells use glycolysis to sustain anabolism for tumor growth. Genetic and functional evidence suggests that abnormalities in the glycine-serine pathway represent an essential process in cancer pathogenesis by promoting energy production and promoting defective purine synthesis.^{234,245,246}

Serine is also a cofactor for the transsulfuration enzyme cystathionine-β-synthase making its availability important for glutathione production.



High Levels

High dietary intake of serine-rich foods, or supplementation, may result in elevated levels.

Due to cofactors needed for serine metabolism, deficiencies of these nutrients can result in elevated serine levels.

Administration of nutrients such as vitamin B₆ or B₁₂ have been shown to lower serine levels, as well as other amino acids.^{72,77}

Given its association with the folate cycle, plasma serine levels may be low or high with homocysteinemia and methylation defects; support with vitamin B₆, B₁₂, folate, or betaine can result in normalized homocysteine as well as serine.²⁴⁷

Low Levels

Low serine may be due to decreased intake, or GI malabsorption and maldigestion.

One pathway of serine biosynthesis requires the vitamin B₆-dependent enzyme phosphoserine aminotransferase. With this, a functional need for vitamin B₆ may contribute to low serine levels.^{248,249}

Given its association with the folate cycle, plasma serine levels may be low or high with homocysteinemia and methylation defects; support with vitamin B₆, B₁₂, folate, or betaine can result in normalized homocysteine as well as serine.²⁴⁷

Ethanolamine

Ethanolamine is an intermediary metabolite in the serine-to-choline sequence. It can be used to synthesize phosphatidylethanolamine (PE), a very important membrane phospholipid. Ethanolamine is not only a precursor, but also a breakdown product of PE.^{250,251} Ethanolamine is abundant in both intestinal and bacterial cell membranes. It plays a significant role in the renewal and proliferation of intestinal cells and intestinal inflammation.²⁵²⁻²⁵⁴ Also, since ethanolamine plays a structural role in skeletal muscle cell membranes, some evidence suggests it may be a marker of skeletal muscle turnover.²⁵⁵

High Levels

The downstream metabolism of ethanolamine is magnesium and manganese dependent. Functional need for these cofactors can contribute to elevated ethanolamine.^{256,257}

Because ethanolamine is found in intestinal epithelial cells and bacterial cell membranes, gut microbiome imbalances have been associated with ethanolamine elevations.^{251-254,258,259}

Low Levels

Decreased precursors, (such as serine), or issues with enzymatic conversion of these precursors may result in lower ethanolamine. This can be clinically problematic given the importance of its role in producing phosphoethanolamine and phospholipids.

Phosphoethanolamine

Phosphoethanolamine is an intermediate in the serine-to-choline sequence. It is both a precursor and byproduct of phospholipid biosynthesis and breakdown. As a precursor to the phospholipid phosphatidylethanolamine, phosphoethanolamine plays a key role in myelination. Elevated phosphoethanolamine reflects brain phospholipid turnover, an indicator of neural membrane synthesis and signal transduction.²⁶⁰ Research into neurologic conditions like Alzheimer's disease and Huntington's disease suggests that depletions of both phosphoethanolamine and ethanolamine accompany neuronal death.²⁶¹

Phosphoethanolamine is also important in cartilage structure and function, especially in bone and teeth.²⁶²

High Levels

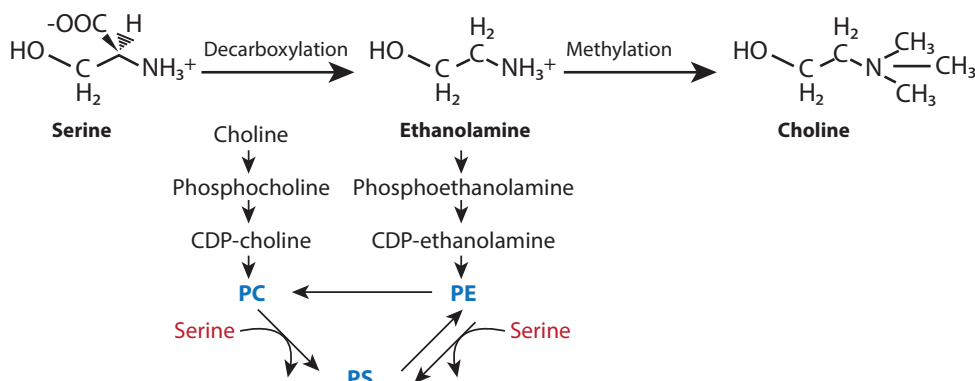
Magnesium and manganese are enzymatic cofactors in the metabolism of phosphoethanolamine. Deficiencies in these nutrients may contribute to elevated levels.^{256,257,262}

The precursor to phosphoethanolamine is ethanolamine. As outlined previously, gut microbiome imbalances can influence ethanolamine levels. With that, elevated phosphoethanolamine has also been associated with gastrointestinal microbiome imbalance.^{263,264}

Clinically, elevated phosphoethanolamine is associated with a rare condition called hypophosphatasia which results in the abnormal development of teeth and bones. Zinc and magnesium deficiencies further complicate this condition.²⁶⁵⁻²⁶⁸

Low Levels

Decreased levels of precursors in the production of phosphoethanolamine, or lack of cofactors needed within the pathway, may contribute to low levels.



