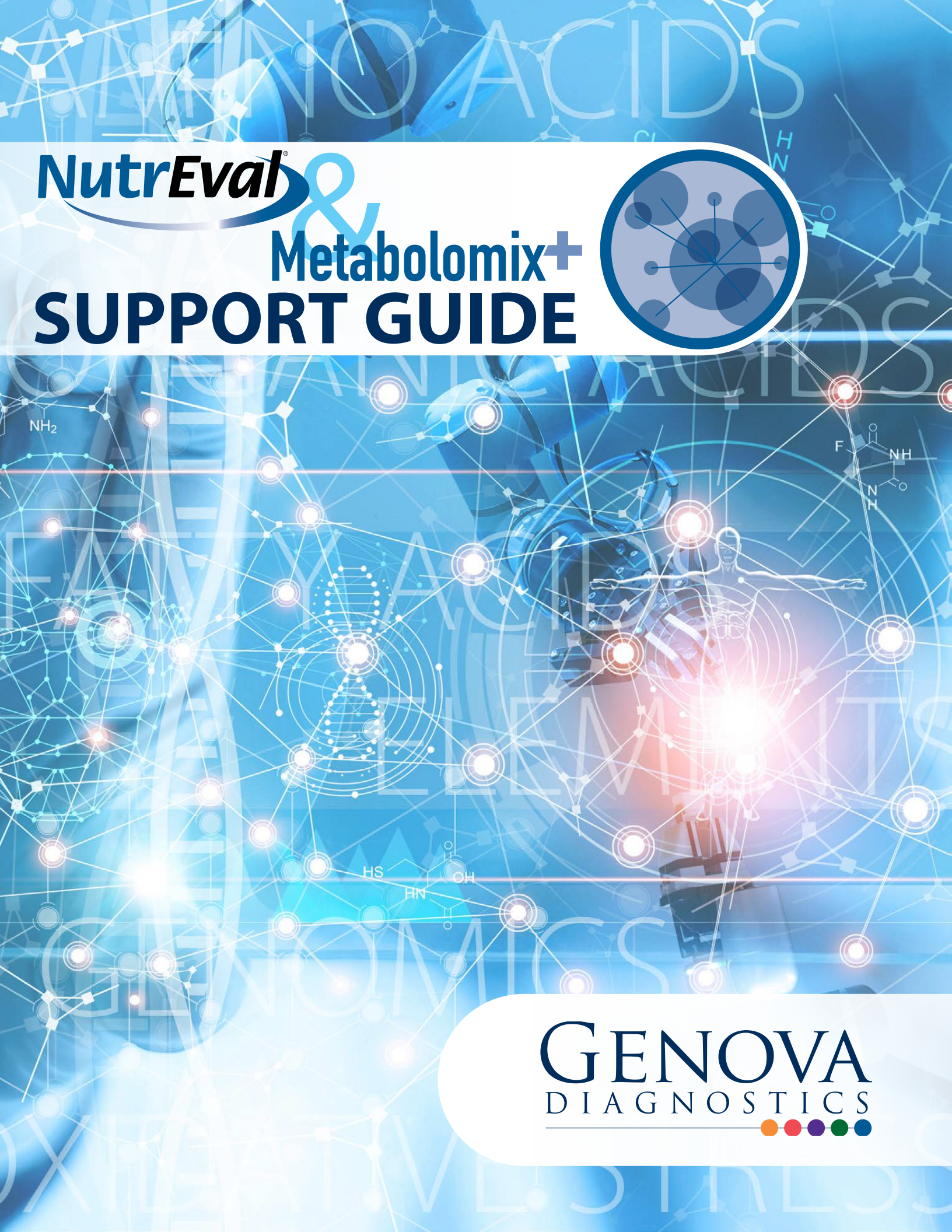
NutrEval® & Metabolomix+ SUPPORT GUIDE



GENOVA DIAGNOSTICS

NutrEval®

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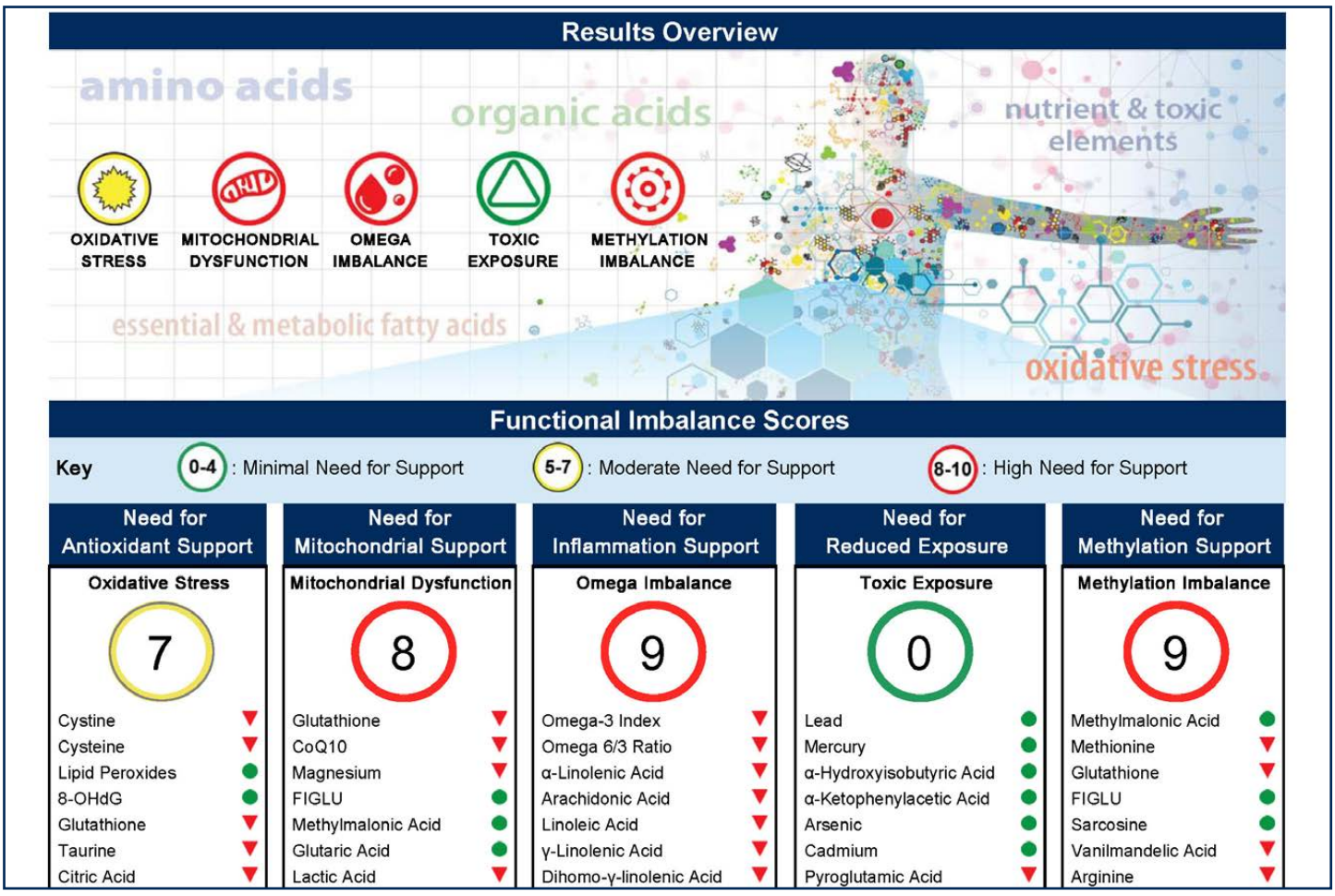
NutrEval Profile

The NutrEval profile is the most comprehensive functional and nutritional assessment available. It is designed to help practitioners identify root causes of dysfunction and treat clinical imbalances that are inhibiting optimal health. This advanced diagnostic tool provides a systems-based approach for clinicians to help their patients overcome chronic conditions and live a healthier life.

The NutrEval assesses a broad array of macronutrients and micronutrients, as well as markers that give insight into digestive function, toxic exposure, mitochondrial function, and oxidative stress. It accomplishes this by evaluating organic acids, amino acids, fatty acids, oxidative stress markers, and nutrient & toxic elements. Subpanels of the NutrEval are also available as stand-alone options for a more focused assessment. The NutrEval offers a user-friendly report with clinically actionable results including:

- Nutrient recommendations for key vitamins, minerals, amino acids, fatty acids, and digestive support based on a functional evaluation of important biomarkers
- Functional pillars with a built-in scoring system to guide therapy around needs for methylation support, toxic exposures, mitochondrial dysfunction, fatty acid imbalances, and oxidative stress
- Interpretation-At-A-Glance pages providing educational information on nutrient function, causes and complications of deficiencies, and dietary sources
- Dynamic biochemical pathway charts to provide a clear understanding of how specific biomarkers play a role in biochemistry

There are various methods of assessing nutrient status, including intracellular, extracellular, direct, and functional measurements. Each method has certain strengths and weaknesses. The NutrEval uses a combination of all these methods and synthesizes the information via an algorithm that determines personalized nutrient needs. The algorithm is based on functional markers shown in the literature to be associated with a need for that particular nutrient.



• Included in profile + Add-on available

Amino Acids, FMV Urine or Plasma	NE FMV	NE PLS	Meta+
β-Alanine	•	•	•
α-Amino-N-butyric Acid	•	•	•
α-Aminoadipic Acid	•	•	•
γ-Aminobutyric Acid	•	•	•
β-Aminoisobutyric Acid	•	•	•
1-Methylhistidine	•	•	•
3-Methylhistidine	•	•	•
Alanine	•	•	•
Anserine	•		•
Arginine	•	•	•
Asparagine	•	•	•
Aspartic Acid	•	•	•
Carnosine	•		•
Citrulline	•	•	•
Creatinine	•	•	•
Cystathionine	•	•	•
Cysteine	•		•
Cystine	•	•	•
Ethanolamine	•	•	•
Glutamic Acid	•	•	•
Glutamine	•	•	•
Glycine	•	•	•
Histidine	•	•	•
Isoleucine	•	•	•
Leucine	•	•	•
Lysine	•	•	•
Methionine	•	•	•
Ornithine	•	•	•
Phenylalanine	•	•	•
Phosphoethanolamine	•	•	•
Phosphoserine	•	•	•
Proline	•	•	•
Sarcosine	•	•	•
Serine	•	•	•
Taurine	•	•	•
Threonine	•	•	•
Tryptophan	•	•	•
Tyrosine	•	•	•
Urea	•	•	•
Valine	•	•	•

Organic Acids	NE FMV	NE PLS	Meta+
α-Hydroxyisobutyric Acid (from MTBE)	•	•	•
α-Keto-β-Methylvaleric Acid	•	•	•
α-Ketoadipic Acid	•	•	•
α-Ketoglutaric Acid	•	•	•
α-Ketoisocaproic Acid	•	•	•
α-Ketoisovaleric Acid	•	•	•
α-Ketophenylacetic Acid (from Styrene)	•	•	•
α-Hydroxybutyric Acid	•	•	•
β-OH-β-Methylglutaric Acid	•	•	•
β-OH-Butyric Acid	•	•	•
3-Hydroxyisovaleric Acid	•	•	•
3-Hydroxyphenylacetic Acid	•	•	•
3-Hydroxyproprionic Acid	•	•	•
3-Methyl-4-OH-phenylglycol	•	•	•
4-Hydroxyphenylacetic Acid	•	•	•
5-OH-indoleacetic Acid	•	•	•
Adipic Acid	•	•	•
D-Arabinitol	•	•	•
Benzoic Acid	•	•	•
Cis-Aconitic Acid	•	•	•
Citramalic Acid	•	•	•
Citric Acid	•	•	•
DHPPA	•	•	•
Formiminoglutamic Acid	•	•	•
Glutaric Acid	•	•	•
Hippuric Acid	•	•	•
Homovanillic Acid	•	•	•
Indoleacetic Acid	•	•	•
Isocitric Acid	•	•	•
Isovalerylglycine	•	•	•
Kynurenic / Quinolinic Ratio	•	•	•
Kynurenic Acid	•	•	•
Lactic Acid	•	•	•
Malic Acid	•	•	•
Methylmalonic Acid	•	•	•
Orotic Acid	•	•	•
Phenylacetic Acid	•	•	•
Pyroglutamic Acid	•	•	•
Pyruvic Acid	•	•	•
Quinolinic Acid	•	•	•
Suberic Acid	•	•	•
Succinic Acid	•	•	•
Tartaric Acid	•	•	•
Vanilmandelic Acid	•	•	•
Xanthurenic Acid	•	•	•

Oxalate Markers	NE FMV	NE PLS	Meta+
Glyceric Acid	•	•	•
Glycolic Acid	•	•	•
Oxalic Acid	•	•	•
Oxidative Stress Analysis			
Glutathione (Whole Blood)	•	•	
Coenzyme Q10 (Ubiquinone)	•	•	
Lipid Peroxides, Urine	•	•	•
8-OHdG, Urine	•	•	•
Essential & Metabolic Fatty Acids Analysis			
α-Linolenic Acid	•	•	+
γ-Linolenic Acid	•	•	+
AA/EPA ratio	•	•	+
Arachidic Acid	•	•	+
Arachidonic Acid	•	•	+
Behenic Acid	•	•	+
Dihomo-γ-linolenic Acid	•	•	+
Docosahexaenoic Acid	•	•	+
Docosapentaenoic Acid	•	•	+
Docosatetraenoic Acid	•	•	+
Eicosadienoic Acid	•	•	+
Eicosapentaenoic Acid	•	•	+
Elaidic Acid	•	•	+
Lignoceric Acid	•	•	+
Linoleic Acid	•	•	+
Linoleic/DGLA ratio	•	•	+
Margaric Acid	•	•	+
Nervonic Acid	•	•	+
Oleic Acid	•	•	+
Omega 3 Index	•	•	+
Omega 6s/Omega 3s ratio	•	•	+
Palmitic Acid	•	•	+
Palmitoleic Acid	•	•	+
Pentadecanoic Acid	•	•	+
Stearic Acid	•	•	+
Tricosanoic Acid	•	•	+
Vaccenic Acid	•	•	+

Nutrient Elements	NE FMV	NE PLS	Meta+
Calcium			+
Chromium			+
Cobalt			+
Copper	•	•	+
Iron			+
Lithium			+
Magnesium	•	•	+
Manganese	•	•	+
Molybdenum			+
Potassium	•	•	+
Selenium	•	•	+
Strontium			+
Sulfur			+
Vanadium			+
Zinc	•	•	+
Toxic Elements•			
Lead	•	•	+
Mercury	•	•	+
Aluminium			+
Antimony			+
Arsenic	•	•	+
Barium			+
Bismuth			+
Cadmium	•	•	+
Cesium			+
Gadolinium			+
Nickel			+
Niobium			+
Platinum			+
Rubidium			+
Thallium			+
Thorium			+
Tin			+
Tungsten			+
Uranium			+
Add-on Testing			
Vitamin D (serum sample)	+	+	
Genomic Add-on Markers			
APO E (C112R + R158C)	+	+	+
COMT (V158M)	+	+	+
MTHFR Combined (A1298C + C677T)	+	+	+
TNFα	+	+	+

What is a functional nutritional assessment?

A functional nutrition assessment looks at metabolic intermediates produced in enzymatic pathways of cellular energy production, detoxification, neurotransmitter breakdown, and amino acid metabolism. Elevated metabolite levels may signal a metabolic inhibition or block. This abnormality may be due to a nutrient deficiency, an inherited enzyme deficit, toxic build-up, or drug effect. It is possible for an individual to have normal blood levels of a vitamin in order to maintain homeostasis, while exhibiting signs of insufficiency/deficiency for that vitamin. For this reason, direct testing of individual nutrients alone does not provide a complete picture.

What is included on the NutrEval profile?

- **Organic Acids** provide insight into key metabolic irregularities that relate to potential nutritional cofactor needs, digestive irregularities, cellular energy production, neurotransmitter metabolism, and detoxification.
- **Oxidative Stress** markers indicate problems in two key areas: antioxidant capacity and oxidative damage. Oxidative stress is relevant to an entire host of clinical conditions. It reflects a need to support with antioxidant intervention along with the reduction of free radical exposure.
- **Amino Acids** are important in many different clinical conditions. The NutrEval looks at essential and nonessential amino acids as well as markers that may indicate poor digestion, absorption, or metabolism of the amino acids.
- **Fatty Acids** reflect intake and metabolism of essential fatty acids which are relevant to many processes include inflammatory balance, cell membrane fluidity, cell signaling, among others. This assessment helps clinicians to determine appropriate nutritional interventions to correct EMFA imbalances.
- **Nutrient & Toxic Elements** provide a window into short-term exposure to various toxins such as mercury, lead, cadmium, and arsenic. Also included are direct evaluations of key minerals such as magnesium, potassium, zinc, copper, and selenium to further determine nutritional adequacy.

When should NutrEval testing being considered?

Metabolism is a complex process revealing how vitamins, minerals, protein, fats, and carbohydrates are used to perform thousands of critical biochemical reactions. Nutrient insufficiencies can lead to biochemical disturbances that affect healthy cellular and tissue function, potentially leading to disease.

Clinical conditions where the NutrEval may offer further insight include:

- Mood disorders[1-4]
- Cardiovascular disease[5-7]
- Diabetes/insulin resistance/metabolic syndrome[8-11]
- Fatigue[12,13]
- Obesity/weight issues/need for dietary guidance[11,14,15]
- Malnutrition[16,17]
- Maldigestion and malabsorption
- Cognitive decline[18,19]
- Athletic optimization[20-23]
- Increased nutrient demand in physical trauma/healing[24,25]

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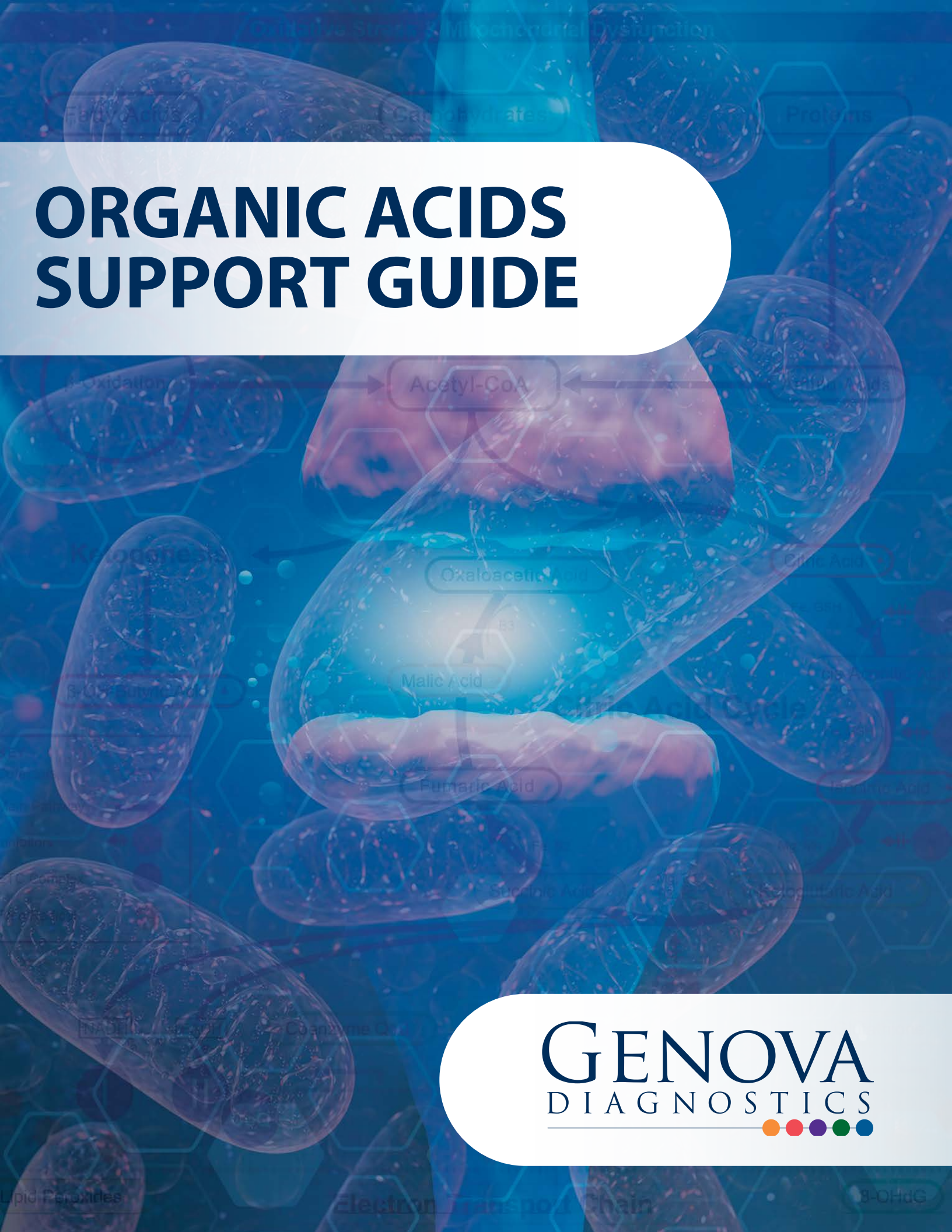
ORGANIC ACIDS SUPPORT GUIDE



GENOVA DIAGNOSTICS

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Organic Acids
NUTRITIONAL

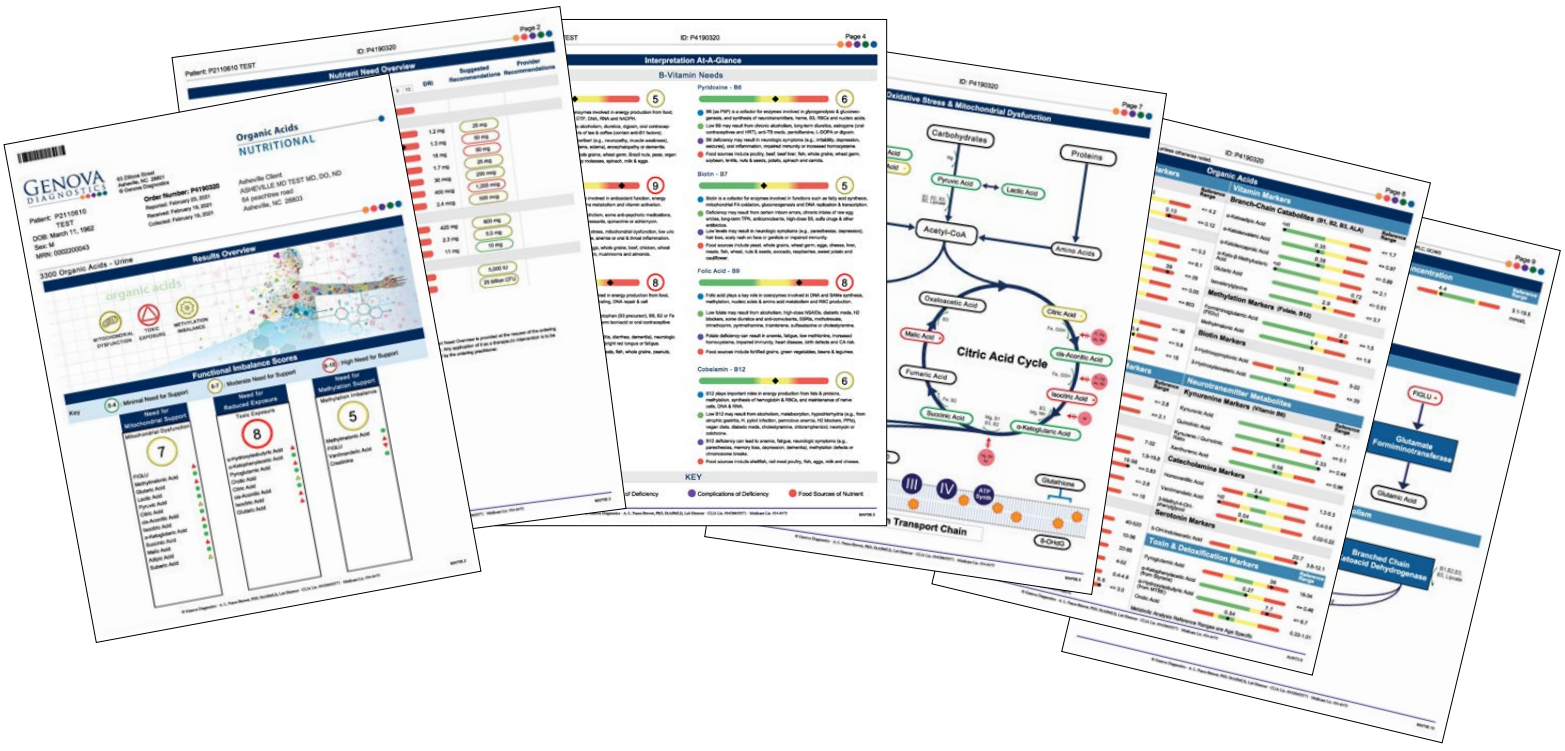
The **Genova Organic Acids** is a functional nutritional assessment of urinary organic acids. Organic acids are a broad class of compounds formed during fundamental metabolic processes in the body. Metabolic reactions produce carboxylic acid compounds derived from the digestion of dietary protein, fat, and carbohydrates. The resulting organic acids are used by the body to generate cellular energy and provide many of the building blocks necessary for cell function. Organic acids are also produced from gut microbiome metabolism, neurotransmitter metabolism, and during detoxification, and provide insight into possible need for support in those areas.