Clinically, reduced plasma levels of phosphoethanolamine have been significantly correlated with depressed mood, diminished interest or pleasure, psychomotor change, psychomotor retardation, and major depressive disorder (MDD), making this a potential biomarker for MDD. Habitual alcohol intake was also related to low phosphoethanolamine levels.²⁶⁰

Phosphoserine

Phosphoserine is the phosphorylated ester of the amino acid serine. The addition of a phosphoryl group to an amino acid, or its removal, plays a role in cell signaling and metabolism.

Phosphoserine is a byproduct of glycolysis and subsequent intermediate to then become serine. The enzyme that catalyzes this step, phosphoserine phosphatase, is magnesium dependent.²⁶⁹

This metabolite is not to be confused with a similar-sounding metabolite, phosphatidylserine; this is a common CNS supplement and essential for neuronal cell membranes.²⁷⁰

High Levels

Elevated phosphoserine may be due to a functional lack of magnesium needed to catalyze the enzymatic conversion of phosphoserine to serine.²⁶⁹

Low Levels

The enzyme which connects glycolysis to the formation of phosphoserine (phosphoserine aminotransferase) requires vitamin B₆ as a cofactor. Lack of vitamin B₆ can result in lower phosphoserine levels.²⁴⁹ Supplementation with vitamin B₆ was shown to alter plasma amino acids resulting in increased phosphoserine.⁴²

Sarcosine

Sarcosine is an amino acid made within the methylation cycle when S-adenosylmethionine (SAM) is conjugated with glycine. It can also be made by catabolism of dimethylglycine (DMG). There are many dietary sources of sarcosine including eggs, legumes, nuts, and meats.²⁷¹ Sarcosine is also available as an over-the-counter supplement, and it is widely used in cosmetic formulations (toothpaste, creams, and soaps) and detergents.²⁷¹

In the methylation cycle, sarcosine is created by the GNMT enzyme, which functions to control SAM excess. Some clinicians use sarcosine elevation as a marker of 'excess methyl supplementation' or 'over-methylation.' Currently, there is no literature to support this hypothesis, but rather it is based on physiology.²⁷²

Sarcosine can also be produced through the breakdown of DMG.²⁷³

Sarcosine is a natural glycine transport inhibitor in the CNS, enhancing N-methyl-D-aspartate (NMDA) receptors. NMDA synaptic receptors are not only important for basic CNS functions (breathing, motor function), but also learning, memory, and neuroplasticity. Decreased NMDA function results in cognitive defects, and overstimulation causes excitotoxicity.²⁷⁴ Abnormalities in these receptors are implicated in many diseases and targeted for pharmacologic therapy.²⁷⁵ Sarcosine has been shown to be a co-agonist for NMDA receptors. For this reason, there are many studies evaluating sarcosine as an adjunct treatment for psychiatric diseases, such as schizophrenia, which is characterized by decreased NMDA function. In addition, using sarcosine to enhance NMDA function can improve depression-like behaviors.²³³ Since DMG is essentially sarcosine with an extra methyl group, research shows that they have similar effects.²⁷³

Some studies have evaluated urinary and serum sarcosine's use as a prostate cancer progression marker; however, the data is mixed.²⁷⁶⁻²⁷⁸ These studies are based on nonspecific metabolomic profiling, which followed random metabolite elevation patterns.

High Levels

Elevated sarcosine may be seen with methyl donor supplementation.^{272,279} Dietary intake of sarcosine-rich foods (i.e. eggs, legumes, nuts, and meats) and environmental sources (i.e. toothpaste, creams, and soaps) may result in elevated levels.²⁷¹

Nutrient cofactor deficiencies within the methylation cycle (folate, vitamin B₁₂, and vitamin B₂) can contribute to elevated levels.^{272,280} In fact, folate therapy has been used to normalize sarcosine.^{62,281,282}