What is a functional assessment?

The quantitative measurement of specific organic acids in the urine offers a functional assessment of nutrient status. Enzymes that are responsible for metabolizing organic acids are vitamin and mineral dependent. With this, elevations in organic acids can speak to a functional need for these nutrients on a cellular and biochemical level, even despite normal serum levels.[1-7] Recommendations for nutrient supplementation based on elevated organic acid results are generated using a literature-based proprietary algorithm.

The Organic Acids report categorizes results into major metabolic areas:

- Malabsorption and Dysbiosis Markers
- Cellular Energy and Mitochondrial Markers
- Vitamin Markers
- Neurotransmitter Metabolites
- Toxin and Detoxification Markers
- Oxalate Markers



Malabsorption and Dysbiosis Markers

The compounds of bacterial and yeast origin are byproducts of bacterial and fungal activity in the GI tract.[8,9] Many of these bacterial metabolites can result from the fermentation of dietary phenols and flavonoids. Therefore, in the absence of dysbiosis, high levels of these phenolic metabolites can reflect a healthy intake of antioxidant-rich foods.[10]

Malabsorption and dysbiosis markers are usually evaluated as a group for overall trends rather than individually. When multiple markers are elevated, a stool test may provide further information regarding dysbiosis or other GI dysfunction.

MALABSORPTION MARKERS

Indoleacetic Acid

Indoleacetic acid (IAA), or indole-3-acetate, is produced by the bacterial fermentation of the amino acid tryptophan.[11] IAA can be formed from several common gut microbes such as *Clostridia* species, *Escherichia coli,* and *Saccharomyces* species.[12-14]

High Levels:

Elevated IAA in the urine suggests incomplete digestion and absorption of tryptophan in the intestine, allowing colonic bacteria to convert tryptophan to IAA. Elevations may also reflect an overgrowth of bacteria acting on tryptophan.

Clinical Associations:

IAA elevations and altered tryptophan metabolism have been associated with systemic inflammation, psychologic and cognitive function, autism, and chronic diseases such as cardiovascular disease.[15-17] Hartnup's disease, a genetically-linked dysfunction in the transport of free-form amino acids across the intestinal mucosa, can cause severe elevations of urinary IAA.[18]

Phenylacetic Acid

Phenylacetic acid (PAA) is produced by the bacterial metabolism of phenylalanine. Several bacterial strains are known to produce PAA, including *Bacteroidetes* and *Clostridium* species.[9] Dietary polyphenols may also contribute to PAA elevation.[19]

High Levels:

Elevated PAA in the urine suggests incomplete digestion and absorption of phenylalanine in the intestine, allowing colonic bacteria to convert phenylalanine to PAA. Elevations may also reflect an overgrowth of bacteria, which convert phenylalanine to PAA. Several bacterial strains are known to produce PAA, including *Bacteroidetes* and *Clostridium* species.[9] Dietary polyphenols may also contribute to PAA elevation.[19]

Clinical Associations:

There is a clinical correlation between decreased urinary PAA and depressive symptoms.[20-22]

DYSBIOSIS MARKERS

Dihydroxyphenylpropionic Acid (DHPPA)

Dihydroxyphenylpropionic Acid (DHPPA), also known as **3,4 dihydroxyphenylpropionic acid**, is a byproduct of the fermentation of dietary phenols by several bacteria, including some *Clostridia* spp. and others. Although once thought to identify the presence of specific dysbiotic bacteria, ongoing research suggests there are several bacterial species potentially involved.

High Levels:

Elevated DHPPA levels may reflect dietary intake of polyphenols. They may also suggest dysbiosis or bacterial overgrowth, increasing dietary polyphenol conversion.

3-Hydroxyphenylacetic Acid and 4-Hydroxyphenylacetic Acid

3-Hydroxyphenylacetic acid and **4-hydroxyphenylacetic acid** are produced by the bacterial fermentation of amino acids, much like IAA. [9,12]

High Levels:

Amino acids that are not digested and absorbed can be metabolized by bacteria in the gut to form these organic acids. Clinicians often use these markers to reflect protein malabsorption or dysbiosis. However, dietary intake of polyphenols such as wine, grapes, green tea, and grape seed extract can also contribute to increased levels.[23-26]

Clinical Associations:

These organic acid byproducts may exhibit free radical scavenging properties, which lends to further support for use of these organic acid markers as an indication of antioxidant consumption.[27-29]

Much like IAA and PAA, there is an inverse correlation between these markers and depressive symptoms.[20-22]

Benzoic Acid and Hippuric Acid

Benzoic acid and hippuric acid are formed from the bacterial metabolism of polyphenols. Urinary benzoic acid may also come from ingestion of food preservatives such as sodium benzoate. Hippuric acid is made when sodium benzoate is conjugated with glycine.[30]

High Levels:

Increased metabolism by imbalanced gut flora may increase levels. Additionally, dietary intake of polyphenols or food preservatives can also increase levels of these organic acids.

Clinical Associations:

Elevated levels of urinary hippuric acid have been associated with several clinical conditions that may be linked to dysbiosis.[31,32] For example, elevated urinary hippurate was associated with an increase in blood pressure, likely due to the direct effect of gut-microbial products on blood pressure. However, in other studies low hippuric acid excretion has also been attributed to dysbiosis, which supports its use as a biomarker for general microbial alterations.[33]

YEAST/FUNGAL DYSBIOSIS MARKERS

D-arabinitol

D-arabinitol is a sugar alcohol produced specifically by *Candida* spp.[34,35] The majority of the published literature shows a correlation between serum or urinary D-arabinitol levels and systemic invasive candidiasis in immunocompromised individuals.[35] Several articles have suggested that D-arabinitol is a useful marker for diagnosis of candidiasis in this patient population as well as potentially be a prognostic indicator in a broad range of conditions. While discrete literature evaluating the clinical application to GI candidiasis has not been conducted, D-arabinitol has been used as a functional indicator of relevant clinical *Candida* overgrowth owing to the existing body of literature. Given that only certain *Candida* species produce D-arabinitol, it may serve as an indirect assessment for subclinical candidiasis.

High Levels:

Elevated D-arabinitol may indicate *Candida* overgrowth. Probiotics were shown to reduce urinary D-arabinitol levels in children with autism.[36] A direct evaluation via stool testing should be considered as an appropriate follow-up to elevated D-arabinitol and a clinical suspicion of GI candidiasis.

Citramalic Acid and Tartaric Acid

Citramalic acid and tartaric acid are yeast metabolites that are also influenced by dietary intake of fruits, wine, and sugars.[37-41]

High Levels:

Though often used by clinicians to gain insight into yeast overgrowth, it should be noted that fruit intake can influence levels. High levels may simply reflect a high dietary fruit intake. A high intake of sugars feeds gastrointestinal yeast, which can promote yeast overgrowth. When these markers are elevated, and dietary influences have been ruled out, a stool test may be warranted to evaluate the presence of yeast in the GI tract.

As noted, the malabsorption and dysbiosis marker levels can also be influenced by common foods, supplements, or preservatives; correlation with the patient's dietary intake is encouraged. [25,26,37-40,42-61]

Urinary Metabolite	Common Dietary Sources
Indoleacetic acid	High tryptophan intake, green/black tea
Phenylacetic acid	Wine/grapes
Dihydroxyphenylpropionic acid	Whole grains, chocolate, coffee, green/black tea, olives/olive oil, citrus fruits (animal studies)
3-Hydroxyphenylacetic acid & 4-hydroxyphenlyacetic acid	Wine/grapes, cranberries, green/black tea, berries, orange juice, grape seed extract
Benzoic acid/Hippuric acid	Orange juice, elderberry, huckleberry, food preservative, berries, other flavonoids
Citramalic acid	Apples, cranberries, sugar beets
Tartaric acid	Wine/grapes, chocolate, food additive/preservative

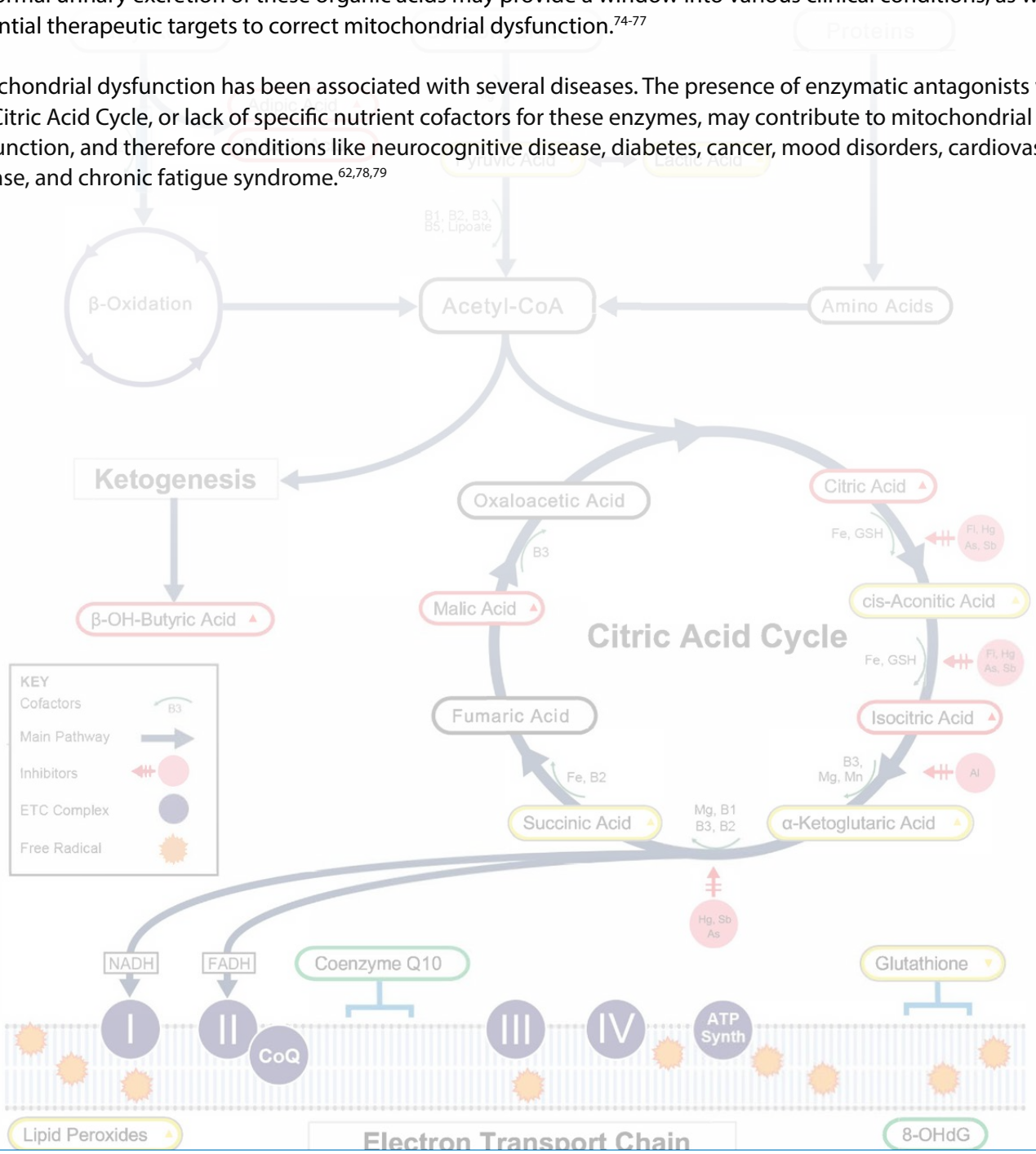
Cellular Energy and Mitochondrial Markers

The cellular energy and mitochondrial metabolite markers reflect the body's ability to process dietary macronutrients to feed the Citric Acid Cycle and subsequent energy production. Abnormalities throughout the Citric Acid Cycle, as well as in fatty acid oxidation, glycolysis, and protein metabolism may reflect enzymatic dysfunction and functional nutrient insufficiencies.

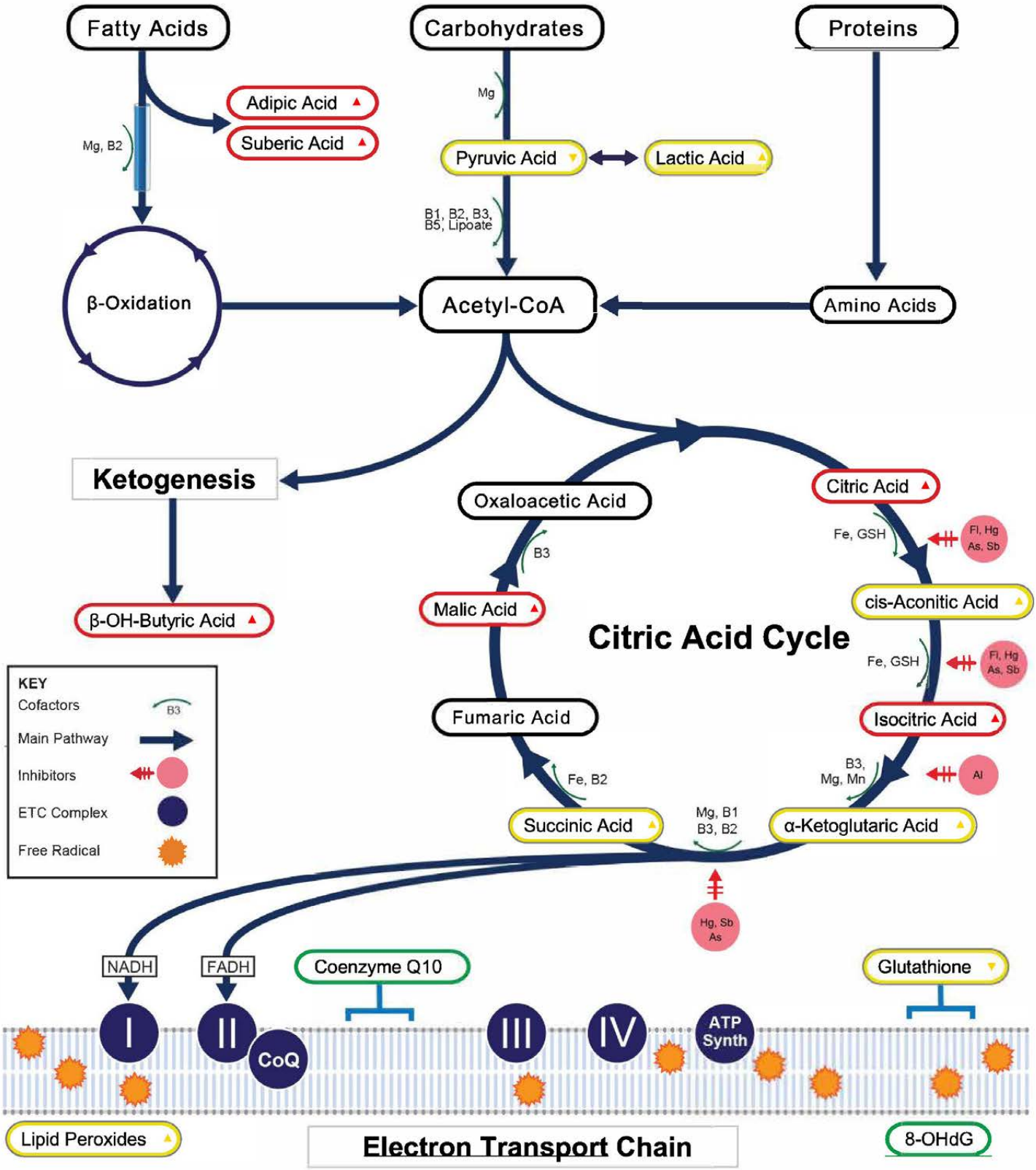
Various factors can alter mitochondrial enzymes such as nutrient and vitamin deficiency, toxins, genetic polymorphisms, and underlying disease. The enzymes catalyzing the transformation of these Citric Acid Cycle intermediates require a variety of nutrient cofactors, such as iron, niacin, magnesium, manganese, thiamin, riboflavin, pantothenic acid, and lipoic acid.[62-72] Toxic exposures and metals including, but not limited to, mercury, arsenic, and lead can interfere with mitochondrial function.[62,63,73]

Abnormal urinary excretion of these organic acids may provide a window into various clinical conditions, as well as potential therapeutic targets to correct mitochondrial dysfunction.[74-77]

Mitochondrial dysfunction has been associated with several diseases. The presence of enzymatic antagonists within the Citric Acid Cycle, or lack of specific nutrient cofactors for these enzymes, may contribute to mitochondrial dysfunction, and therefore conditions like neurocognitive disease, diabetes, cancer, mood disorders, cardiovascular disease, and chronic fatigue syndrome.[62,78,79]



Oxidative Stress & Mitochondrial Dysfunction



FATTY ACID METABOLISM

Adipic and Suberic Acid

Dietary fatty acids are metabolized into fuel sources using beta-oxidation. Fatty acid conversion into Acetyl-CoA requires transport across the mitochondrial membrane via the carnitine shuttle.[80] When beta-oxidation is impaired, fats are metabolized using an alternate pathway called omega-oxidation. Omega-oxidation results in elevated levels of dicarboxylic acids such as adipic acid and suberic acid.

Impaired beta-oxidation occurs in carnitine deficiency or enzymatic dysfunction due to lack of nutrient cofactors.[81,82] Vitamin B_2 and magnesium play a role in optimizing beta-oxidation.[83-88]

High Levels:

Elevated levels of adipic and suberic acid may reflect insufficient carnitine or lack of nutrient cofactors for proper beta-oxidation.[86,88,89]

Clinical Associations:

Increased omega-oxidation metabolites can be seen in ketosis, insulin resistance, diabetes, fasting, or low carbohydrate intake. Elevations of suberic and adipic acid can lead to further mitochondrial dysfunction by injuring the cell membrane and producing free-radical damage.[80,90,91]

CARBOHYDRATE METABOLISM

Lactic Acid and Pyruvic Acid

Lactic Acid and Pyruvic Acid are byproducts of glycolysis. Carbohydrates, which contain glucose, are broken down through glycolysis to form pyruvate and two ATP molecules. Pyruvate can also be generated through the catabolism of various amino acids, including alanine, serine, cysteine, glycine, tryptophan and threonine.[92] Magnesium is an important cofactor for a number of glycolytic enzymes necessary to produce pyruvate.[93] Optimally, pyruvic acid is oxidized to form Acetyl-CoA to be used aerobically via the Citric Acid Cycle to produce energy. In an anaerobic state, lactic acid is formed instead.

High Levels:

An elevated pyruvic acid would reflect an inability to form Acetyl-CoA to feed the Citric Acid Cycle. Pyruvate uses the pyruvate dehydrogenase complex to form Acetyl-CoA. A different enzyme, pyruvate carboxylase, is responsible for the conversion of pyruvate into oxaloacetate. Nutrient cofactors, such as vitamin-B_1, B_2, B_3, B_7, magnesium, and lipoate are needed to support the pyruvate dehydrogenase and pyruvate carboxylase enzymes.[62,64,94-97] Insufficiency in any of these nutrients can raise levels of pyruvic acid. In vitro studies have shown there are some toxins that can also affect these enzymes, such as antimony, mercury, and cadmium.[98,99] Pyruvate elevations can also be seen with a high intake of carbohydrates, as well as rare genetic forms of pyruvate dehydrogenase deficiency.[92]

Any anerobic or low oxygen state, including pulmonary disease, anemia, sleep apnea, among others can lead to elevations of lactic acid. Elevations of urinary lactic acid can also be the result of strenuous exercise, insulin resistance, dysglycemia, and alcohol dependence.[100-104] Zinc is an essential component in the enzymes which regulate glycolysis, such as lactate dehydrogenase (LDH). LDH converts lactate back to pyruvate in the liver via the Cori cycle.[92,105,106] Elevations may be seen with a functional need for zinc.

Low Levels:

Low levels of pyruvic acid might imply low carbohydrate intake, lack of magnesium cofactors for glycolytic enzymes, or lack of insulin.[93,107]

Clinical Associations:

Pyruvate metabolism abnormalities play important roles in cancer, heart failure, and neurodegeneration.[92]

α-hydroxybutyric Acid

α-hydroxybutyric acid (2-hydroxybuturic acid [2-HB]) is a marker that relates to oxidative stress. 2-HB is an organic acid produced from α-ketobutyrate via the enzymes lactate dehydrogenase (LDH) or α-hydroxybutyrate dehydrogenase (HBDH). These enzymes are catalyzed by NADH. Oxidative stress creates an imbalance in NADH/NAD ratios, which leads directly to the production of 2-HB. Being that 2-HB's precursor α-ketobutyrate is a byproduct in the glutathione (GSH) synthesis pathway, an increased demand for GSH may ultimately result in increased 2-HB. Increased oxidative stress associated with insulin resistance increases the rate of hepatic glutathione synthesis. Plasma 2-HB is highly associated with insulin resistance and may be an effective biomarker for prediabetes.[108,109] A study on type 2 diabetics showed that GSH infusion restored the NADH/NAD balance and resulted in improvement of insulin sensitivity and beta cell function.[110]

High levels:

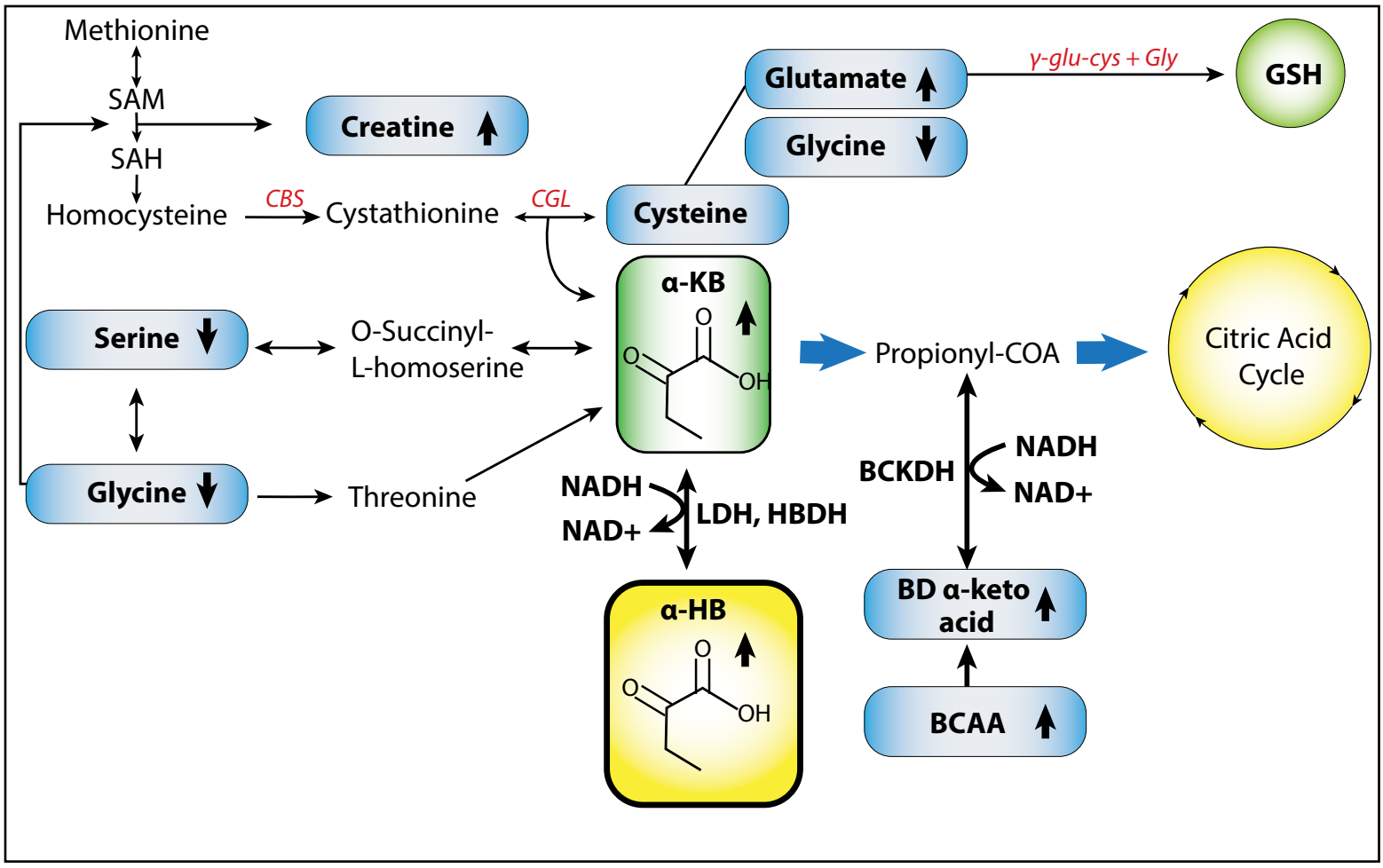
Higher circulating levels of 2-HB are associated with insulin resistance and prediabetes.[108,109]

Elevated α-hydroxybutyric acid may be seen with oxidative stress. Evaluate oxidative stress markers such as lipid peroxides and 8-hydroxydeoxyguanosine (8-OHdG) and ensure adequate antioxidant intake and glutathione status.