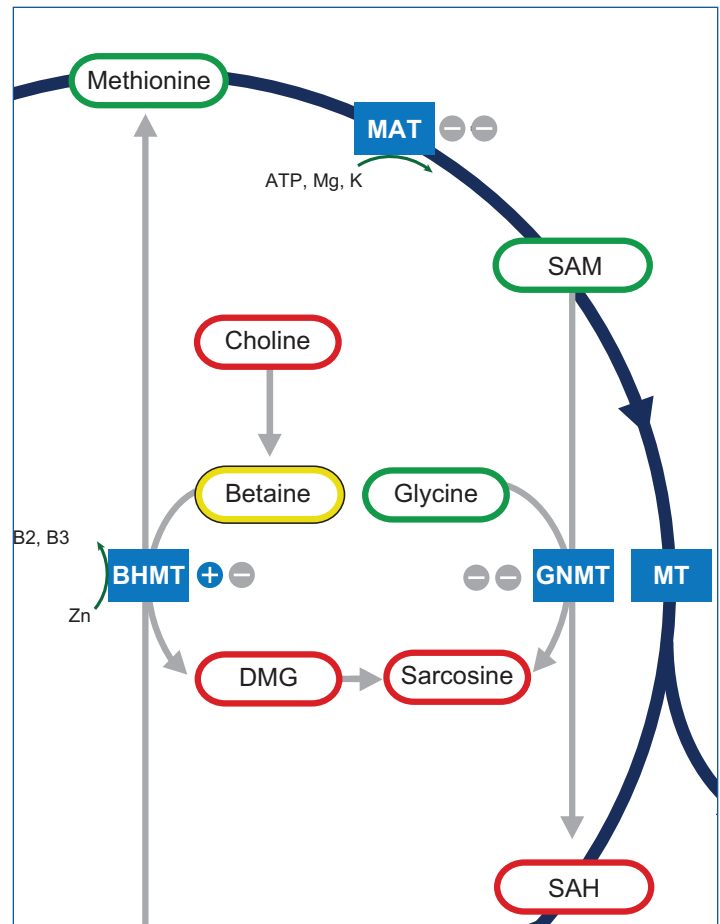
Upregulation or a SNP in the GNMT enzyme within the methylation cycle may contribute to sarcosine elevations.²⁸³

Sarcosine has no known toxicity, as evidenced by the lack of phenotypic expression of inborn errors of sarcosine metabolism.²⁸⁴

Low Levels

The clinical significance of low sarcosine is unknown.

This group of markers relates to the intake of meat, poultry and fish, and may be decreased in vegetarians/vegans.



Dietary Peptide Related Markers

Anserine (dipeptide)

Anserine (beta-alanyl-3-methyl-histidine) is a urinary biomarker from the consumption of poultry and fish.^{46,208,285-287} It is a dipeptide consisting of the amino acids 1-methylhistidine and beta-alanine.⁴⁶ The enzyme carnosine-N-methyl transferase catalyzes the transfer of a methyl group of S-adenosylmethionine (SAM) on carnosine to form anserine.^{288,289}

Anserine acts as an antioxidant, free radical scavenger, and pH buffer. It can reduce blood sugar and affect renal sympathetic nerve activity and blood pressure.^{288,290}

Anserine is measured in FMV urine only.

High Levels

High intake of poultry and fish can cause elevated levels of anserine. Additionally, because anserine is a dipeptide, elevated levels may also reflect incomplete protein digestion into its constituent molecules of beta-alanine and 3-methyl-histidine.

Carnosinase is a zinc- and manganese-dependent enzyme that hydrolyzes both carnosine and anserine.^{288,289} Functional need for zinc and manganese may elevate both markers.

Low Levels

Anserine can be decreased with low protein intake, as seen in vegetarian and vegan diets.

Carnosine (dipeptide)

Carnosine (beta-alanyl-L-histidine) is a urinary biomarker which comes from the consumption of beef, pork, and to a lesser extent, poultry.^{46,208,285,287} It is a dipeptide consisting of the amino acids histidine and beta-alanine and is concentrated in skeletal and heart muscle, brain, and kidneys. Carnosine has antioxidant properties, antiglycation effects, enhanced calcium sensitivity, and pH buffering activity during high-intensity exercise.²⁹¹ It also has neuroprotective properties and may play an important role in Alzheimer's disease and other neurodegenerative diseases.²⁹²⁻²⁹⁵ Carnosine is also protective against secondary diabetic renal complications.^{290,293,296}

Plasma levels are non-detectable in fasting individuals; after beef consumption, postprandial plasma carnosine levels tend to rise then decrease to non-detectable levels within hours of consumption.²⁹⁷ In urine, levels reach a peak after 5 hours, but carnosine is completely excreted within 20-25 hours following the meal.^{46 298}

Carnosine has an affinity to chelate zinc, copper, cobalt, nickel and cadmium.^{289,294} The combination of zinc chelated with L-carnosine has been used therapeutically in the treatment of gastric ulcers.^{288,289}

Carnosine is measured in FMV urine only.