Hard physical exercise can result in lactic acidosis and accumulation of 2-HB.[111]

Low levels:

There are no known clinical associations with low levels of α-hydroxybutyric acid.



β-hydroxybutyric Acid

β-hydroxybutyrate is a ketone body. During periods of fasting, exercise, and metabolic disease, ketone bodies are generated in the liver and become an energy source instead of glucose.

High Levels:

Low carbohydrate intake and ketogenic diets may increase urinary levels of beta-hydroxybutyrate. The severity of ketosis is not accurately reflected by the degree of ketonuria. Only a small amount of the body burden of ketones is excreted in the urine; most must be oxidized in extrahepatic tissues using and depleting available oxygen.

Clinical Associations:

In the absence of dietary influence, elevations are sometimes used as an early indicator of diabetes, impaired glucose tolerance, and worsening glycemic control.[112-114]

β-hydroxy-β-methylglutaric Acid

β-hydroxy-β-methylglutaric acid (HMG) is a precursor to cholesterol and coenzyme Q10 (CoQ10) synthesis. It is a product of hydroxymethylglutaryl-coenzyme A (HMGCoA). HMGCoA- reductase is a rate limiting enzyme in cholesterol production. Medications that interfere with this enzyme may result in elevated HMG and subsequent low levels of cholesterol and CoQ10.[115] CoQ10 is important for cellular energy production in the mitochondrial respiratory chain.

High Levels:

Urinary β-hydroxy-β-methylglutaric acid is often elevated in patients taking statin medications and red yeast rice. CoQ10 supplementation has been shown to help ameliorate statin-associated myopathies.[116]

There are also inborn errors of metabolism which can elevate HMG. These affect the HMGCoA reductase enzyme with varying degrees of onset and clinical manifestations such as neurodevelopmental disorders and cardiomyopathy.[117]

ENERGY METABOLISM (CITRIC ACID CYCLE)

Citric Acid, Isocitric Acid, and cis-Aconitic Acid

A two-carbon group from Acetyl-CoA is transferred to oxaloacetate to form citric acid. Citric acid is then converted to isocitric acid through a cis-aconitic intermediate using the enzyme aconitase. Aconitase is an iron-sulfate protein that controls iron homeostasis.[118]

High Levels:

Iron deficiencies and overload at the systemic or cellular levels can negatively impact the aconitase enzyme and overall mitochondrial health and function.[119] Due diligence with iron assessment is recommended when levels of these organic acids are abnormal. Glutathione may also be an important means of modulating aconitase activity during oxidative stress.[120] Various toxins may influence mitochondrial enzymes and contribute to mitochondrial dysfunction, such as fluoride, aluminum, mercury, arsenic, and tin.[121-124]

Low Levels:

Low levels of these analytes may reflect insufficient precursors, or suboptimal glycolysis or fatty acid oxidation.

α-Ketoglutaric Acid

Isocitric Acid is converted to **α-ketoglutaric acid** using the enzyme isocitrate dehydrogenase. Alpha-ketoglutarate is a rate-determining intermediate in the Citric Acid Cycle[125] and provides an important source of glutamine and glutamate that stimulates protein synthesis and bone tissue formation, inhibits protein degradation in muscle, and constitutes an important metabolic fuel for cells of the gastrointestinal tract.[125] Alpha-ketoglutaric acid is then converted to Succinyl CoA using the enzyme alpha-ketoglutarate dehydrogenase. This enzyme complex is very similar to the pyruvate dehydrogenase complex with similar nutrient cofactor needs.

High Levels:

Elevations can be seen with nutrient cofactor deficiencies needed for the enzymatic conversion of α-ketoglutarate such as vitamin B_3, zinc, magnesium, and manganese. Higher levels are seen in mitochondrial oxidative phosphorylation disorders and mitochondrial dysfunction.[126] Genetic abnormalities with the enzyme itself can also limit conversion of alpha-ketoglutarate, causing elevations.[127]

Low Levels:

Low levels of α-ketoglutarate may reflect lack of precursors higher up from enzymatic dysfunction due to lack of nutritional cofactors, genetic defects, or toxin exposures.

Succinic Acid

Succinyl CoA becomes succinic acid using succinyl CoA synthetase. This reaction produces NADH which directly provides electrons for the electron transport chain or respiratory chain.[127]

Succinic acid requires the enzyme succinate dehydrogenase to become fumarate. This enzyme is iron-based and requires vitamin B_2 to support flavin adenine dinucleotide (FAD) as a redox coenzyme.[128] Succinate dehydrogenase plays a critical role in mitochondrial metabolism. Impairment of this enzyme's activity has been linked to a variety of diseases such as cancer and neurodegenerative diseases.[129]

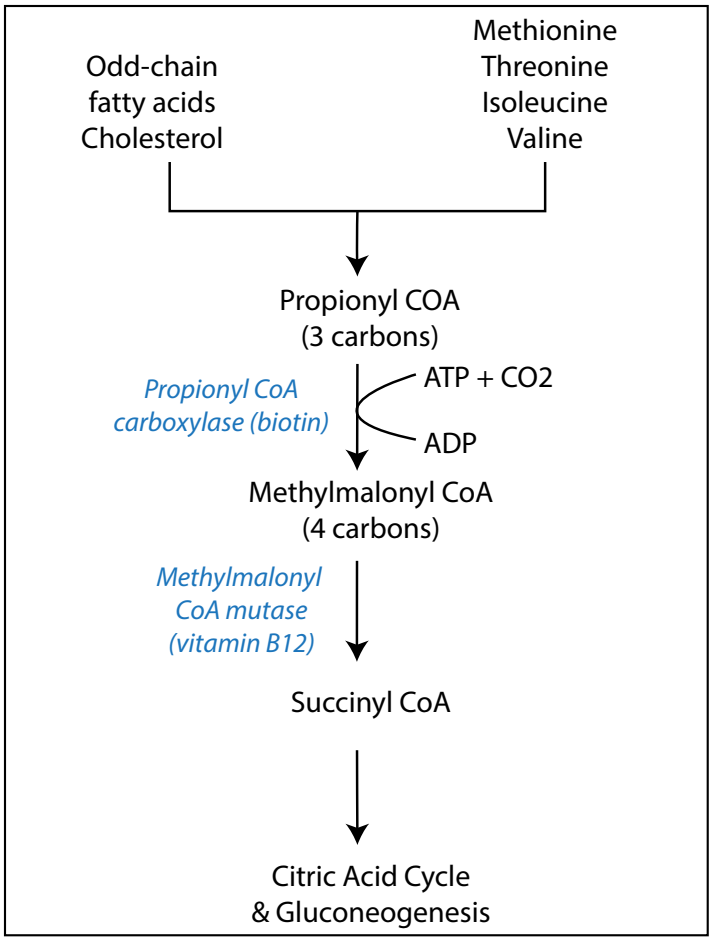
High Levels:

Elevated levels of mitochondrial succinate are seen in nutritional cofactor insufficiencies of succinate dehydrogenase or primary enzymatic defects. Succinate can also be formed peripherally by microbes in the GI tract. The major producers of succinate in the gut are bacteria belonging to the Bacteroidetes phylum. However, it is typically detected at low rates in the gut lumen because it is rapidly converted to propionate, a major short chain fatty acid.[130]

Several studies indicate that elevations in both succinate and fumarate play a role in oncogenesis by causing DNA damage and hypermethylation.[131]

Low Levels:

Low levels of succinic acid can be seen with poor dietary intake or absorption of branched-chain amino acids. Branched-chain amino acids are catabolized to acetyl-CoA or succinyl-CoA to feed the Citric Acid Cycle. Additionally, vitamin B_{12} deficiency can induce a defect in the conversion of methylmalonyl-CoA to succinyl-CoA at the distal end of the valine and isoleucine pathways which can then decrease succinyl-CoA.[132]



Malic Acid

Fumaric acid uses the fumarase enzyme to become malic acid. Malate dehydrogenase catalyzes the conversion of malic acid into oxaloacetate. Two forms of this enzyme exist in eukaryotes. One operates within the mitochondria to contribute to the Citric Acid Cycle; the other is in the cytosol where it participates in the malate/aspartate shuttle.[133] Riboflavin is an important cofactor for this enzyme and overall mitochondrial energy production and cellular function.[134]

At the end of each Citric Acid Cycle, the four-carbon oxaloacetate has been regenerated, and the cycle continues.

High Levels:

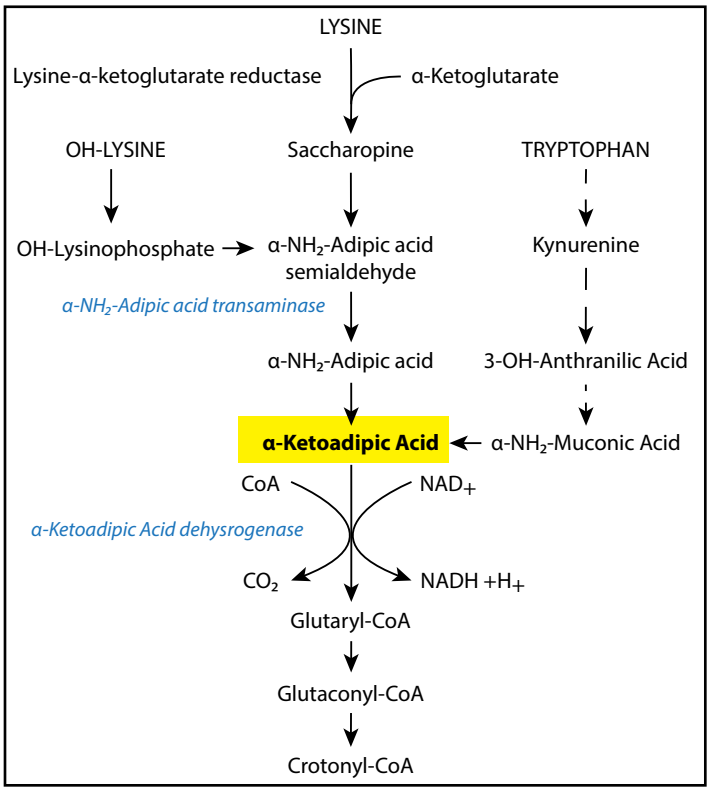
High levels of malic acid can be seen if its dehydrogenation to oxaloacetic acid is reduced from lack of vitamin B_3 as NAD. Malic acid also has many food sources, such as vegetables, as well as fruits like apples and pears. It is also an additive and preservative in beverages, throat lozenges, and syrups.[135]

There are groups of organic acids commonly used to assess the status of specific B-vitamins. By measuring organic acids that are known to rely on specific nutrients for enzymatic metabolism, clinicians can gain insight into functional vitamin needs.

BRANCHED-CHAIN CATABOLITES

α-Ketoadipic Acid

α-Ketoadipic Acid (AKAA; 2-Oxoadipic acid, 2-Ketoadipic acid) is an organic acid formed from α-aminoadipic acid (which originates with lysine) and also from α-aminomuconic acid (derived from tryptophan).[136] AKAA metabolizes to form glutaryl-CoA via oxidative decarboxylation. The cofactors needed in this step are Coenzyme A, NAD, thiamine pyrophosphate (vitamin B_1), lipoic acid, and vitamin B_2.[189]



High Levels:

Elevations in urinary AKAA may reflect enzymatic dysfunction due to nutritional cofactor needs.[72] Mitochondrial oxidative phosphorylation disorders are also associated with higher levels of AKAA.[126]

α-Ketoisovaleric Acid, α-Ketoisocaproic Acid α-Keto-β-Methylvaleric Acid

Of the essential amino acids, there are three branched-chain amino acids (leucine, isoleucine, and valine). Unlike most amino acids, the initial step of branched-chain amino acid (BCAA) metabolism does not take place in the liver. They increase rapidly in systemic circulation after protein intake and are readily available for use. Skeletal muscle is where most of the initial catabolism of BCAA takes place using branched-chain aminotransferase enzymes to form α-ketoacids, which are then released from muscles back into the blood to be further metabolized, mainly in the liver.[137] BCAA act as substrates for protein synthesis, energy production, neurotransmitter production, glucose metabolism, immune response, and many other beneficial metabolic processes.[137]

- **α-Ketoisovaleric Acid (AKIV)** is produced from the essential amino acid valine. It then metabolizes to become succinyl Co-A. AKIV is glucogenic.
- **α-Ketoisocaproic Acid (AKIC)** is produced from leucine and further metabolizes to form acetyl-CoA and acetoacetate. AKIC is ketogenic.
- **α-Keto-β-Methylvaleric Acid (AKBM)** comes from isoleucine, and further metabolizes to form acetyl-CoA and succinyl-CoA. AKBM is therefore both glycogenic and ketogenic.

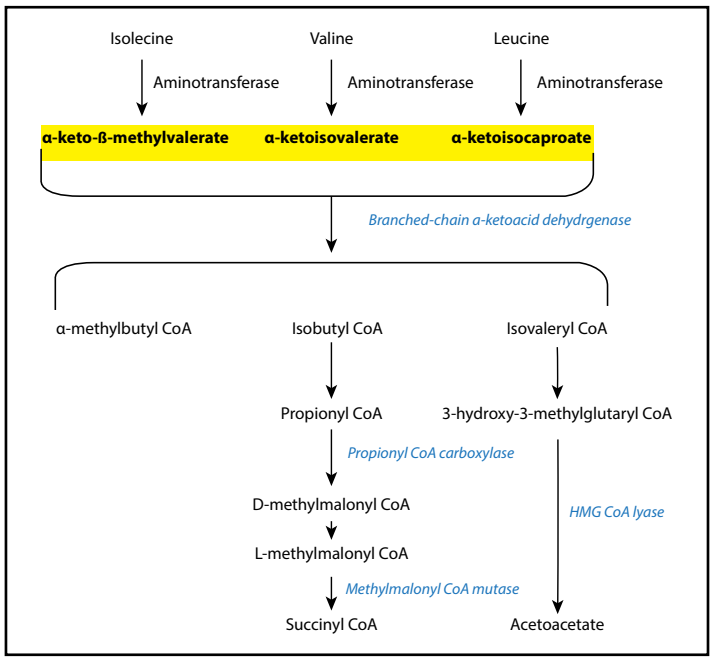
These α-ketoacids then require an enzyme complex called branched-chain α-keto acid dehydrogenase (BCKD) for further metabolism.[138] This enzyme complex requires multiple vitamin cofactors, such as vitamin B_1, B_2, B_3, B_5, and lipoic acid.[72,139-141]

High Levels:

Urinary elevations of these ketoacids can be the result of functional need for the vitamin cofactors to support BCKD.[72,141]

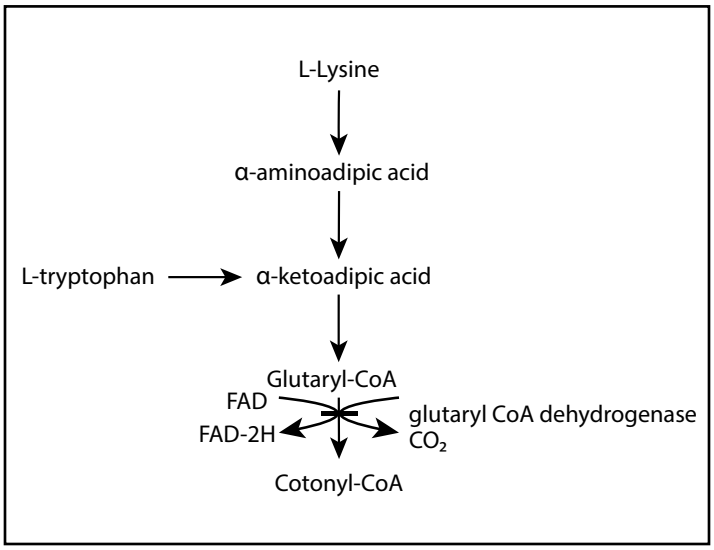
A genetic defect of the α-keto acid dehydrogenase enzyme complex is responsible for maple syrup urine disease, which results in very elevated levels of AKIC, AKIV, AKMB.[137]

Elevated plasma levels of branched-chain amino acids have been associated with insulin resistance as a result of decreased catabolism for energy production. This metabolic disturbance may be compounded by further nutrient deficiencies limiting the activity of the BCKD enzyme.[142,143]



Glutaric Acid

Glutaric Acid is formed from the essential amino acids lysine and tryptophan through the intermediaries of alpha ketoadipic acid and glutaryl-CoA. Glutaryl-CoA is further metabolized to glutaconyl- and crotonyl-CoA by an enzyme called glutaryl-CoA dehydrogenase. This enzyme requires riboflavin (vitamin B_2) as a cofactor.



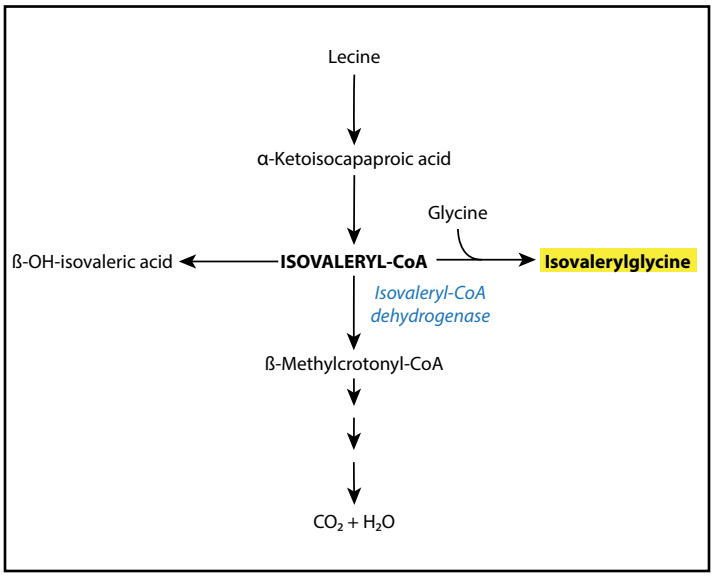
High Levels:

Elevations of urinary glutaric acid may reflect enzymatic insufficiency requiring vitamin B_2 or mitochondrial electron transport dysfunction.

Deficiencies of the enzyme glutaryl-CoA dehydrogenase, and multiple acyl-CoA dehydrogenase deficiency (MADD), are well-studied inborn errors of metabolism which result in significant glutaric aciduria. However, milder forms of this rare mitochondrial disorder exist and can result in adult-onset presentations. Late-onset forms can present as atypical beta-oxidation disorders with exercise intolerance, muscle weakness, and CNS dysfunction.[144,145] In these cases, riboflavin, carnitine, and CoQ10 have been used therapeutically.[145-147]

Isovalerylglycine

Isovalerylglycine is produced from leucine catabolism. It is further metabolized via isovaleryl-CoA dehydrogenase. This enzyme requires vitamin B_2 as a cofactor.[148,149]



High Levels:

Acyl-CoA dehydrogenase enzymes are not only involved in branched-chain amino acid metabolism, but also beta-oxidation of fatty acids.[89] Enzymatic dysfunction and elevations in isovalerylglycine are seen when there is a functional nutrient cofactor need and in certain inborn errors of metabolism. However, elevations of isovalerylglycine are also seen in problematic mitochondrial fatty acid beta-oxidation.[150,151]

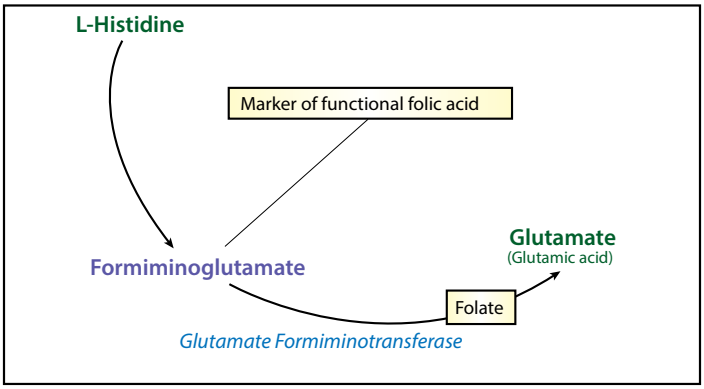
Carnitine, glycine, vitamin B_2, and antioxidants have been used therapeutically to treat abnormal levels of isovalerylglycine.[152-154]

There is an association between elevated isovalerylglycine and anorexia nervosa. The mechanism is believed to be due to poor thyroid conversion of vitamin B_2 into active FAD, which normalized in some patients after a refeeding program.[155]

METHYLATION MARKERS

Formiminoglutamic Acid

Formiminoglutamic Acid (FIGlu) is an intermediary organic acid in the conversion of the amino acid histidine to glutamic acid. This enzymatic conversion requires tetrahydrofolic acid.[156]



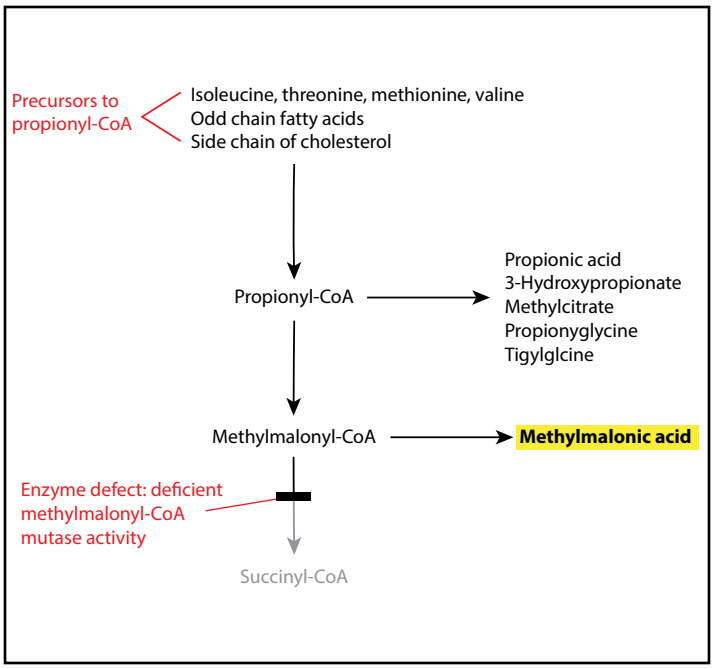
High Levels:

FIGlu elevations in urine have been used as a marker for folate deficiency dating back to the 1950's.[2,157] In addition to folate deficiency, elevated urinary FIGlu may also reflect vitamin B_{12} status since folate recycling requires vitamin B_{12} as a cofactor and both are critical steps in the methylation cycle.[158]

There are multiple clinical associations with elevated urinary FIGlu, including acute and chronic alcohol use, pregnancy, and oral contraceptive use.[159-162]

Methylmalonic Acid

Methylmalonic Acid (MMA) is formed from propionyl-CoA via methylmalonyl-CoA. Major dietary sources of propionyl-CoA include valine, isoleucine, methionine, threonine, and odd chain fatty acids.[163] Methylmalonyl-CoA is converted to succinyl-CoA to feed the Citric Acid Cycle via the enzyme methylmalonyl-CoA mutase. This enzyme is very vitamin B_2 dependent. In B_{12} deficiency, methylmalonyl-CoA is hydrolyzed to methylmalonic acid.[164]



High Levels:

The most common cause of MMA in the urine is vitamin B_{12} deficiency. However, a rare deficiency of the methylmalonyl-CoA mutase enzyme is another. Any underlying condition which results in vitamin B_{12} deficiency should be considered, such as reduced intestinal absorption, chronic alcoholism, or strict vegan diets.[164]

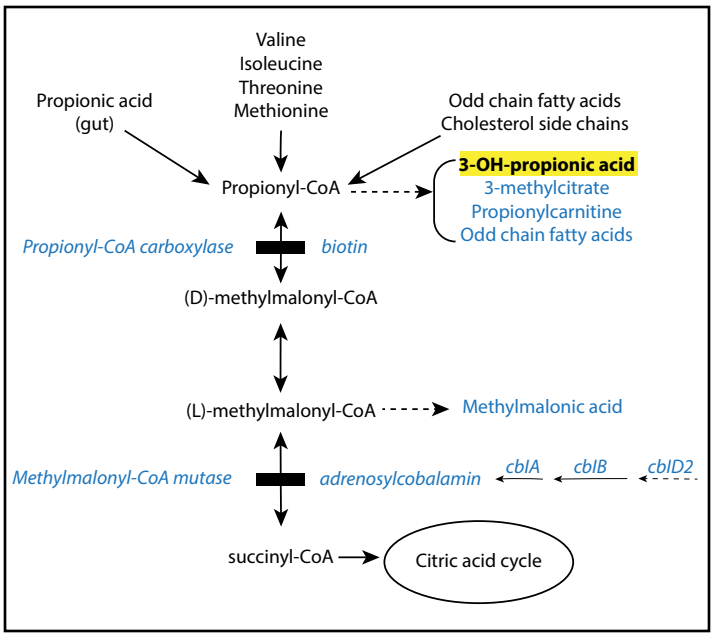
Methylmalonic acid, as a functional biomarker, is considered a more sensitive index of B_{12} status when compared to serum B_{12}.[165-170] Urinary MMA correlates with serum MMA, making the simple urine test a useful screening tool for B_{12} deficiency in at-risk populations, such as the elderly or patients with GI dysfunction.[167,171]

Vitamin B_{12} therapy lowers MMA. Monitoring this metabolite may help prevent the consequences of B_{12} deficiency, such as cognitive decline and neuropathy.[169,172,173]

BIOTIN MARKERS

3-Hydroxypropionic Acid

3-Hydroxypropionic Acid (3-HPA) is a major urinary metabolite of propionic acid. Propionic acid is derived from dietary branched-chain amino acids, odd-chain fatty acids, and can be produced in the gut by bacterial fermentation of fiber. The biotin-dependent enzyme propionyl CoA carboxylase is responsible for metabolizing propionic acid to methylmalonyl CoA, which is subsequently isomerized to succinyl CoA. Decreased activity of this enzyme shunts propionyl CoA into alternative pathways which form 3-HPA.



High Levels:

As noted, biotin is a cofactor in the propionyl-CoA-carboxylase enzyme.[174] Reduced activity of this enzyme due to functional biotin deficiency can cause elevations of the urinary organic acid 3-hydroxypropionic acid. However, in isolation, it may not be as sensitive a marker as 3-hydroxyisovaleric acid to diagnose marginal biotin deficiency.[175]

There are inborn errors of metabolism associated with this organic acid. When the propionyl-CoA-carboxylase enzyme is deficient, the result is propionic acidemia and elevated urinary 3-hydroxypropionic acid. Some isolated case reports reveal the possibility of a later onset in this enzyme deficiency.[176]

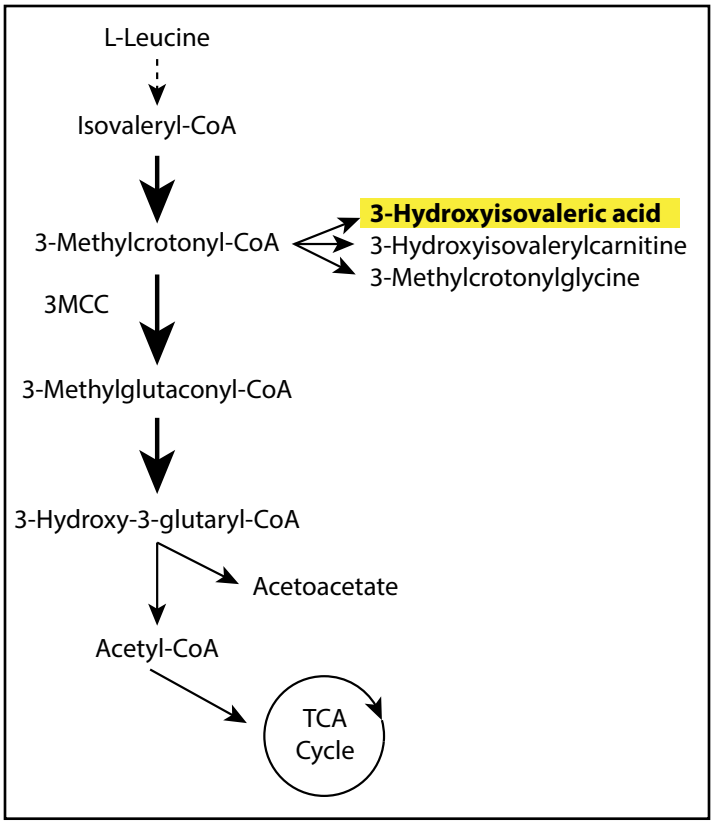
Because of the relationship between propionyl-CoA and methylmalonyl CoA, 3-HPA elevations have also been observed in inborn errors causing methylmalonic acidemia.[177]

Low Levels:

Low levels of urinary 3-hydroxypropionic acid may be seen with decreased amino acid and fatty acid precursors from maldigestion, malabsorption or impaired fatty acid oxidation. Because the propionic acid precursor is also made in the GI tract, decreased fiber intake or antibiotic use can result in lower urinary 3-hydroxypropionic acid as well.[178] In fact, low protein diets and antibiotics are used acutely to treat inborn errors of metabolism which cause propionic acidemia.[179]

3-Hydroxyisovaleric Acid

3-Hydroxyisovaleric Acid (3-HIA) is formed from the metabolism of the branched-chain amino acid leucine. Methylcrotonyl-CoA carboxylase catalyzes an essential step in this pathway and is biotin dependent. Reduced activity of this enzyme leads to an alternate pathway of metabolism resulting in 3-hydroxyisovaleric acid.



High Levels:

The urinary excretion of 3-HIA has been shown to be an early and sensitive indicator for marginal biotin deficiency.[175]

Elevated levels of 3-HIA in pregnant women reflect reduced or marginal biotin status.[180] Smoking and anticonvulsant medication can also increase this metabolite as a reflection of accelerated biotin metabolism and therefore marginal deficiency.[181,182]

Neurotransmitter Metabolites

These organic acid compounds are down-stream metabolites of neurotransmitter synthesis and degradation.[73] Many of the neurotransmitter metabolites in urine primarily reflect peripheral metabolism, as in the enteric nervous system. Elevations in these organic acids can represent altered neurotransmitter metabolism. This can be due to enzymatic nutrient cofactor needs, or genetic predispositions. Toxins, chronic illness, and stress can also influence results.[183,184]

KYNURENINE MARKERS

Kynurenic Acid and Quinolinic Acid

Kynurenic acid and Quinolinic acid are tryptophan metabolites formed through the kynurenine pathway. Tryptophan is the amino acid precursor to serotonin; its major route for catabolism is the kynurenine pathway. Important products of the kynurenine pathway include xanthurenic acid and kynurenic acid, which can further metabolize into quinolinic acid.

The historical importance of this pathway has mainly been as a source of the coenzyme NAD+, which is important for all redox reactions in the mitochondria. However, it is now understood that kynurenic and quinolinic acid have physiologic implications. This alternate pathway is upregulated in response to inflammation and stress, which can lead to deficient serotonin production.[185]

Kynurenic acid has shown some neuroprotective properties in the brain, since it can stimulate NMDA receptors. However, its importance on the periphery is still not fully elucidated. Some studies outline anti-inflammatory, analgesic, antiatherogenic, antioxidative, and hepatoprotective properties to peripheral kynurenic acid.[186-188] The correlation to levels of urinary excretion needs further study.

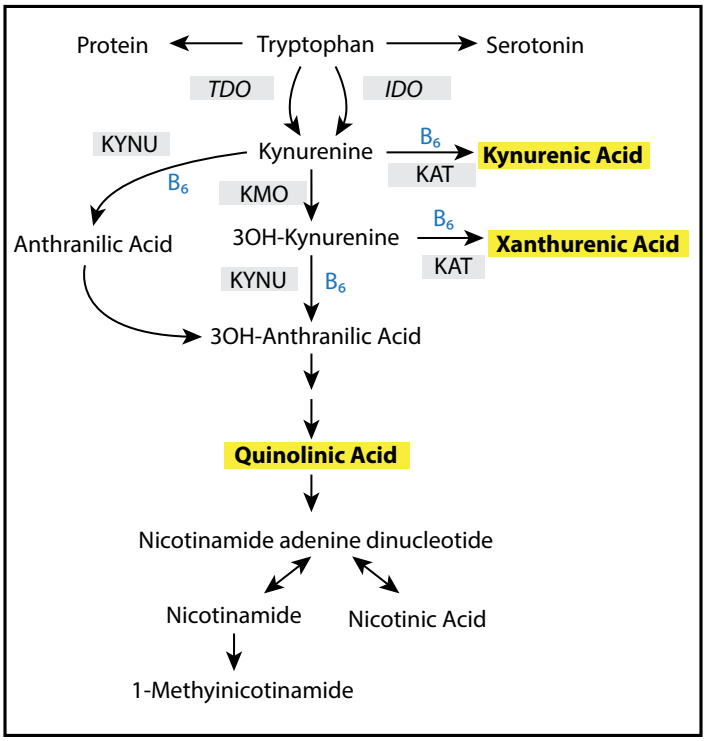
Quinolinic acid, in and of itself, can be inflammatory and neurotoxic.

High Levels:

The kynurenine pathway is particularly sensitive to vitamin B_6 deficiency, which can elevate urinary kynurenic acid (and xanthurenic acid).[189-191] Vitamin B_2 is also an important vitamin cofactor in the enzymatic conversion reactions within the pathway.[192] Because a major-end product of this pathway is also NAD+, elevations in kynurenic and quinolinic acid may also reflect vitamin B_3 need.

Oral contraceptives and estrogen therapy have been implicated in increasing quinolinic acid excretion both from altered tryptophan metabolism directly, as well as vitamin B_6 insufficiency.[193]

Many of the intermediates and products in the kynurenine pathway are implicated in numerous neurological and psychiatric diseases, such as depression. Alterations in this pathway also have some connection to the development of insulin resistance, diabetes, tumor growth and proliferation, and inflammatory myopathies.[194-198]



Kynurenic/Quinolinic Acid Ratio

Because of the specific inflammatory component of quinolinic acid, as well as the potentially protective role of kynurenic acid peripherally (as outlined above), laboratories measure the ratio of kynurenic acid to quinolinic acid. This ratio can act as a measure of disturbed kynurenine pathway metabolism. It suggests that tryptophan is catabolized via the kynurenine pathway, rather than the serotonin pathway. There is literature regarding a low kynurenic/quinolinic ratio association with neurotoxicity and major depressive disorder.[199,200]

Xanthurenic Acid

Xanthurenic acid is produced as part of the kynurenine pathway of tryptophan catabolism, along with kynurenic and quinolinic acid, as previously outlined.

High Levels:

Because this pathway is heavily dependent on vitamin B_6, elevations of xanthurenic acid can reflect a functional need for vitamin B_6.[201] Kynurenine pathway metabolites may also become elevated when there are needs for vitamin B_3.[202,203]

Elevations in urinary xanthurenic acid are seen with increased intake of tryptophan, and in high estrogen states. Pregnancy and oral contraceptive use is associated with elevated levels of urinary xanthurenic acid where a functional nutrient need for B-vitamins is pronounced.[5,204]

Abnormalities in the kynurenine pathway have been associated with many clinical conditions including immune suppression, cancer, and inflammatory conditions.[201]
Administration of vitamin B_6 can decrease xanthurenic acid excretion.[205,206]

CATECHOLAMINE MARKERS

Homovanillic Acid

Homovanillic acid (HVA), or 3-methoxy-4-hydroxyphenylacetic acid, is a metabolite of dopamine. Although dopamine is an important brain neurotransmitter, a substantial amount of dopamine is produced in the GI tract.[207]

In neurotransmitter production, dopamine is formed from phenylalanine and tyrosine using several enzymes which require nutrient cofactors such as iron, tetrahydrobiopterin, and pyridoxal phosphate.[208] Dopamine then becomes norepinephrine using the enzyme dopamine beta-hydroxylase, which requires copper and ascorbic acid for optimal activity.[209]

Dopamine can be metabolized to homovanillic acid using both monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).[207] MAO requires a vitamin B_2 (FAD) cofactor, while the COMT enzyme requires SAM, magnesium, and vitamin B_6.[210,211]

High Levels:

Elevations of homovanillic acid can be seen with lack of vitamin cofactors for enzymes within the metabolism of dopamine or the production of norepinephrine. Quercetin supplementation can elevate plasma HVA and perhaps urinary excretion.[212] Dietary flavanols, such as tomatoes, onions, and tea are also known to elevate urinary HVA.[213]

Like VMA, urinary HVA is elevated in conditions such as neuroblastoma and neural crest tumors.[214,215] And, since dopamine regulates emotional and motivational behavior, changes in dopamine levels, and subsequent HVA levels, have been studied in the overall stress response, PTSD, mood disorders, and autism.[216-221]

Low Levels:

Low levels of urinary HVA imply deficient production of dopamine due to decreased amino acid precursors or lack of vitamin cofactors throughout the production cycle. It may also reflect impaired methylation of dopamine to HVA. Low dopamine turnover and low HVA levels are seen in some mood disorders and as an effect of various antidepressants.[222,223]

Vanilmandelic Acid

Vanilmandelic acid (VMA) is formed in the liver by the oxidation of 3-methoxy-4-hydroxyphenylglycol.[224] As a downstream metabolite of tyrosine-derived catecholamines, levels of VMA can reflect the overall synthesis and metabolism of catecholamines.[225] Whether norepinephrine or epinephrine are metabolized into VMA or 3-methoxy-4-OH-phenylglycol (MHPG) depends on the presence and specificity of various available aldehyde reductase and dehydrogenase enzymes.[226]

High Levels:

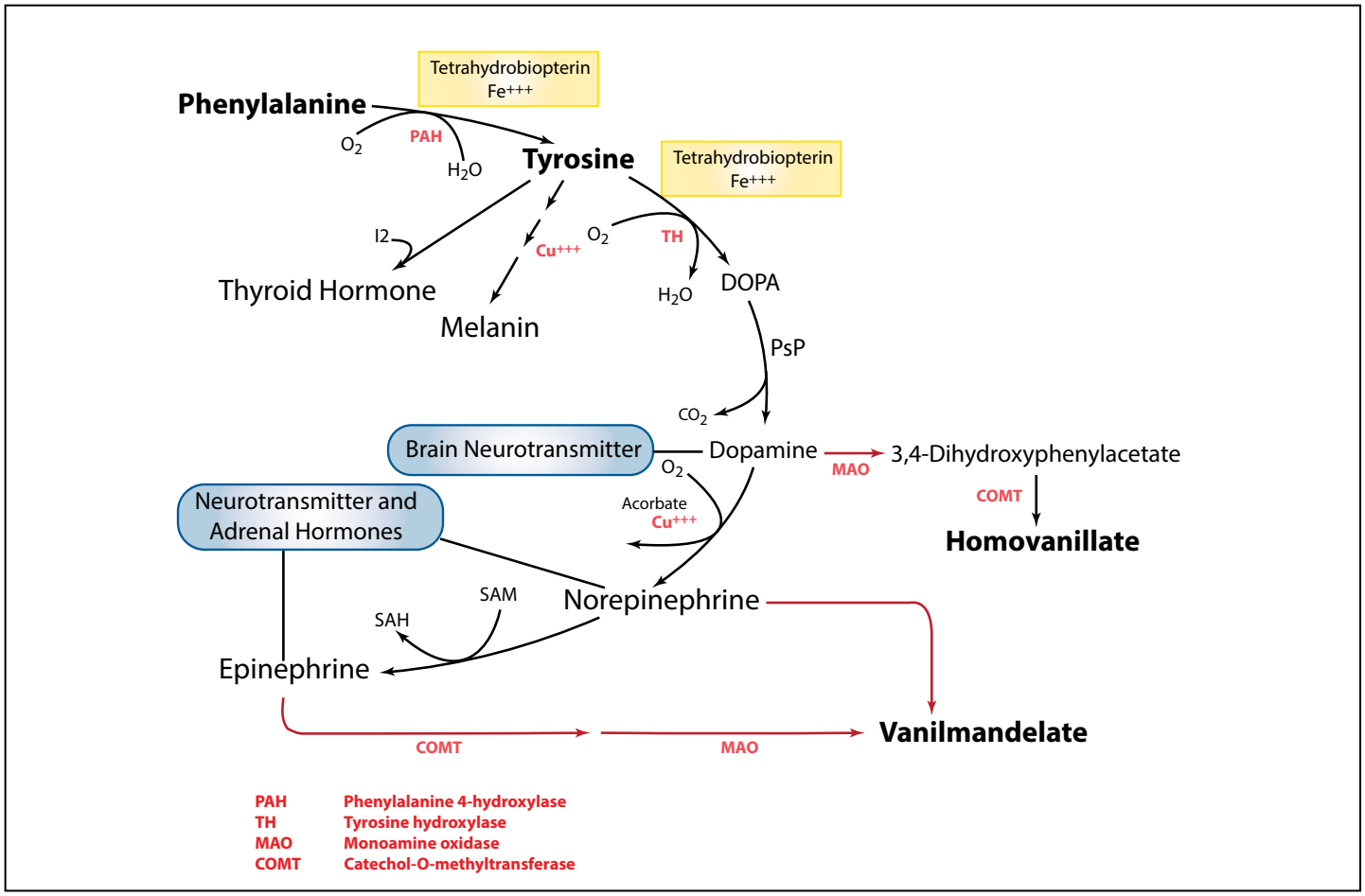
Centrally-acting medications, such as antidepressants and stimulants used for ADHD can elevate overall catecholamines and therefore urinary metabolites.[227,228] Urinary levels have been shown to correlate with generalized anxiety disorder.[229] VMA is sometimes used in the work up of pheochromocytoma, neural crest tumors, renovascular hypertension, and neuroblastoma in the right clinical context.[230-233] Elevations in catecholamine urinary metabolites have been shown to correlate with the physiologic stress response, exercise, and PTSD.[234-237]

Low Levels:

Low levels of catecholamine metabolites can reflect insufficient amino acid precursors for neurotransmitter production, nutrient cofactor insufficiencies for enzymatic conversion, and genetic abnormalities in enzyme function. Methylation is required for neurotransmitter creation and metabolism. Thus, methylation defects or lack of methylation cofactors may contribute to abnormal levels. Copper is an important cofactor for dopamine beta-hydroxylase, which forms norepinephrine from dopamine. In copper deficiency, norepinephrine formation can be impaired and potentially lower VMA levels.

Manganese released into the synaptic cleft may influence synaptic neurotransmission. Dietary manganese deficiency, which may enhance susceptibility to epileptic functions, appears to affect manganese homeostasis in the brain, probably followed by alteration of neural activity.[238]

There are studies which evaluate the neurotoxicity of manganese. Elevated levels of VMA and HVA have been seen in manganese toxicity from occupational exposure which induces a CNS condition similar to Parkinson's disease.[239,240]



3-Methyl-4-Hydroxy-Phenylglycol

3-Methyl-4-OH-Phenylglycol (MHPG) is a byproduct of the central nervous system's norepinephrine (NE) metabolism. MHPG metabolizes to vanilmandelic acid (VMA) in the liver using the enzymes alcohol dehydrogenase and aldehyde dehydrogenase. Urinary MHPG was originally thought to represent CNS sympathetic output, but is now known to be principally derived from peripheral neuronal NE metabolism.[241]

MHPG has been widely studied as a marker to predict response to medications used in mood disorders or as a biomarker to monitor pharmacotherapies.[242-245]

High Levels:

The role of hepatic alcohol and aldehyde dehydrogenase explains the clinical observations that ethanol consumption decreases the excretion of VMA, while increasing MHPG.[246,247]

Because norepinephrine is involved in the pathophysiology of hot flashes in postmenopausal women, MHPG levels have been studied in this patient population.[248,249] Interestingly, folic acid was found to interact with receptors causing subjective improvement in symptoms.[250]

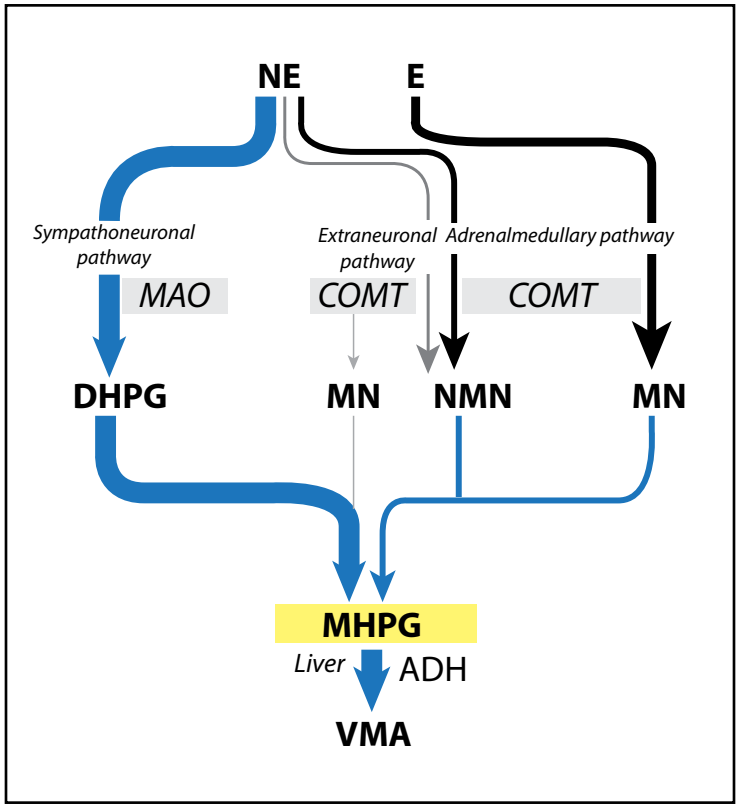
Sleep deprivation can act as a stimulus to the peripheral sympathetic nervous system, which can influence central nervous noradrenergic neurotransmitter levels and elevate MHPG.[251] As a central nervous system metabolite, levels can correlate with central catecholaminergic disturbances, as in anxiety and seizures.[252,253] Elevated MHPG levels have also been associated with the stress response.[254]

Pheochromocytomas are rare, mostly benign tumors of the adrenal medulla which can secrete catecholamines causing a wide array of sympathetic symptoms. These tumors contain MAO and COMT. They can therefore produce MHPG. However, because peripheral sympathetic nerves can also contribute to high MHPG, using MHPG for diagnosis of pheochromocytoma limited. VMA is also not very sensitive for diagnosis of pheochromocytoma because it can be made in the liver from MHPG. Although neither organic acid is diagnostic of pheochromocytoma, it is possible to see elevations of these analytes in the disease.[255]

Low Levels:

Since catecholamines are made from dopamine, low levels of the MHPG metabolite can result from low levels of dopamine, dopamine amino acid precursors, nutrient enzymatic cofactor deficiencies in dopamine metabolism, and overall methylation defects.

Low levels of MHPG have been correlated to mood and behavioral disorders, anorexia, and ADHD.[256-258]



SEROTONIN MARKERS

5-Hydroxyindolacetic Acid

5-Hydroxyindolacetic acid (5-HIAA) is a downstream metabolite of serotonin, which is formed from the essential amino acid tryptophan. Most blood serotonin and urinary 5-HIAA comes from serotonin formation outside of the CNS, primarily the liver and enterochromaffin cells in the gastrointestinal tract. Serotonin is further metabolized by monoamine oxidase to become 5-HIAA.[259]

High Levels:

Elevations, as well as low levels of urinary 5-HIAA, can reflect underlying intestinal microbial balance.[260] Serotonin produced by intestinal enterochromaffin cells is necessary for GI motility.[261] Because of this, antidepressants such as tricyclics and serotonin selective reuptake inhibitors have been used in treating IBS.[262] Enterochromaffin cells and their serotonin signaling are influenced by overall inflammatory responses to bacteria in the GI tract.

Diets rich in tryptophan and serotonin have been shown to increase urinary 5-HIAA. Bananas, plantains, kiwi, pineapple, nuts, and tomatoes, among other foods, can cause elevations of this urinary metabolite.[259]

The excretion of 5-HIAA seems to vary among individuals who supplement with 5-hydroxytryptophan (5HTP).[259] Carcinoid tumors are well-differentiated neuroendocrine tumors derived from the enterochromaffin cells in the GI tract and lung. These tumors secrete vasoactive peptides, especially serotonin which causes flushing and diarrhea. Urinary 5-HIAA levels are elevated in patients with carcinoid syndromes.[263]

It should be noted that certain medications may cause false abnormalities in urinary 5-HIAA, and/or interfere with electrochemical detection on chromatography. These include guaifenesin, aspirin, and acetaminophen.[259,264-267] Many medications can alter serotonin levels and therefore impact urinary 5-HIAA levels. Due diligence is recommended to investigate medications as a possible etiology of abnormal levels.[259,267,268]

Abnormalities, both high and low, in urinary 5-HIAA can be caused by methylation defects, as well as vitamin and mineral nutrient cofactor deficiencies.

Low Levels:

Decreased 5-HIAA levels can reflect low tryptophan intake, or malabsorption/maldigestion of tryptophan. Medications, like MAO inhibitors, decrease serotonin turnover and decrease 5-HIAA.[269] Low levels of urinary 5-HIAA have been observed in cardiovascular disease, metabolic syndrome, IBS patients, and those with mood disorders and migraines.[270-272]

These urinary markers can reflect exposure to environmental toxins, or up-regulation of detoxification pathways in response to exposures. When these markers are elevated, the recommendation is to identify, minimize, and remove exposures. Clinicians may consider the use of antioxidants and nutritional support of detoxification pathways. For further information on environmental toxins, the following websites may be helpful:

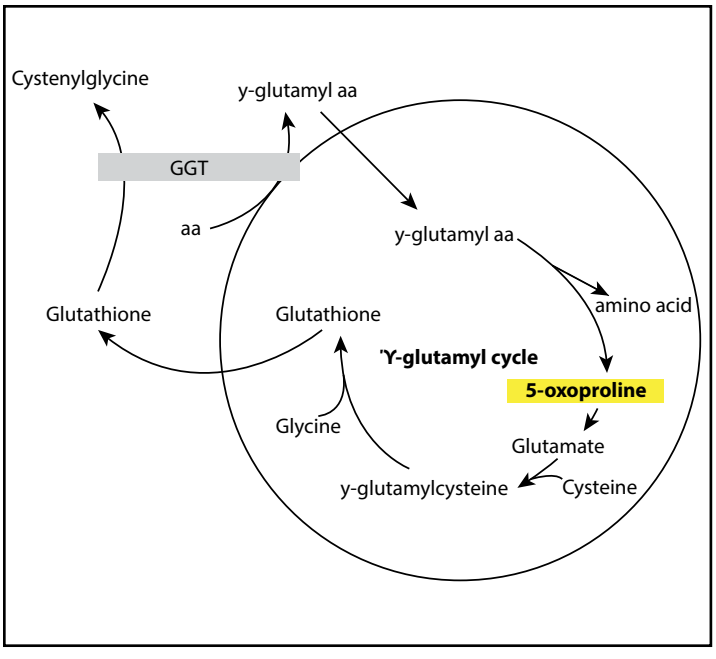
Environmental Working Group: https://www.ewg.org/
Agency for Toxic Substances and Disease Registry: https://www.atsdr.cdc.gov/

Pyroglutamic Acid

Pyroglutamic acid (5-oxoproline) is produced and utilized in the gamma-glutamyl cycle. This cycle is needed to assist in the production and recycling of glutathione (GSH), a powerful antioxidant.

Glutathione is a tripeptide, consisting of glutamate, cysteine, and glycine. Using the gamma-glutamyl cycle, GSH is divided into cysteinyl glycine and a gamma-glutamyl molecule which attaches to another amino acid for transport across a membrane or into a cell. Gamma-glutamyl transferase then splits off that attached amino acid, and the glutamate becomes pyroglutamic acid (5-oxoproline). Cysteinyl glycine is also broken down and transported into the cell as cysteine and glycine.

The entire GSH molecule needs to be reformed intracellularly from pyroglutamic acid by recombining cysteine, glycine, and glutamic acid using GSH synthetase.[273,274] This enzymatic reformation requires cofactors such as ATP and magnesium.[275]



High Levels:

Elevations in pyroglutamic acid can reflect lack of precursors (glycine, cysteine, glutamine) or nutrient cofactors for GSH recycling (magnesium). Most specifically, pyroglutamic acid has been proposed as a measure of glycine availability.[276,277]

Oxidative stress, in general, can upregulate the detoxification pathways and result in elevated pyroglutamic aciduria.[278,279] Significant toxic exposures, such as medication toxicities, can deplete ATP, interrupting GSH recycling and causing elevations in pyroglutamic acid. In rare cases, this can result in metabolic acidosis.[280-282]

Deficiency in glutathione synthetase has also been described in literature as presenting with pyroglutamic aciduria.[283]

Low Levels:

Because pyroglutamic acid formation is dependent on glutathione entering the gamma-glutamyl cycle, an insufficient amount of GSH or its precursors and necessary cofactors can result in low pyroglutamic acid.

α-Ketophenylacetic acid (from Styrene)

α-Ketophenylacetic Acid, also known as p**henylglyoxylic acid (PGA)**, is a urinary metabolite of styrene, toluene, xylenes, and ethylbenzene. It acts as a urinary marker of recent exposure via inhalation, contact, oral, and others.[284] The biologic half-life of styrene in humans is fairly short and corresponds with the disappearance of PGA from the urine.[285,286]

Styrene is widely used for synthesis of polymers such as plastics, rubbers, and surface coating. It is also used in the pharmaceutical industry. Styrene is commonly applied in the manufacturing of paints, pigments, and glues. Co-exposure to other solvents, like toluene and ethyl acetate is common in workplaces where styrene is a concern.[287] Since toluene and xylene are components of unleaded gasoline, workers at gas stations are at potential risk of exposure, as well as the general population.[288]

Styrene exposure may interfere with peripheral metabolism of thyroid hormones by inhibiting conversion of T4 to T3.[289] It may also affect DNA repair capacity and damage.[290] There are also clinical associations with insulin resistance, oxidative stress, and inflammation.[291]

α-Hydroxyisobutyric Acid (from MTBE)

α-Hydroxyisobutyric Acid is a major urinary metabolite of the industrial solvent methyl tert-butyl ether (MTBE). MTBE was a gasoline additive discontinued in the early 2000's used to reduce automobile emissions. Due to significant ground water leakage from storage tanks, ongoing exposure to MTBE exists in ground water. There is also data available on levels of MTBE in ambient air.[292] Urinary α-hydroxyisobutryic acid is a marker of recent MTBE exposure.[293,294]

Although, MTBE was initially designated as "non-carcinogenic", recent studies suggest some interesting clinical associations. Exposure to MTBE has been linked to type 2 diabetes as a result of disrupted zinc homeostasis and glucose tolerance.[295] There are also clinical associations with autism, DNA oxidative damage, and methylation defects.[296-299] Studies on cancer, reproductive abnormalities, nonalcoholic fatty liver, and neurotoxicity have been either negative or inconclusive thus far.[300-302]

Orotic Acid

Orotic Acid is an organic acid which serves as an intermediate in nucleotide synthesis and is linked to arginine metabolism as a urea cycle marker for nitrogen balance.[303]

It is formed from aspartic acid and carbamoyl phosphate.[304] Carbamoyl phosphate plays an important role in the body because it brings nitrogen into the urea cycle for detoxification and disposal. Carbamoyl phosphate enters the urea cycle to react with ornithine to form citrulline. When ammonia levels significantly increase or the liver's capacity for detoxifying ammonia into urea decreases, carbamoyl phosphate leaves the mitochondria and instead enters the pyrimidine pathway. This stimulates orotic acid biosynthesis and subsequent urinary excretion.[305]

Orotic acid can also be found in the diet. The richest dietary sources include cow's milk and dairy products. Most urinary orotic acid is synthesized in the body as an intermediate in the nucleotide synthesis.[306] Although it is also linked with abnormalities in arginine metabolism as a urea cycle marker for nitrogen balance, orotic acid plays no direct role in the urea cycle, yet is increased in urea cycle disorders.[303] Hyperammonemia is characteristic of all urea cycle disorders; orotic acid is only elevated in a few.[303]

High Levels:

Elevations of orotic acid are seen in with hereditary deficiencies of urea-cycle enzymes, ammonia overload as seen in high protein diets, and abnormalities in arginine metabolism.[303,305]

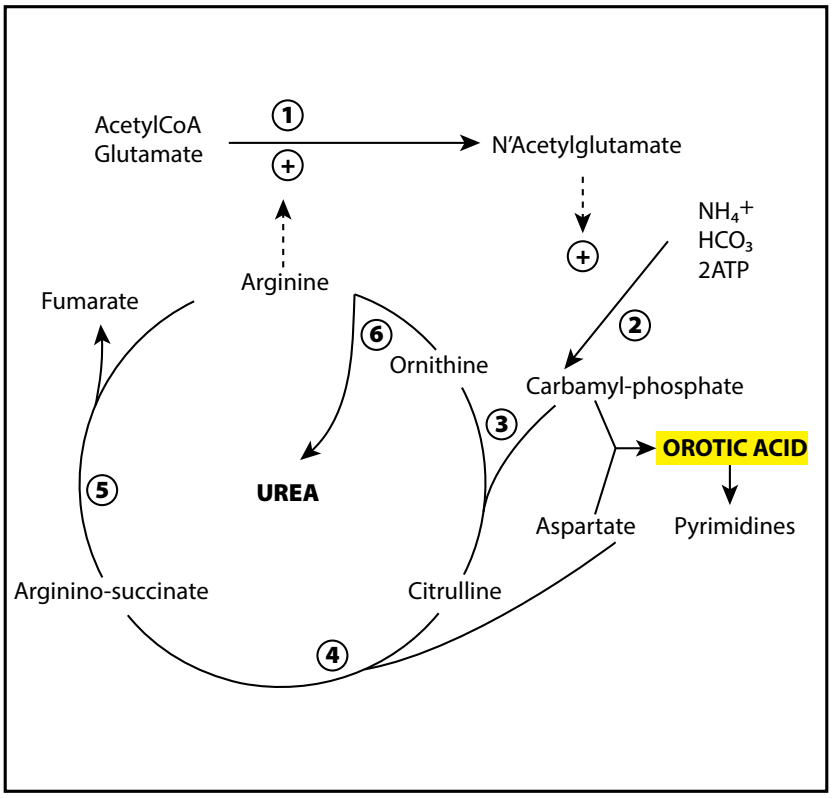
Any hepatotoxin or underlying liver condition can affect ammonia metabolism and increase orotic acid. There are studies that show elevations in orotic acid after drinking alcohol, which then declined with abstinence.[307]

Orotic acid excretion is increased by allopurinol and 6-azauridine seemingly related to action of these drugs on pyrimidine synthesis.[308]

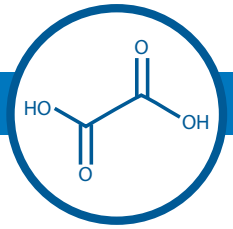
There are animal studies which show a link between orotic aciduria and hypertension. Orotic acid can induce endothelial dysfunction by contributing to vascular and systemic insulin resistance which impacts nitric oxide production, leading to hypertension.[309] Random case studies also show an association between megaloblastic anemia and orotic aciduria as a result of hereditary defects in pyrimidine synthesis.[310]

Low Levels:

There is no clinical significance to low levels of urinary orotic acid.



Oxalate Markers

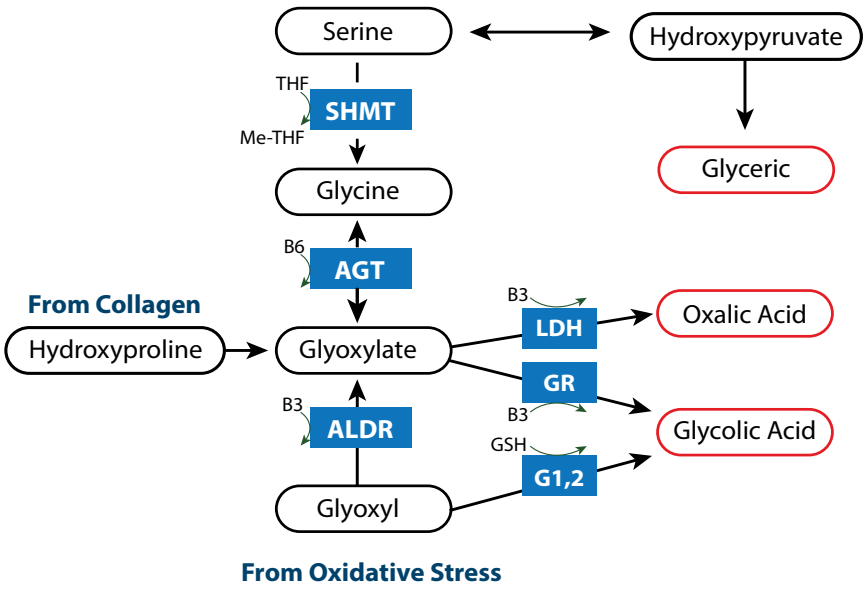


The oxalate markers are a collection of 3 organic acids that are metabolic end-products of the glyoxylate pathway (see diagram). They consist of glyceric acid, glycolic acid, and oxalic acid. As a collection of biomarkers, the oxalate markers may provide insight into abnormal metabolism in the glyoxylate pathway which ultimately could result in higher levels of oxalic acid. The oxalates may have specific clinical relevance to patients suffering from recurrent kidney stones, as high levels of oxalic acid are a strong risk factor in kidney stone development.[311] Also, there is evidence to support the notion that increased levels of oxidative stress and/or metabolic dysfunction may ultimately contribute to dysfunctional oxalate metabolism leading to higher excretion of oxalic acid.[312]

Higher systemic levels of oxalic acid are found in inborn errors of disease which contribute to a condition known as oxalosis where calcium-oxalate crystals can be deposited in systemic tissues.[313] This most commonly occurs in the kidney, however there is evidence of deposition in other tissues to a lesser degree. The accumulation of calcium oxalate deposits in the absence of hereditary disease is termed "dystrophic oxalosis" and is not well studied in the literature. However, calcium oxalate deposits have been reported in atherosclerotic plaques, lymph nodes, myocardium, ocular tissues, as well as various endocrine organs in a small number of studies.[312]

As will be discussed, there are many factors than can influence the glyoxylate pathway, ultimately predisposing individuals to higher oxalic acid levels. It is known that this puts a person at risk for urolithiasis, however what is not known is the degree to which oxalic acid levels may contribute to dystrophic oxalosis. To date, there is no evidence to support any connection between the fungal mycobiome and disordered oxalate metabolism. Therefore, utilization of these markers to suggest fungal overgrowth should be discouraged.

The Glyoxylate Pathway



Glyceric Acid

Glyceric acid is an organic acid that stems from the catabolism of the amino acid serine.[314] Severe elevations in glyceric acid are an indication of a rare inborn error of metabolism known as glyceric aciduria. One form of glyceric aciduria is the result of a defect in the enzyme glycerate kinase which removes glyceric acid from the system.[313] While many case studies have linked this disorder with severe developmental abnormalities,[315] there is some debate as to whether glycerate kinase deficiency is the cause or rather a confounding variable.[316]

Another glyceric aciduria is referred to as primary hyperoxaluria type 2 (PH2). This rare genetic condition results in excessive production of oxalates in the system in the form of oxalic acid. Over time, systemic deposition of oxalates in body tissues can occur which is a process known as oxalosis. This disease is characterized by urolithiasis, nephrocalcinosis, and deposition of oxalates in other body tissues.[313]

High Levels

Aside from these rare inborn errors of metabolism, elevated levels of glyceric acid have been demonstrated in a few metabolomic studies. One study demonstrated that glyceric acid was among 3 metabolites that correlated in patients with rheumatoid arthritis.[317] Furthermore, correlation between glyceric acid was amongst a small handful of metabolites that were able to effectively identify patients with schizophrenia and bipolar as compared to controls.[318] These profiles suggest that more subtle metabolic abnormalities may result in elevated urinary glyceric acid excretion.

It is known that a deficiency in the enzyme glyoxylate reductase leads to excessive levels of glyceric acid resulting in primary hyperoxaluria type 2 and oxalosis.[319] This enzyme requires vitamin B_3 in the form of NAD as a cofactor. Whether subclinical elevations in glyceric acid could be an indication of a functional need for vitamin B_3 has not been studied in the literature. Interestingly, niacin has been shown to be effective in clinical trials with patients suffering from schizophrenia. Glycerate kinase requires magnesium as a cofactor to convert glyceric acid. Therefore, magnesium deficiency may play a role in glyceric acid levels.

Lastly, glyceric acid is formed during metabolism of fructose and serine (previously mentioned). The contribution of fructose intake to total urinary glyceric acid excretion has not been fully elucidated. A careful dietary recall should be considered with increased glyceric acid in the absence of suspected metabolic defects.

Low Levels

The clinical relevance of low urinary glyceric acid has not been studied in the peer-reviewed literature. However, knowing that glyceric acid accumulation is the result of breakdown of both serine and fructose, it is possible that low glyceric acid may be caused by low amino acid status and/or low fructose intake.

Glycolic Acid

Glycolic acid is another byproduct of the oxalate pathway and comes from the conversion of glyoxylic acid. Urinary levels of glycolic acid have most commonly been studied in the rare inborn error of metabolism primary hyperoxaluria type 1 (PH1). PH1 is caused by a deficiency of alanine:glyoxylate aminotransferase (AGT) which converts glyoxylic acid into glycine.[320] When this pathway is blocked, due to inborn error, glyoxylic acid ultimately leads to higher production of glycolic acid and oxalic acid.[321]

Clinically, PH1 results in a similar clinical presentation as PH2 with increased oxalic acid excretion and calcium oxalate deposition (oxalosis). This can ultimately progress to renal calcinosis and kidney failure.[322]

Aside from inborn error, a large portion of glycolic acid is derived from metabolism of glycine and hydroxyproline. It has been projected that between 20% and 50% of urinary glycolate comes from hydroxyproline in the form of collagen turnover in the body.[323] Supplementation or recent intake of collagen or collagen-rich foods may influence levels of glycolic acid in the urine.

Another important source of glycolic acid is the molecule glyoxal.[312] Glyoxal is derived, in part, from oxidative stress in the forms of lipid peroxidation and protein glycation.[324] The majority of this glyoxal is converted into glycolic acid utilizing glutathione as a cofactor.[325]

High Levels

Extremely high levels of urinary glycolic acid are suspicious of a metabolic defect in the glyoxylate pathway such as in PH1. However, this rare inborn error is commonly diagnosed early in life. To note, Genova's urinary organic acid testing is not designed for the diagnosis of metabolic inborn errors. However, the enzyme defect responsible for PH1 (AGT) is dependent on vitamin B_6 as a cofactor.[326] The extent to which urinary glycolic acid could be a functional indicator of vitamin B_6 insufficiency has not been studied, however patients with PH1 have shown improvement with B_6 intervention.[327]

Aside from inborn error, higher levels of glycolic acid may be indicative of increased oxidative stress.[312] This is because oxidative stress causes higher levels of glyoxal which is ultimately converted into glycolic acid for excretion utilizing glutathione as a cofactor.[325] Lower levels of glutathione may promote more conversion of glyoxal to oxalic acid (see below).

Lastly, a large proportion of glycolic acid comes from collagen in the form of hydroxyproline.[323] Consumption of foods high in collagen should be considered with unexplained elevations in glycolic acid. The extent to which accelerated turnover of collagen, such as in catabolic conditions, contributed to urinary glycolic acid has not been studied in the literature.

Low Levels

The clinical relevance of low levels of urinary glycolic acid has not been fully explored. Low levels of glycolic acid precursors could potentially explain low levels of this end-product. This could be found in lower overall oxidative stress burden or low collagen turnover. Glycine is also a precursor to the glyoxylase system and could theoretically result in low downstream metabolites, such as glycolic acid.

Oxalic Acid

Oxalic acid is the metabolic end-product of the glyoxylase pathway and is derived from the oxidation of glyoxylate.[321] In the cell, the majority of glyoxylate is converted into glycine or glycolic acid. However, in some instances there may be greater oxidation of glyoxylate to oxalic acid. This leads to increased urinary excretion of oxalic acid. As 80% of kidney stones are calcium-oxalate stones, an increase in oxalic acid is strongly correlated to frequency of urolithiasis.[328]

As mentioned previously, there are inborn errors of metabolism that cause elevated oxalic acid such as primary hyperoxaluria. The dramatically elevated levels of oxalic acid in these conditions lead to renal calculi formation and systemic oxalosis. However, there are other clinical circumstances that can predispose an individual to have higher urinary oxalic acid levels, including recent dietary intake of oxalate-rich roods.

The relationship between diet and urinary oxalic acid levels is complex and dependent on many variables. While the majority of oxalic acid originates from endogenous production, it is estimated that 40% of urinary oxalic acid is derived from the diet, however these levels are largely dependent on the microbiome and intake of dietary calcium.[329] Specifically, the gut bacteria *Oxalobacter formigenes* degrades dietary oxalates and there is a direct correlation between concentrations of this bacteria and lower oxalate levels. The absence of *Oxalobacter formigenes* is also correlated to increased oxalate stone formation.

Food sources that lead to higher oxalic acid excretion include spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries.[311] However, it is well-documented cooking oxalate-rich foods dramatically reduces the oxalate concentration. Furthermore, often these foods are also high in calcium which inhibits oxalate absorption at the intestinal lining.[311]

Aside from dietary intake, oxalic acid concentrations will vary based on a number of factors. As previously mentioned, oxidative stress may play a large role in the formation of oxalic acid. This is because glutathione is responsible for the neutralization of glyoxal created by free radical damage.[325] With lower glutathione levels, glyoxal is more likely to shunt toward glyoxylate and ultimately could become oxalic acid.[325]

High Levels

Elevated urinary oxalic acid can be a result of several factors. First, dietary intake of oxalate-rich foods must be considered, especially in the context of dysbiosis and microbiome deficiency. A GI Effects stool test may be warranted to evaluate the concentration of *Oxalobacter formigenes* alongside other microbiota capable of degrading dietary oxalates. Calcium intake should be assessed as moderate calcium intake has been shown to decrease oxalate absorption and stone formation.