High Levels

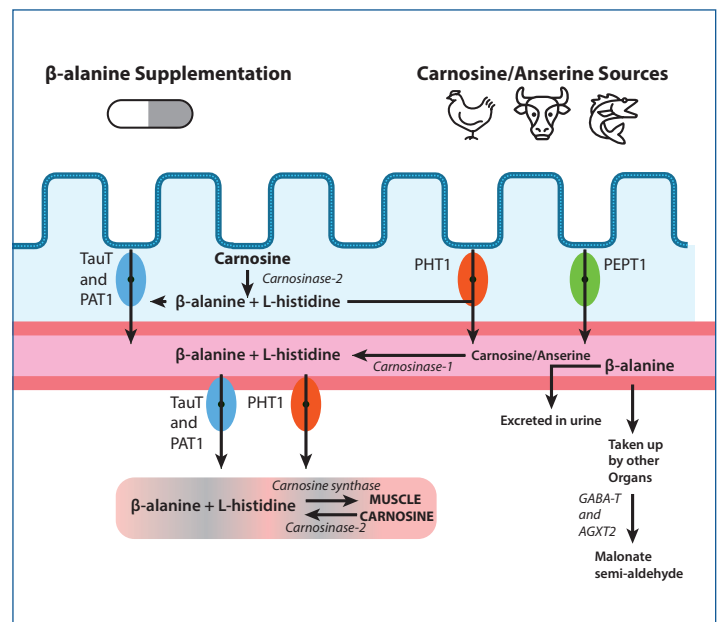
Elevations are likely due to high consumption of meat or beta-alanine supplementation. Since it is a dipeptide, elevations might also signify incomplete protein digestion.

As noted previously, zinc and manganese are important cofactors for the enzyme carnosinase that splits carnosine into the amino acids histidine and beta-alanine.^{288,289,299} Functional need for these nutrients can contribute to elevations of carnosine.

Lastly, carnosinemia/carnosinuria is a rare inborn error of metabolism caused by a deficiency of the enzyme carnosinase.

Low Levels

Carnosine can be decreased with low animal protein intake, as seen in vegetarian or vegan diets.



1-Methylhistidine

1-methylhistidine is derived from the dipeptide anserine (which consists of the amino acids 1-methylhistidine and beta-alanine). Anserine and its derivatives are associated with the consumption of poultry and fish.^{46,208,285-287} Both 1-methylhistidine and 3-methylhistidine have been proposed as markers of meat intake.^{208,209} Note that confusion exists in the literature regarding the numbering of atoms in the imidazole ring of histidine – 1 versus 3 – and thus, there is caution with interpretation and clinical significance of these two markers.^{46,208}

High Levels

Urine and plasma levels of 1-methylhistidine are higher with poultry and fish consumption.^{46,208,300} Since it is a dipeptide, elevations might also signify incomplete protein digestion.

Low Levels (urine)

1-Methylhistidine is decreased with low animal protein intake, as seen in vegetarian and vegan diets.

β-Alanine

β-alanine is a breakdown product of carnosine and anserine, which are dipeptides from meat consumption. Although β-alanine's properties are limited, its relationship to carnosine makes it important. Both have antioxidant properties. And, as previously mentioned, carnosine is critical for pH buffering in skeletal muscle during exercise, but its formation can be limited by enzymatic factors. For this reason, supplementation with β-alanine is sometimes used to enhance carnitine and therefore improve athletic performance.³⁰¹

In addition to diet and supplementation, β-alanine can also be endogenously produced. This occurs via degradation of uracil in the liver but it can also be made by intestinal bacteria such as *E. coli*.³⁰² Since β-alanine comes from meat consumption, endogenous production is the only source in vegetarian and vegan populations. Given their limited diets, vegetarians and vegans have lower levels of β-alanine and muscle carnosine compared to omnivores.²⁹¹

There is also an interesting interplay between taurine and β-alanine. Taurine and β-alanine share the same skeletal muscle transporter, whereby β-alanine can inhibit taurine's uptake into muscle.³⁰³ Elevated beta-alanine can sometimes deplete taurine leading to oxidative stress, causing tissue damage.^{290,304,305} Additionally, these two amino acids compete for the same reabsorption transporters in the kidney. Elevated β-alanine can contribute to renal wasting of taurine.³⁰⁶

High Levels

Levels may be elevated in meat consumption when dipeptides anserine and carnosine are elevated since they both contain β-alanine. Supplementation with β-alanine also results in elevated levels.

Urinary beta-alanine excretion is associated with gut bacterial fermentation and elevated levels may indicate dysbiosis.³⁰² And, as outlined above, elevated β-alanine can contribute to renal wasting of taurine give their unique relationship.

The breakdown and metabolism of β-alanine requires vitamin B₆-dependent enzymes. With that, a functional need for vitamin B₆ can contribute to elevations.³⁰⁷⁻³¹⁰

Lastly, there are very rare inborn errors of metabolism that can cause elevations of β-alanine.

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