Hydroxyproline, a component of collagen, is a potential precursor to glyoxylate (discussed above). Higher consumption of collagen-rich foods and supplements may contribute to elevations in urinary oxalic acid.[323] It is also estimated that 5-20% of urinary oxalic acid excretion stems from collagen turnover in the body.[323]

Ascorbic acid intake has been evaluated as a contributor toward oxalate levels because ascorbic acid is metabolized into oxalic acid. While individuals who are predisposed toward stone formation appear to have increased urinary oxalic acid excretion after ascorbic acid loads,[330] in general the research has shown that vitamin C intake is not associated with urinary oxalic acid or kidney stone risk.[331,332]

Oxidative stress is another factor potentially driving the formation of oxalic acid (as discussed previously). Clinically, evaluating glutathione and lipid peroxide levels may be helpful to determine the need to support with antioxidants. Not only may antioxidants, such as glutathione, assist in neutralizing the oxalate precursor glyoxal, but they may also assist in prevention of calcium oxalate deposition to urothelium and subsequent renal damage.[333,334] Also, metabolic syndrome may preclude risk toward increased formation and excretion of oxalic acid whereas weight, BMI, and insulin resistance have all demonstrated positive correlations with urinary oxalic acid.[328] Whether these associations are due to oxidative stress disturbances is yet to be determined.

Lastly, micronutrient insufficiencies may also play a role in oxalic acid levels. Glyoxylate is mostly converted to glycine through the enzyme AGT, which utilizes vitamin B_6 as a cofactor (discussed above). Vitamin B_6 therapy has been used in the setting of primary hyperoxaluria with varying degrees of success. Also, intake of vitamin B_6 has been shown to decrease risk of kidney stones in some, but not all, investigations.[331]

Urinary Creatinine

Urinary creatinine is commonly used as a laboratory standardization when evaluating urinary analytes.[335-337] Creatinine excretion is influenced by muscle mass and body habitus since creatinine formation occurs in muscle. Dietary intake of proteins containing arginine and glycine (precursors of creatine) and creatine supplementation can elevate levels.[338] Hydration status may also play a role in urinary creatinine levels.

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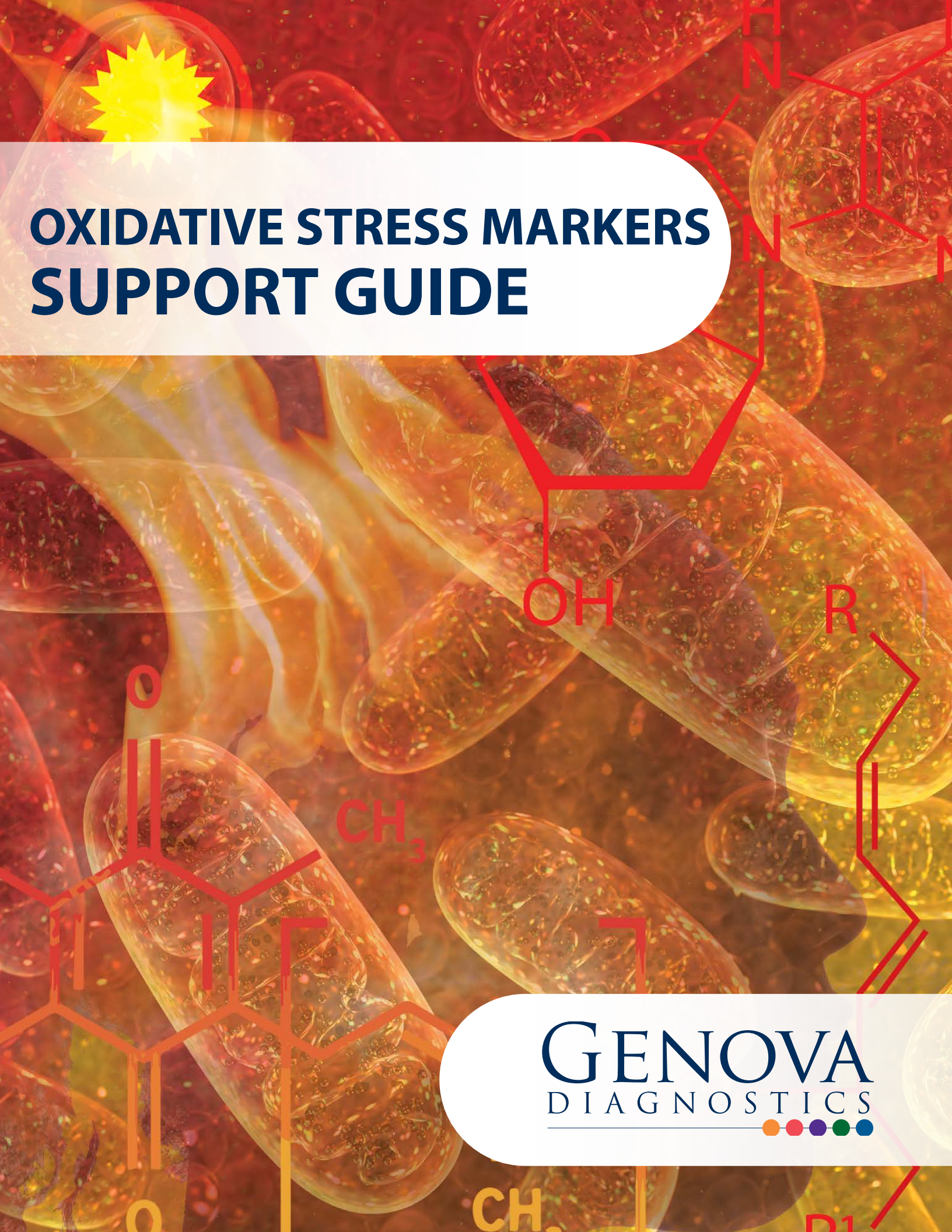

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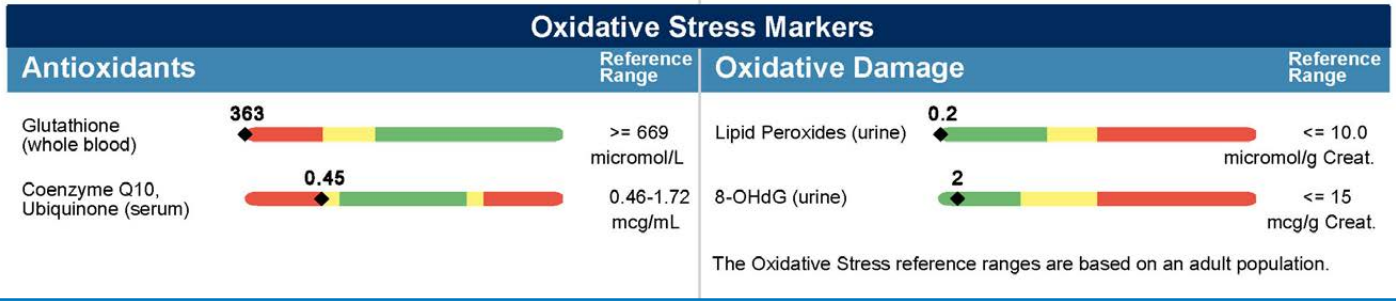
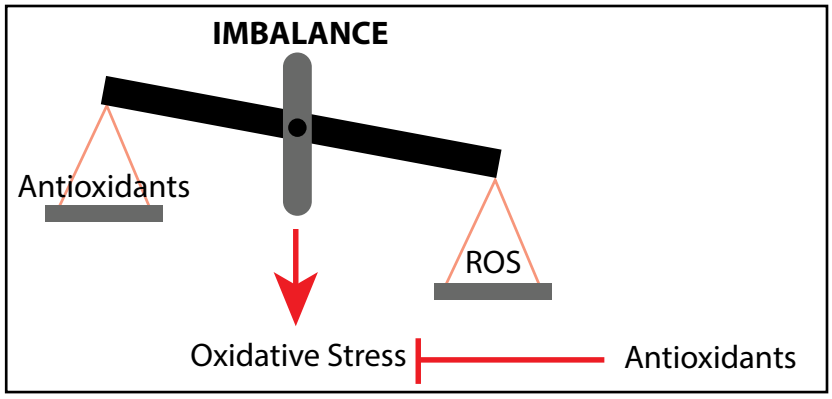
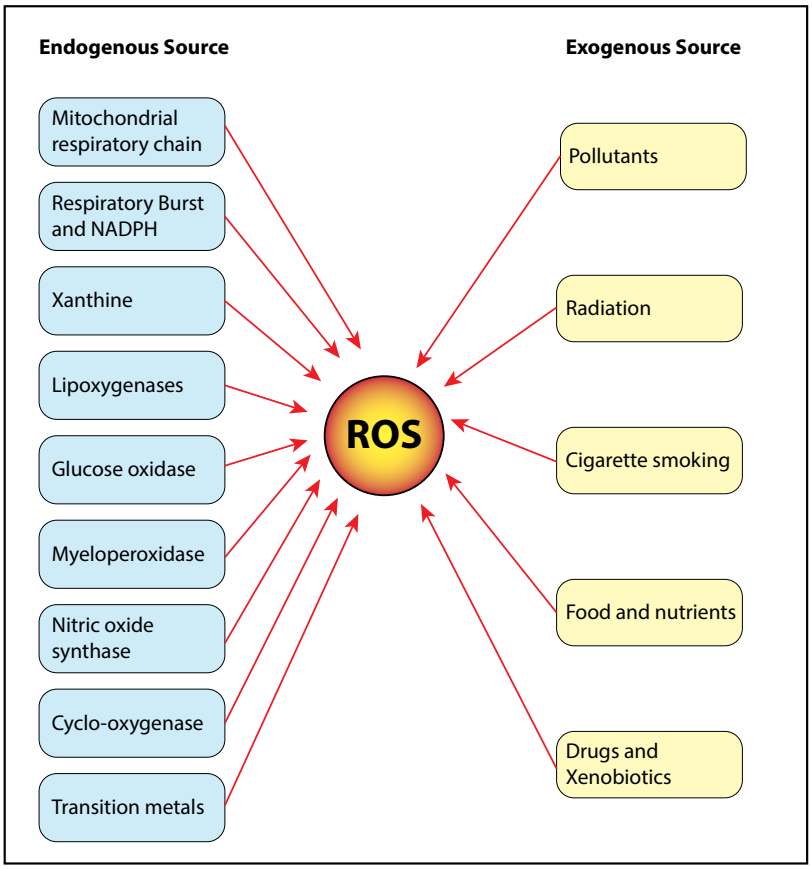


Oxidative Stress Markers

Normal metabolic processes such as cellular respiration, immune system activation, and detoxification result in the production of prooxidative substances including reactive oxygen species (ROS) and reactive nitrogen species. Additionally, external environmental factors such as toxic metal and chemical exposures, smoking, poor diet and certain medications can promote free radical production. Oxidative stress occurs when the production of prooxidative substances outweighs the body's ability to remove them, thus shifting this equilibrium in the direction of oxidation. The instability of free radicals causes them to extract electrons from neighboring molecules in a chain reaction, resulting in cellular damage. Reducing agents, including dietary antioxidants, nutritional supplements, and antioxidant enzymes provide protection against free radical damage. Oxidative stress has an integral relationship with the inflammatory cascade, which produces ROS, and is considered a driving force in the aging process. Oxidative stress has been implicated in a growing list of disorders, including cancer, arthritis, cardiovascular disease, inflammation, diabetes, autoimmune diseases, and neurodegenerative diseases.[1-6]



Sources of oxidative stress



Glutathione (whole blood)

Glutathione (GSH) is a tripeptide comprised of three amino acids (cysteine, glycine, and glutamic acid). Glutathione is the body's most potent intracellular antioxidant. It exists intracellularly in either an oxidized or reduced state.

GSH acts as an antioxidant and detoxifying agent. Excessive formation of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), is toxic to the cell. Hence, the metabolism of these free radicals are critical, and they are tightly controlled.[7]

Availability of the amino acid cysteine is known to be rate-limiting for glutathione synthesis, and it is widely known that cysteine supplementation (in the form of N-acetylcysteine) can increase GSH levels. Alpha lipoic acid maintains GSH levels via reducing cystine to cysteine as well as inducing de novo GSH synthesis.[8] Recent literature has also suggested that adequate glycine levels are critical in maintaining glutathione levels, and glycine availability may modulate the production of glutathione.[9]

Glutathione's antioxidant function is accomplished largely by GSH peroxidase-catalyzed reactions. GSH neutralizes hydrogen peroxide and lipid peroxide, resulting in water and alcohol. By accepting a free radical electron, GSH is then oxidized. GSH continues to donate and accept electrons, forming a redox cycle to counter free radicals.[10]

Glutathione is also involved in phase II detoxification by conjugating hormones, toxins, and xenobiotics to make them water soluble for excretion.[11]

There are many foods which contain significant GSH sources including, but not limited to, asparagus, avocado, watermelon, ham, and pork.[12]

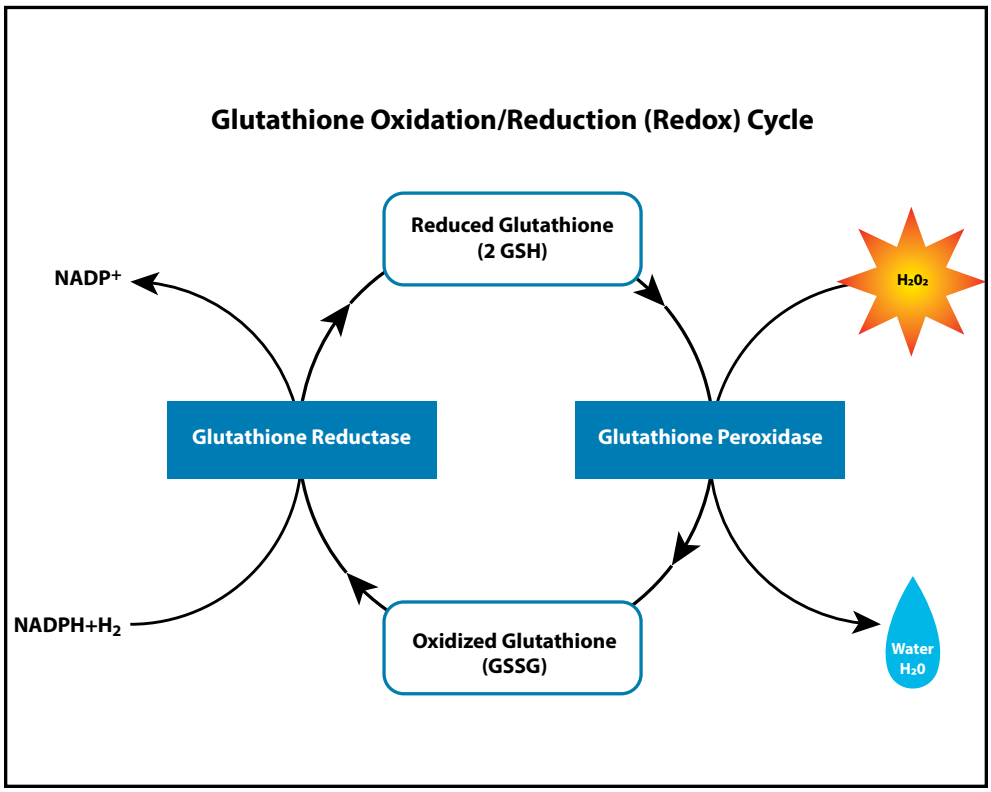
High levels

There is a transient increase in GSH plasma levels after intravenous supplementation and oral GSH ingestion, which may be useful under oxidative stress to counter free radical damage.[13]

Low levels

Nutritional deficiencies in GSH precursors (cysteine, glycine, glutamine) can result in low GSH. Genetic polymorphisms (SNPs) can also affect the production of GSH. Without adequate GSH levels, oxidative stress and free radicals contribute to aging and disease. GSH deficiency and problems with GSH synthesis have been implicated in many diseases such as cancer, neuropsychiatric dysfunction, Parkinson's disease, HIV, liver disease, and cystic fibrosis.[7]

GSH inclusion in oral over-the-counter supplements may be of limited value, since the reduced state will not be maintained when exposed to normal atmospheric conditions and room temperature. Liposomal GSH has been shown to be an excellent alternative to raise GSH levels.[14,15] Additionally, increasing amino acid dietary intake and supplementation with sulfur-containing products (N-acetyl cysteine) and foods (cruciferous vegetables, such as asparagus, broccoli, cauliflower, Brussels sprouts) will support GSH synthesis.[7] The latter requires a healthy gastrointestinal ecosystem.



Lipid peroxides

Lipid peroxides are a class of reactive oxygen species (ROS) that preferentially oxidize polyunsaturated fatty acids (PUFAs) linoleic, arachidonic, and docosahexaenoic acids (omega-6 PUFAs). Lipid peroxides exert their toxic effects via two mechanisms. One is by altering the assembly, composition, structure and dynamics of cell membrane lipid bilayers. The second is by producing more reactive oxygen species or by degrading into reactive compounds capable of damaging DNA and proteins. The central nervous system is particularly prone to lipid peroxidation due to the high quantity of ROS as a byproduct of ATP synthesis in a lipid-enriched environment.[16] Circulating LDLs can be affected by lipid peroxidation and are implicated in diseases including atherosclerosis, metabolic syndrome, and diabetes.[17-19] Genova uses the TBARS (thiobarbituric acid reactive substances) approach for determination of lipid peroxidation; the main indicator of which is malondialdehyde (MDA). MDA is a degradation product of lipid peroxides.

Ferroptosis is an iron-dependent form of cell death that is characterized by the accumulation of lipid peroxides. It is distinct from other cell death modalities, including apoptosis, classic necrosis, autophagy, and others.[20] Synthesis of PUFAs and their incorporation into phospholipid membranes is required for ferroptosis. This process is triggered by the loss of glutathione peroxidase 4 (GPX4), a lipid repair enzyme.[16,21] Depriving cells of cysteine, an amino acid precursor of glutathione can also induce ferroptosis. Clinically, ferroptosis has been associated with degenerative diseases (Alzheimer's, Huntington's, and Parkinson's diseases), carcinogenesis, stroke, intracerebral hemorrhage, traumatic brain injury, ischemia-reperfusion injury, and kidney degeneration.[22]

High levels

Elevated lipid peroxides indicate damage to lipids and lipid membranes. The enzyme glutathione peroxidase is responsible for reducing lipid peroxides and uses glutathione as a cofactor.[16,21] Lower levels of glutathione may contribute to lipid peroxidation.[23,24] Vitamin E mitigates toxicity from lipid peroxides.[16,21] Coenzyme Q10 was shown to reduce lipid peroxides and symptoms in patients with fibromyalgia and headaches.[25] Green tea catechins decreased lipid peroxide concentrations in patients with Alzheimer's disease.[26] Pre-clinical studies demonstrate inhibition of ferroptosis with iron chelators, polyphenols including curcumin, EGCG from green tea, baicalein, and lipophilic antioxidants including vitamin E.[16,27]

8-hydroxydeoxyguanosine (8-OHdG)

8-hydroxy- 2'-deoxyguanosine (8-OHdG) is a byproduct of oxidative damage to guanine bases in DNA.[28] It is used as a biomarker for oxidative stress and carcinogenesis. It has been studied to estimate DNA damage after exposure to carcinogens including tobacco smoke, asbestos fibers, heavy metals, and polycyclic aromatic hydrocarbons.[29] 8-OHdG levels are positively associated with markers of inflammation and evening cortisol, indicating that increased physiological or psychosocial stress is associated with increased oxidative damage.[30,31]

High levels

Elevated 8-OHdG indicates oxidative damage to DNA. Diseases including cardiovascular disease, COPD, cancer, thyroid disease, and diabetes have been associated with excessive concentrations of 8-OHdG.[2,28,32-38] Minimizing exposure to xenobiotics and cigarette smoke, stress management, and increasing antioxidant intake may prevent further oxidative damage.[30,31,39] Increased physical activity is associated with a reduction in urinary 8-OHdG levels.[40] Green tea catechins decreased 8-OHdG concentrations in patients with Alzheimer's disease.[26]

Coenzyme Q10 (CoQ10)

CoQ10 (ubiquinone) is synthesized in almost all cells and membranes. It is vital for electron transfer within the mitochondrial respiratory chain to create energy in the form of ATP. It is an important lipophilic intracellular antioxidant. Endogenous production of CoQ10 decreases with age. Low levels are implicated in age-related and chronic disease due to mitochondrial dysfunction and/or low antioxidant activity.[41]

Supplementation with CoQ10 has been shown to prevent, and provide improvement in, neurologic conditions like Huntington's disease, migraines, and Parkinson's disease. It's been extensively studied and used in metabolic and cardiovascular diseases such as congestive heart failure, hypertension, and diabetes.[41-44] Supplementation is also associated with improved proinflammatory cytokine TNF-α levels.[45]

Coenzyme Q10 decreases with use of statin medications used to lower cholesterol via inhibiting the enzyme HMG-CoA reductase. This enzyme is responsible for cholesterol as well as CoQ10 biosynthesis. This resultant CoQ10 deficiency may contribute to the development of myopathy and muscle symptoms seen commonly with statin use. Treatment with CoQ10 has been found to ameliorate these symptoms and improve well-being and functioning in daily life[41,46]

High levels

In general, elevated CoQ10 is seen in patients who are supplementing, however, there is no known upper level for toxicity. CoQ10 tends to be well-tolerated with a low toxicity profile. Elevated plasma CoQ10 levels have been associated with hypothyroidism.[47]

Low levels

CoQ10 deficiency occurs with age and levels can be depleted with certain medications. Low levels of CoQ10 may prompt a need for supplementation. Decreased circulating levels of CoQ10 have been associated with neurodegenerative diseases, fibromyalgia, diabetes, cancer, mitochondrial diseases, muscular diseases, hyperthyroidism, and heart failure.[42,44,47]

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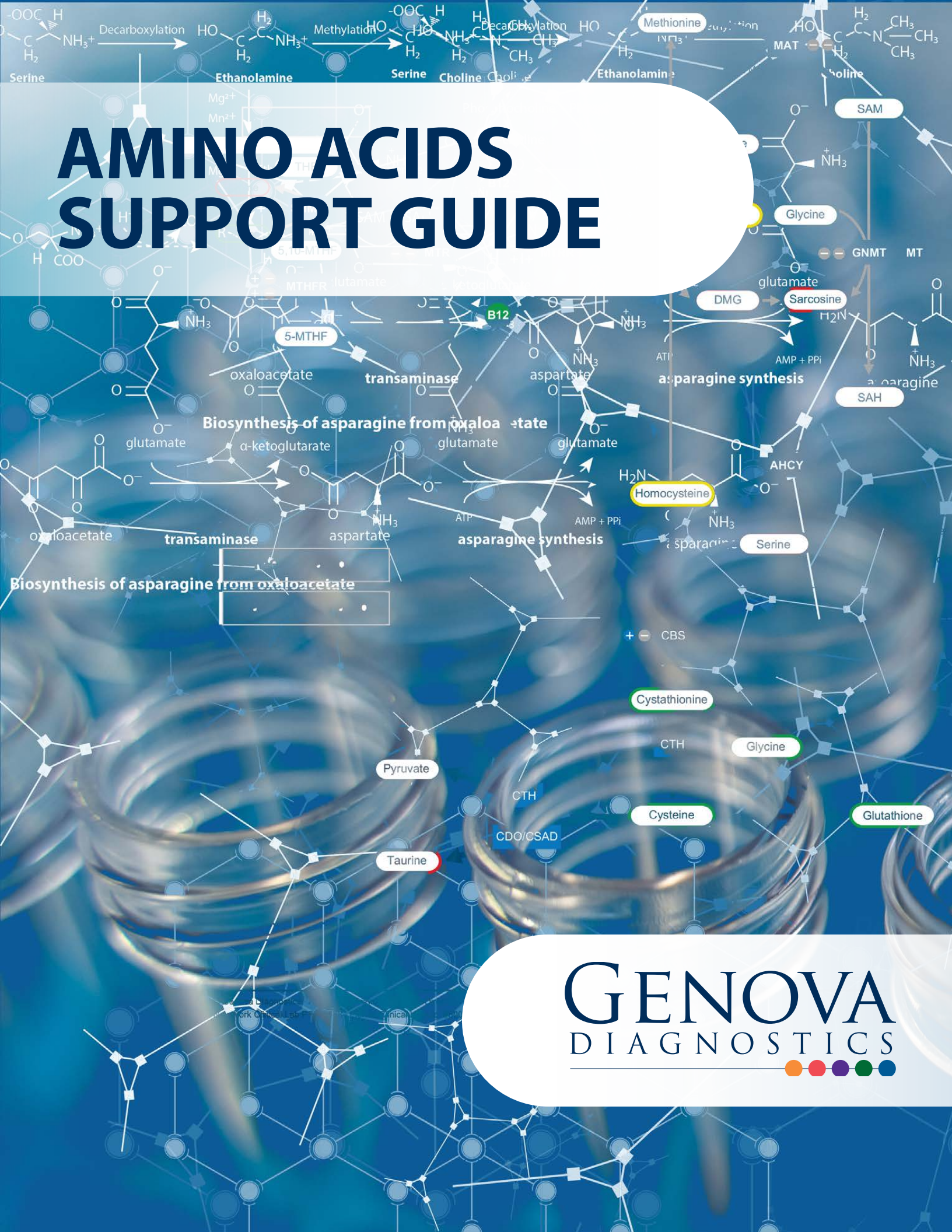

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Amino Acids Analysis

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 disorders[1]
- Weight issues/Dietary guidance[2,3]
- Malnutrition (often observed in the elderly or those with poor protein intake)[4,5]
- Gut maldigestion/malabsorption
- Fatigue[6-9]
- Athletic optimization[10,11]
- Increased nutrient demand in physical trauma/healing[8,12]
- Kidney disease[13]
- Liver disease
- Obesity/Insulin resistance/Type 2 Diabetes[14,15]
- Autism[16-18]

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

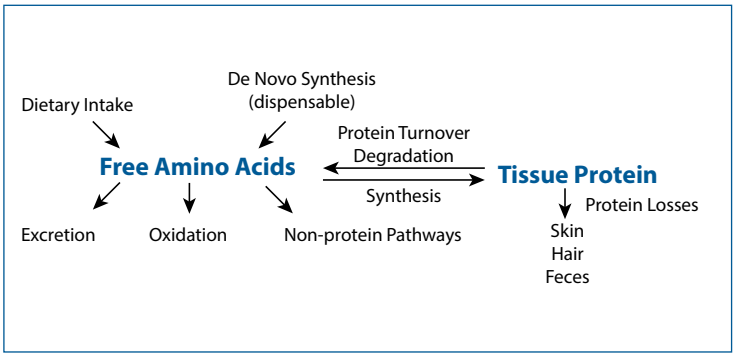
Different analytes are measurable in blood versus urine and selection of sample type depends on the clinical concern. Recent food intake briefly increases plasma amino acid levels, which is why a fasting sample is recommended. Short-term fasting does not result in depletion of plasma amino acids, but 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]

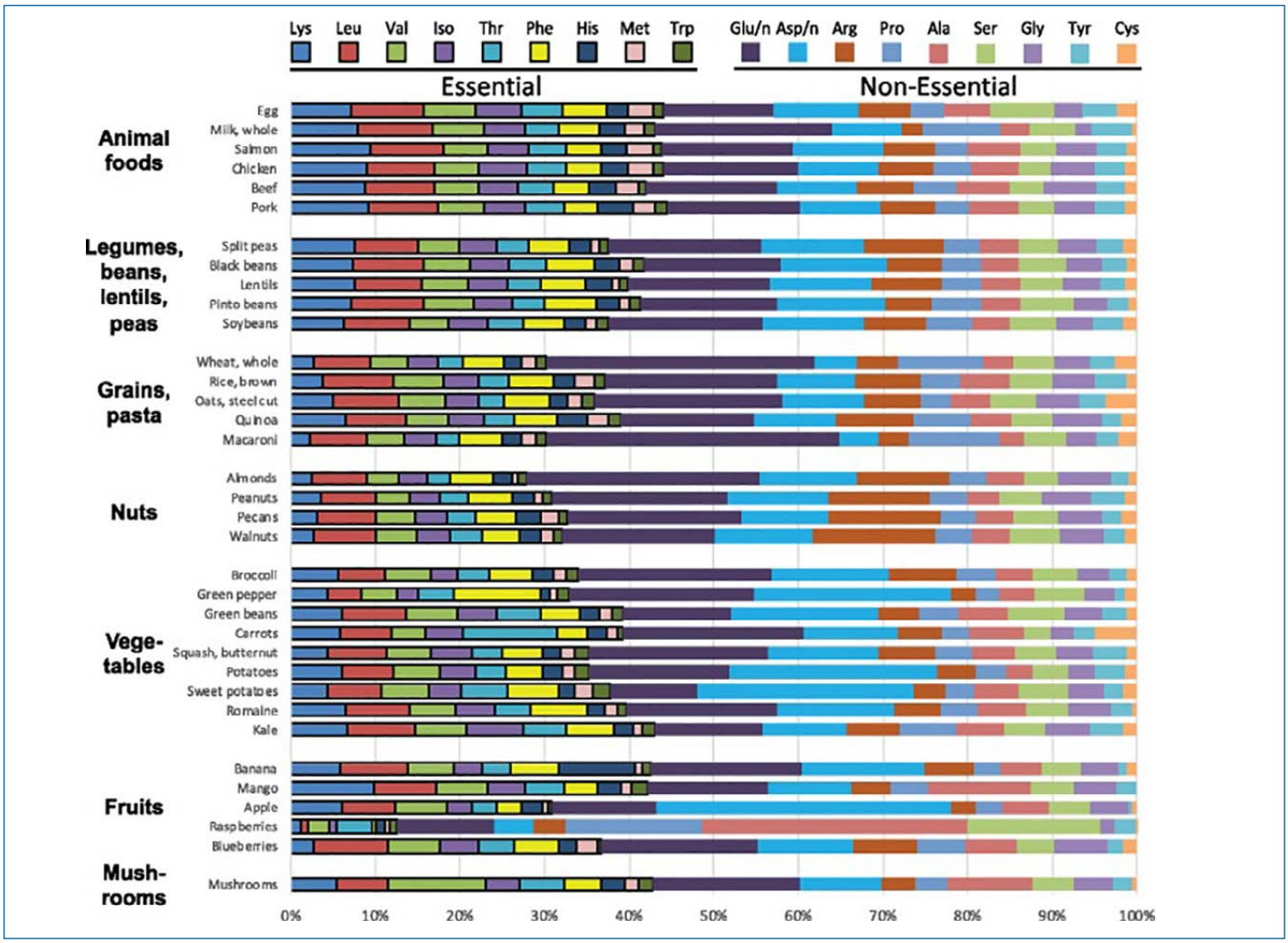
Amino Acid Composition

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 absorption[29]

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 including[31-36]

- 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 B_6 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 histadine.[46] Other food sources include poultry, fish, nuts, seeds, and grains.

Histidine and histamine have a unique relationship. The amino acid histadine becomes histamine via a vitamin B_6-dependent enzyme called histidine decarboxylase.[47] With this, decreased amounts of histidine and insufficient vitamin B_6 can subsequently lead to a decrease in histamine concentration. This may impair digestion, since histamine binds to H_2 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, histadine 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 histadine are seen in high protein diets. And, as outlined above, vitamin B_6 is needed to convert histadine to histamine, therefore a functional need for vitamin B_6 may elevate levels of histadine.[47]

There is also a relationship between histadine and folate metabolism. Histadine metabolizes to glutamic acid with FIGLU as an intermediary and tetrahydrofolate as a cofactor. Therefore, elevated histadine can be seen with vitamin B_{12} and folate insufficiencies. Urinary levels have been shown to normalize with folate administration and plasma levels have been altered in supplementation with vitamin B_{12}.[53-56]

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

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 histadine levels are clinically significant because as outlined above, histadine 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 B_6, vitamin B_1, 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 B_3, and vitamin B_6, 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 B_3 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 B_6 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 B_{12} and folate are needed to remethylate homocysteine into methionine, functional need for these cofactors may contribute to low methionine levels.[89]

Lastly, vitamin B_3 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 B_3 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 B_1, irregular renal excretion of taurine can be seen in functional vitamin B_1 insuffiencies.[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 B_6-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 B_3 deficiency has been associated with lower levels of threonine, and other amino acids.[78]

Tryptophan

Tryptophan is involved in serotonin production via vitamin B_6-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 B_3) in the liver and deficiencies of vitamin B_6, 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 B_3. This B_3 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 B_6 deficiency, and increasing age can all inhibit the conversion of tryptophan to 5-HTP and elevate tryptophan.[125] Lack of nutrient cofactors (vitamin B_6, 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]

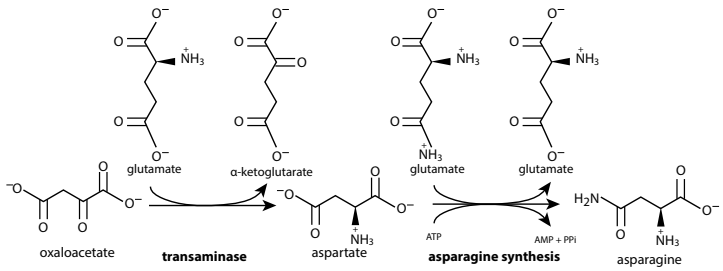
Low Levels

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 B_6 and vitamin B_3 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]



Biosynthesis of asparagine from oxaloacetate

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 B_6, 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 B_{12} may also be a cofactor in the peripheral utilization of cysteine; therefore functional deficiencies of vitamin B_{12} 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 B_6 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 prodution.[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 B_6- dependent enzyme; therefore vitamin B_6 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 B_6-

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 B_1, B_3, and B_6. Functional deficiencies in these cofactors can contribute to elevated levels. Administration of these nutrients can lower glutamate levels.[42,72,78]

Low Levels

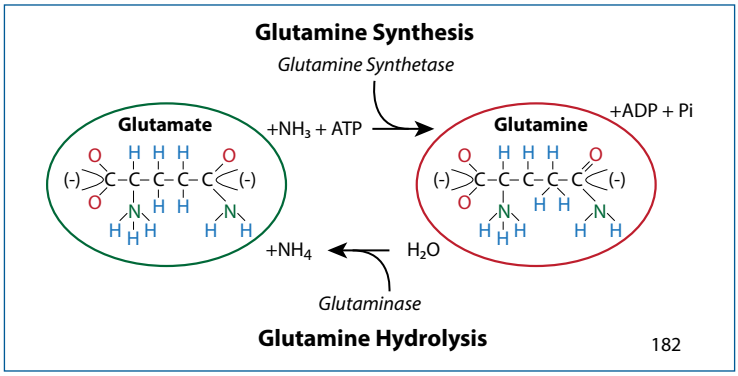
Low protein intake, GI malabsorption, and maldigestion can all contribute to low levels of amino acids. As above, there are many endogenous pathways which create glutamate, each with vitamin and mineral cofactors. Lack of those cofactors should also be considered.

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 B_1.[182] Functional deficiencies of vitamin and mineral cofactors can also elevate levels. There is literature to suggest vitamin B_1 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 B_1, can result in elevated levels. Furthermore, administration of vitamin B_1 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 B_1, vitamin B_6, 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 B_3 deficiency has been associated with altered levels of amino acids.[78]

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 B_6.[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 B_2, B_6, B_{12}, 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 it's 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 B_6 as a cofactor.[194]

High Levels

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

A functional need for vitamin B_6 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 it's precursors. Additionally, a functional deficiency in vitamin B_6 has been associated with lower levels of α-ANB and levels can increase with B_6 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 B_6-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 B_6 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 B_6 and iron.

Cystathionine is then converted to cysteine, and eventually goes on to either make glutathione or feed the Kreb's 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 B_6, 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 B_{12} 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 B_6 as a cofactor, deficiencies in vitamin B_6 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

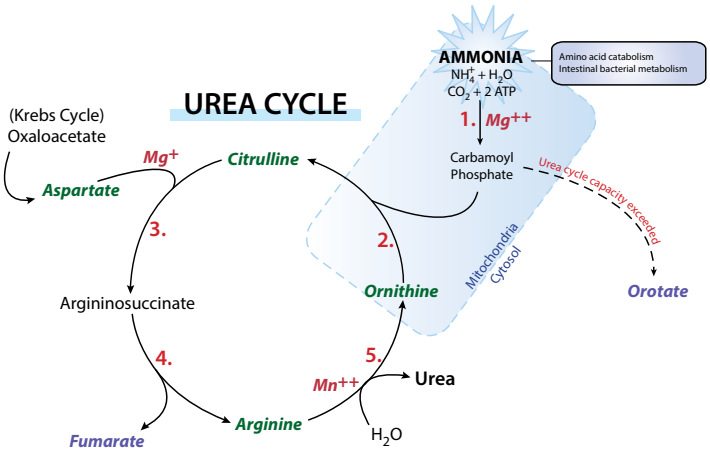
Urine and plasma levels of 3-methylhistidine can be higher with meat consumption.[46,208] And, as noted above, elevations have been seen in catabolism or fasting states.

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, arginosuccinate. 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]



FAULTY ENZYME — producing —	HYPERAMMONEMIA AND
1. *Carbamoyl Phosphate Synthase I*	Low Urinary Orotate
2. *Ornithine Transcarbamoylase*	Oroticaciduria
3. *Argininosuccinate Synthase*	Citrullinemia
4. *Argininosuccinate Lyase*	Argininosuccinic Aciduria
5. *Arginase*	Hyperargininemia

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 repleting 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 arginosuccinate 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 B_1) 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 B_6) 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 B_6) as a cofactor. OAT deficiency is a rare congenital disorder characterized by gyrate atrophy of the choroid and retina, and is treated with vitamin B_6 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 B_6 and magnesium.[181,223-226]

Elevations may also result from supplementation of citrulline or ornithine.[39,227]

Administration of thiamine (vitamin B_1) 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

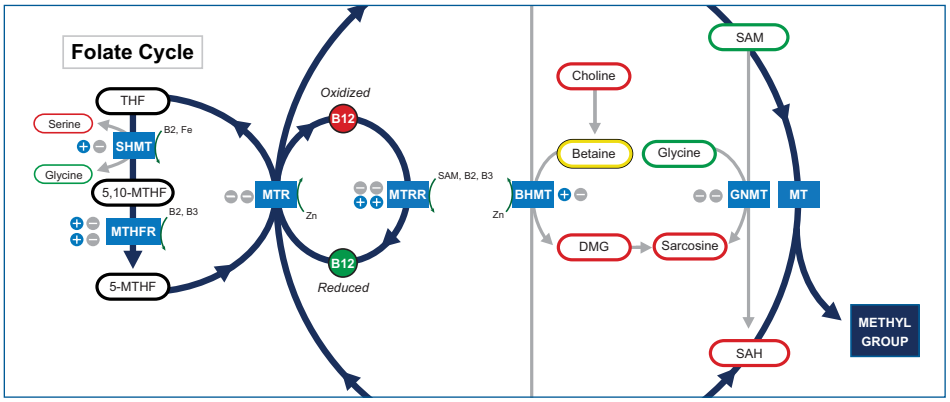
Elevated glycine may be due to dietary intake (i.e. meat, fish, legumes, and gelatin) or supplementation.

Enzymatic SNPs or cofactor deficiencies in glycine production and metabolism (vitamin B_6, B_{12}, 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.

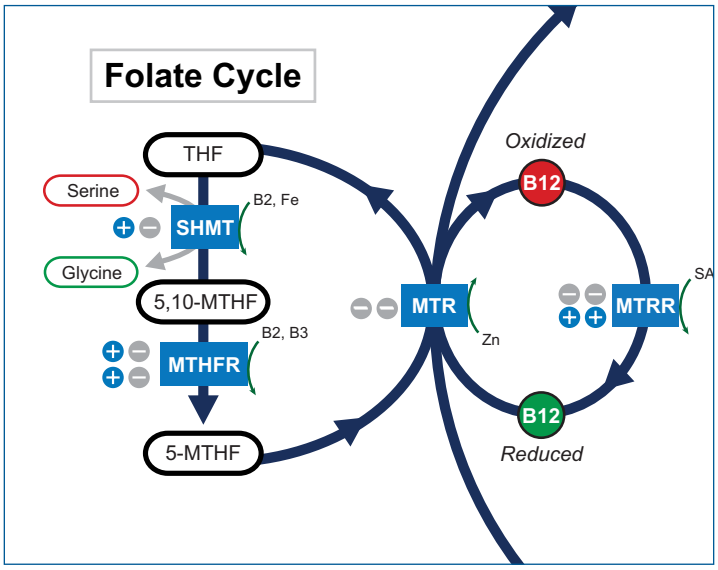
Glycine's function as an antioxidant plays an important role in disease processes and is incorporated into glutathione, an important antioxidant. Therefore, low levels have significant clinical impact. Antioxidants such as vitamins A and E can help mitigate damage from oxidative stress.[240]



Serine

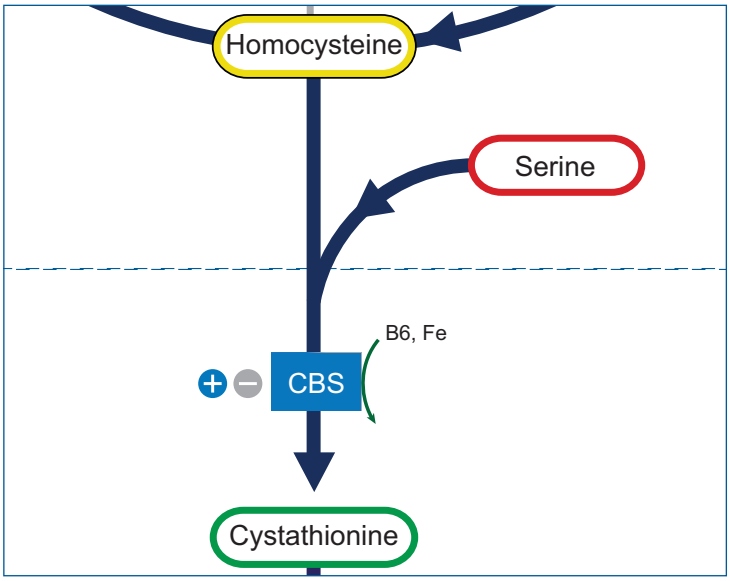
Serine is a nonessential amino acid used in protein biosynthesis and can be derived from four possible sources: dietary intake, degradation of protein and phospholipids, biosynthesis from glycolysis intermediate 3-phosphoglycerate, or from glycine.[241] Serine is found in soybeans, nuts, eggs, lentils, 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 B_6 or B_1 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 B_6, B_{12}, 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 B_6-dependent enzyme phosphoserine aminotransferase. With this, a functional need for vitamin B_6 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 B_6, B_{12}, 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

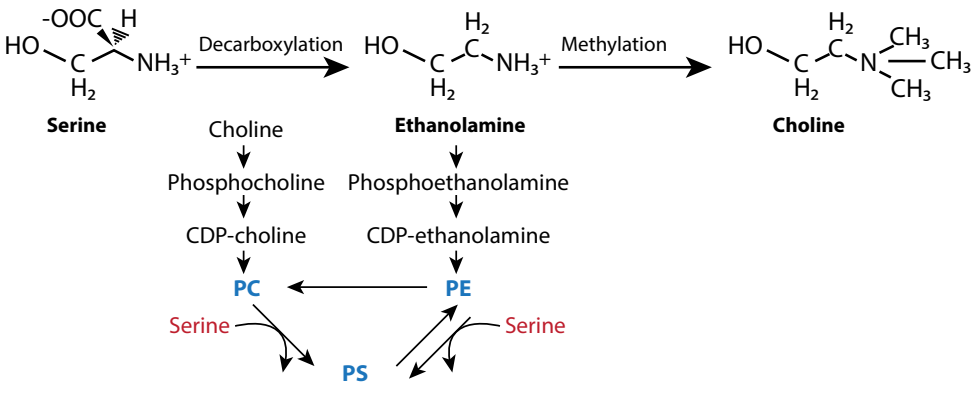
Magnesium and manganese are enzymatic cofactors in the metabolism of phosphoethanolamine. Deficiencies in these nutrients may contribute to elevated levels.[256,257,262]

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 a 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 B_6 as a cofactor. Lack of vitamin B_6 can result in lower phosphoserine levels.[249] Supplementation with vitamin B_6 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.

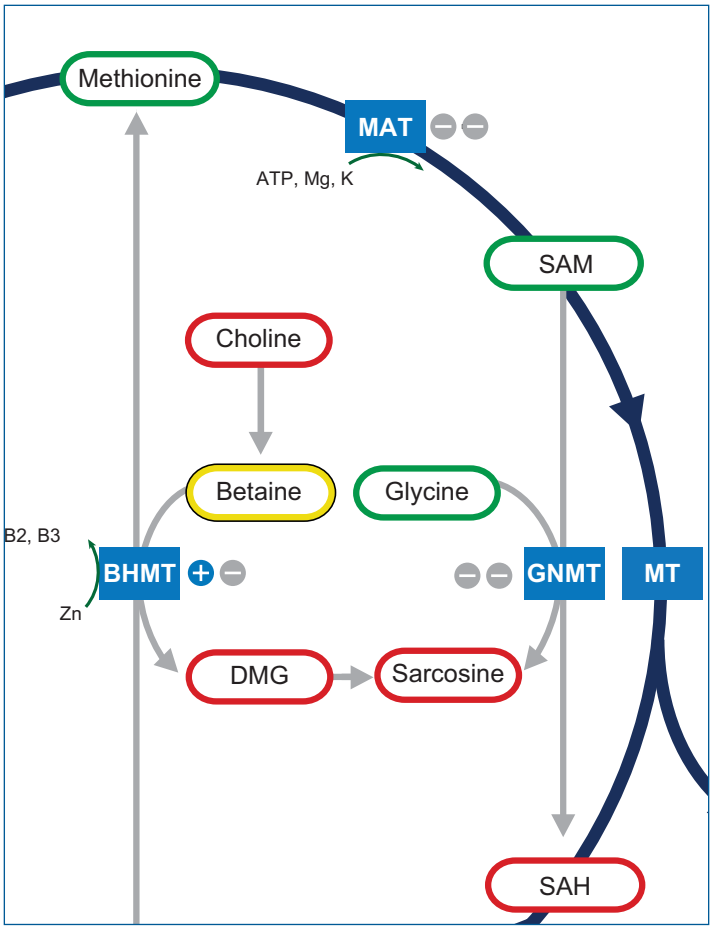
High Levels

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 B_{12}, and vitamin B_2) 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-histadine.

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

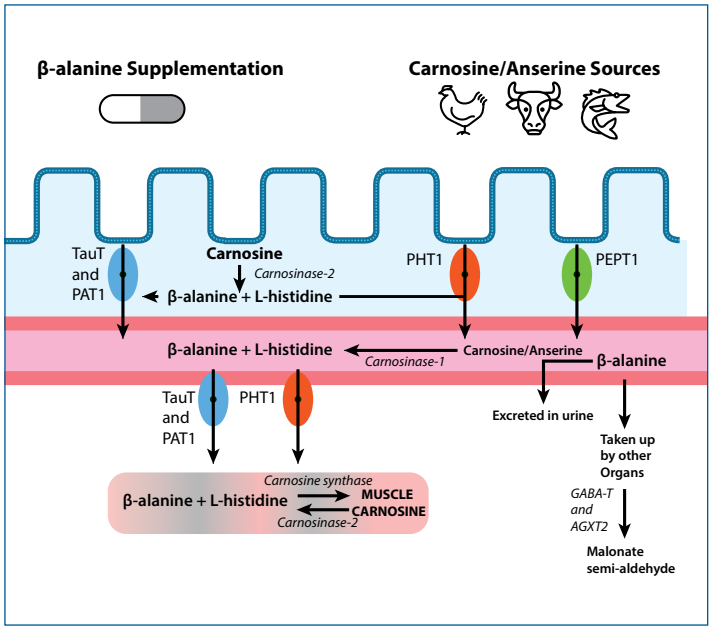
Elevations are likely due to high consumption of meat or beta-alanine supplementation. Since it is a dipeptide, elevations might also signify incomplete protein digestion.

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 B_6-dependent enzymes. With that, a functional need for vitamin B_6 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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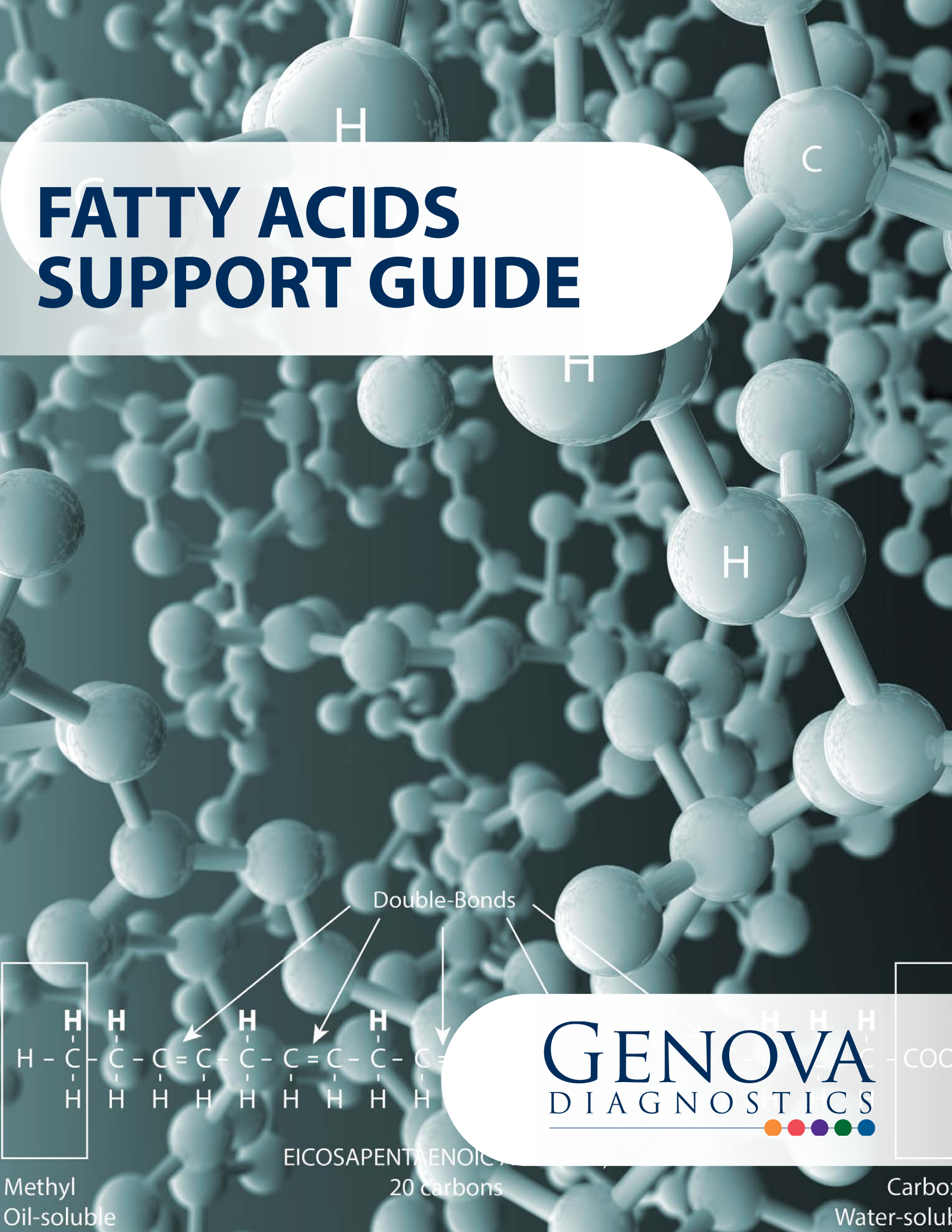

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Essential & Metabolic Fatty Acids

NUTRITIONAL

Dietary fat is emerging as one of the most important nutritional modifiers for overall health. There are many health implications which make measuring fatty acids vitally important. Relying on dietary recall may not be accurate since fatty acids can not only be obtained from the diet, but also created endogenously. Imbalances in fatty acids have been implicated in many clinical conditions including but not limited to:[1-18]

- **Cardiovascular disease**
- **Chronic inflammatory conditions**
- **Autoimmune diseases**
- **Osteoporosis**
- **Cognitive decline**
- **Mood disorders**
- **Neurologic disease**
- **Cancer**
- **Diabetes**
- **Eczema and psoriasis**
- **Metabolic syndrome**
- **Polycystic ovary syndrome**
- **Chronic obstructive pulmonary disease**
- **Asthma**

MEASUREMENT IN PLASMA VERSUS RED BLOOD CELL

Plasma and erythrocyte assessments are commonly used to assess fatty acid imbalances. Because the red blood cell life averages 90-120 days, it reflects a longer status than plasma. On the NutrEval, fatty acids are measured in the red blood cell as a weighted percentage of the cell membrane. Blood spot evaluation is whole blood, including RBC and plasma. This may reflect both short- and longer-term status, though internal data reveals good correlation between the two.

It should be noted that when dealing with percentages, the amount of each fatty acid can influence the others. For example, fish oil supplementation may increase the overall omega-3 percentage, which may lower the omega-6 percentage.

WHAT IS A FATTY ACID?

Fatty acids are simple in structure: a carbon backbone with a carboxyl group (COO) at one end, and a methyl group (CH3) at the other. They are used as energy storage units, structural components of cell membranes, and precursors to eicosanoids, which are important signaling molecules in the inflammatory cascade. Fatty acids are made through the digestion of dietary fat or by endogenous production.

'Essential' fatty acids must come from dietary intake and cannot be made in the body. Dietary fat is digested and broken down into fatty acids which are then absorbed into circulation. In circulation, they can undergo beta-oxidation to become Acetyl-CoA to be used as energy in the Citric Acid cycle. They can also join in circulation to form triglyceride molecules.

Endogenous production of nonessential fatty acids happens one of three ways: synthesis, elongation, and desaturation.

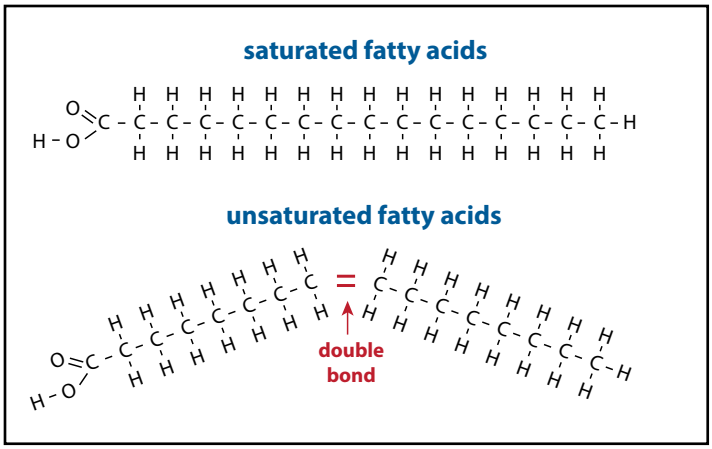
a. Fatty acids can be **synthesized** from carbohydrates. Dietary carbohydrates are metabolized to Acetyl-CoA which itself can form fatty acids outside of the mitochondria in the cytosol. Also, insulin can covert excess glucose into triglycerides in the liver and adipocytes.[19]
b. **Elongation** is the adding of carbon molecules to an existing fatty acid to produce a longer fatty acid using an elongase enzyme.
c. **Desaturation** is the process of adding double bonds to dietary fatty acid carbon backbones. The enzymes for this process are called delta-desaturases, further classified based on where the bond is being added. For example, adding a double bond between carbons 9 and 10 uses delta-9 desaturase.[20]

FATTY ACID STRUCTURE AND NOMENCLATURE

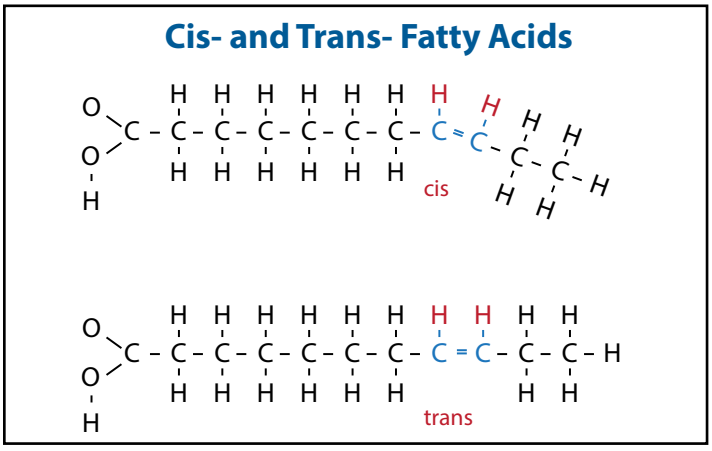
Understanding the nomenclature of fatty acids can seem complex since there are several different naming conventions used by various laboratories and throughout literature. The basic structure of a fatty acid lays the groundwork by which it is named.

As mentioned previously, fatty acids consist of a carbon backbone with a carboxyl group at one end, and a methyl group at the other. That methyl group is referred to as omega (ω) or (Ω). The letter n is also frequently used. The length of each carbon backbone can range from 6 to 22, and sometimes longer. This is what differentiates them as short-chain, medium chain, long-chain, or very-long-chain fatty acids. The number of carbons delineating each of these (i.e. long vs. very-long-chain) varies somewhat in literature.[21]

A presence or absence of double bonds between the carbons reflects the degree of saturation. When the carbon backbone contains no double bonds, it is called saturated – filled with hydrogen as hydrocarbon chains. Unsaturated fatty acids contain one or more double bonds within that carbon backbone. Monounsaturated fatty acids contain one double bond while polyunsaturated fatty acids contain 2 or more. Because fatty acids are cell membrane structural components, the degree of saturation can play a role in membrane fluidity.[22]

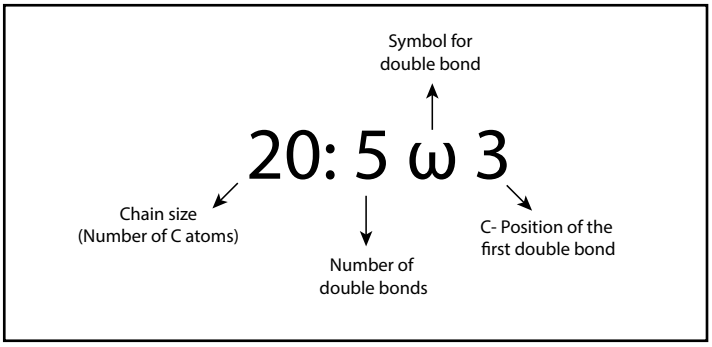


The placement of hydrogen molecules around the double bonds of the carbon backbone causes structural variances which can reflect important differences in the form, function, and energetics of the molecule. When the hydrogen molecules on either side of the double bond are in the same configuration, and on the same side, it is termed a cis configuration. Most fatty acids are in this cis configuration. This results in a type of bending to the chain since the atoms repel each other slightly. It prevents them from stacking together and becoming solid in room temperature. When the hydrogen atoms are on opposite sides, it is referred to as a trans fatty acid. Trans fats can naturally occur in small amounts in foods such as dairy and meat. However, they more commonly originate from food processing and partial hydrogenation. Hydrogenation involves chemically adding hydrogen molecules to eliminate double bonds to make the chain saturated. This process is used in the food industry to prolong shelf-life and increase the fat's melting point to make it more suitable for frying.[23] Trans fats produced in this industrial processing are harmful and risk inducing.[24-26]

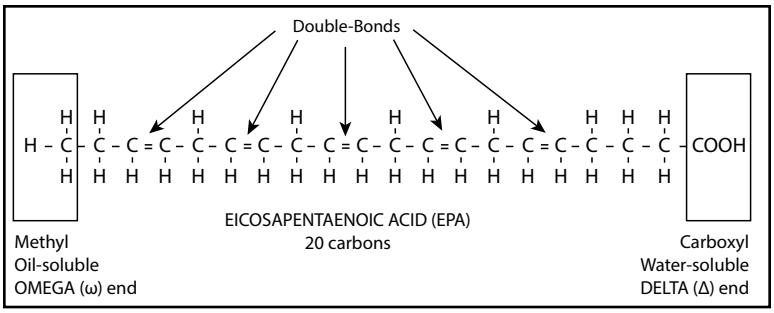


In mono- and polyunsaturated fats, it is the position of the first double bond between carbon molecules as it relates to the methyl group end which then further delineates them. For example, eicosapentaenoic acid (EPA) contains several double bonds, the first of which is at the third carbon; this makes EPA a polyunsaturated, omega -3 (ω-3) fatty acid. Oleic acid contains one double bond at the 9th carbon, which makes it a monounsaturated, omega-9 (ω-9) fatty acid.

Additionally, some use a numerical abbreviation and others describe the double bonds in relation to the carboxyl end of the carbon chain, rather than the methyl/ omega end. Using the above examples, EPA has twenty carbons with five double bonds, starting at the third carbon, numerically it can be represented as 20:5n3.



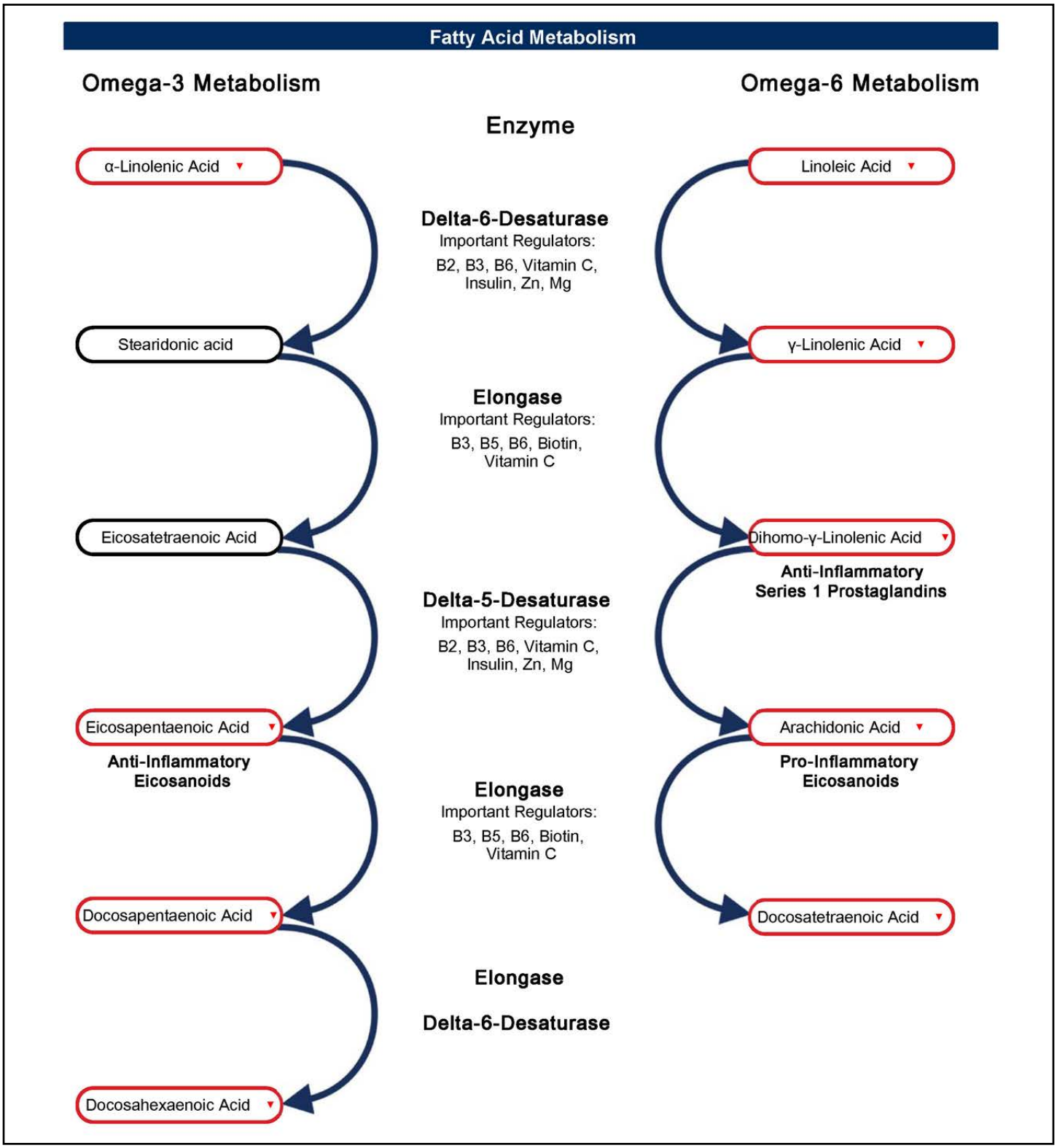
When naming fatty acids in relation to the carboxyl group, acidic end, the delta (Δ) symbol is used, followed by the numeric placement of double bonds. Therefore, EPA can be represented as 20:5 Δ5,8,11,14,17.



ESSENTIAL FATTY ACID METABOLISM

Dietary fatty acids can be converted into energy, stored, incorporated into cell membranes, or produce other fatty acids. There are only two essential dietary fatty acids: α-linolenic acid (omega-3) and linoleic acid (omega-6). All other fatty acids can either be obtained in the diet or be made from the essentials.

As discussed above, elongase and desaturase enzymes convert the essential fatty acids into others by adding carbon molecules to the backbone, or by inserting double bonds. Omega-3 and omega-6 fatty acids are competitive in their use of desaturase and elongase enzymes.[27]



Omega-3 Fatty Acids

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been linked to healthy aging throughout our lifespan - from fetal development to prevention of Alzheimer's disease. Omega-3 fatty acids are anti-inflammatory and used in cell membrane production, function, and overall gene expression.[28]

Most standard American diets are deficient in common n-3 food sources such as flax, oily fish, nuts, and green leafy vegetables. Deficiencies in n-3 have been correlated with many clinical conditions such as neurodevelopmental and behavioral disorders, cardiovascular disease, cognitive decline, mood disorders, skin abnormalities, visual changes, and cancer.[29-35]

Omega-3 Fatty Acids Low Levels:

The reference ranges for omega-3 fatty acids are one-tailed since their health benefits are well-studied and deficiencies are associated with many clinical conditions. Low levels can be seen with decreased dietary intake of n-3 containing foods. Gastrointestinal malabsorption or maldigestion should also be considered.

Additionally, desaturase and elongase enzymes are used to metabolize and create n-3 fatty acids from the essential alpha-linolenic acid. Lack of vitamin or mineral cofactors for these enzymes, or single nucleotide polymorphisms (SNPs) can contribute to lower, or sometimes higher levels of each. Nutrients such as zinc, vitamin B_6, vitamin B_3, and magnesium are important cofactors for fatty acid metabolism. Other enzymatic influences such as alcohol, cortisol, and adrenocorticoids can also influence these enzymes.[36,37]

It should also be noted that there is competition between the omega-3 and omega-6 fatty acids for use of the desaturase and elongase enzymes which may alter levels of fatty acid metabolites.[27]

Alpha-Linolenic Acid

Alpha-linolenic acid (ALA) is an essential n-3 fatty acid and must be obtained in the diet. Sources include green leafy vegetables, oily fish, flaxseed, soybean oil, canola oil, walnuts, and chia seeds.[38,39] ALA has an 18-carbon backbone with 3 double bonds starting at the third carbon molecule (18:3n3). It is an important precursor to make eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), though these can also be obtained in the diet. Most dietary ALA is used to generate energy and only a small portion is converted to EPA and DHA.[40]

High Levels

Increased dietary intake of ALA-rich foods or supplementation can elevate levels.

The delta-6 desaturase enzyme is used to convert ALA into other downstream fatty acids. Lack of vitamin and mineral cofactors or genetic single nucleotide polymorphisms (SNPs) in the enzyme may slow the enzyme and contribute to elevations. Some studies suggest that the conversion rates of ALA to downstream fatty acids are gender dependent. There may be direct estrogen effects to desaturase and elongase enzymes whereby women of reproductive age show substantially greater conversion rates.[37,41]

Higher levels of ALA are beneficial and its positive effects have been studied in several clinical conditions such as cardiovascular disease, diabetes, cancer, neurodegenerative diseases, and autoimmunity.[39,41-44]

Although there is limited toxicological data for ALA, no serious adverse effects have been reported. Research is inconclusive regarding increased risk of prostate cancer in association with high dietary ALA intake.[41,45]

Eicosapentaenoic Acid

Eicosapentaenoic acid (EPA) is an omega-3 fatty acid with 20 carbons and 5 double bonds (20:5n3). EPA can either be made from the downstream metabolism of ALA or it can be obtained in the diet. Food sources include oily fish such as salmon, mackerel, cod, and sardines. In addition to diet and ALA desaturation, EPA is also available as a fish oil supplement. The desaturation of ALA to EPA is not a very efficient process, therefore dietary intake or supplementation is important.[27,40]

As a precursor for prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 eicosanoids, EPA carries special importance in the inflammatory cascade. EPA can also lower plasma triglyceride levels without raising low-density lipoprotein cholesterol levels. Some studies suggest that in cardiovascular disease, EPA may decrease plaque vulnerability, prevent progression, and decrease macrophage accumulation. It is also vasodilatory which can lower blood pressure.[46]

High Levels

Elevations in EPA can be due to high dietary intake of EPA-containing foods as outlined above and from supplementation with fish oil.

Lack of vitamin and mineral cofactors, or SNPs in the elongase enzyme, may also contribute to elevations. It should also be noted that there is competition for the elongase and desaturase enzymes between the omega-3 and omega-6 fatty acids which may affect levels of fatty acid metabolites.

High levels of EPA and its downstream metabolite DHA have been used in treatment for many clinical conditions. Studies show benefit in cardiovascular disease, depression, cognitive decline, autoimmune diseases, skin diseases, inflammation, cancer, and metabolic syndrome.[47-53]

Because of EPA's anti-platelet effects, over-supplementation was once thought to increase bleeding risk, especially if taken with other anticoagulants. However, new literature finds no increased risk of bleeding in patients taking fish oil supplementation while undergoing surgery and invasive procedures. In fact, some literature demonstrates a reduced need for blood transfusion in these patients.[54-56]

Docosapentaenoic Acid

Docosapentaenoic acid (DPA) is an omega-3 fatty acid with 22 carbons and five double bonds (22:5n3). It is formed from its precursor, EPA, by way of the elongase enzyme which adds two carbons. It can be supplemented or obtained in the diet from foods such as marine oily fish. Not only is DPA found in most fish and marine foods but it is also present in lean red meat from ruminant animals.[57]

DPA is often overlooked and overshadowed by the significant amount of research on its precursor EPA and downstream metabolite docosahexaenoic acid (DHA). Both EPA and DHA are widely studied and commonly available as fish oil supplements. However, DPA is also found to have significant clinical importance.

DPA inhibits cyclooxygenase-1 and is a potent inhibitor of platelet aggregation. It also has been shown to suppress lipogenesis to regulate lipolysis in favor of increased lipid oxidation for energy. It's beneficial role in cardiovascular disease has also been studied. DPA can be retroconverted to EPA. Some research suggests that DPA can function as a reservoir or buffer of the other omega-3 fatty acids.[57]

High Levels

Elevated DPA is seen with high intake of marine fish and other food sources, as well as with supplementation. High levels are less likely due to problems with downstream metabolism to DHA since it's been shown to retro convert to EPA as a regulator and reservoir of the omega-3 fatty acids.

DPA's anti-inflammatory effects have been studied in many conditions such as inflammatory bowel disease, peripheral vascular disease, cardiovascular disease, cognitive decline, and stroke.[58-63]

Docosahexaenoic Acid

Docosahexaenoic acid (DHA) is an omega-3 fatty acid with 22 carbons and 6 double bonds (22:6n3). It can be obtained from the diet, supplemented, or created by conversion from DPA using elongase and desaturase enzymes. DHA is present in fatty fish such as salmon, tuna, and mackerel, and low levels of DHA can be found in meat and eggs.[64]

Both individually or in combination with EPA, DHA is widely supplemented due to the enormous amount of research available regarding its anti-inflammatory role in many clinical conditions such as cardiovascular disease, cognitive decline, autoimmune disease, fetal development, visual disturbances, cancer, and metabolic syndrome.[28,29,33,64,65]

High Levels

Elevations in DHA can be seen in high omega-3 dietary intake and in patients who are supplementing with fish oil.

In addition to the clinical implications outlined above, having adequate levels of DHA is important for neuroprotection, blood pressure regulation, protection from cardiac arrythmia, inflammation, and tumorigenesis.[66]

Much like EPA, oversupplementation with DHA was initially thought to increase bleeding, especially in patients also taking anticoagulants. However, literature is showing that fish oil containing EPA and DHA does not increase perioperative bleeding in patients undergoing invasive procedures. In fact, higher levels are associated with lower risk of bleeding in these patients.[67]

Percentage Omega-3s

When assessing fatty acids in RBCs, Genova measures a weighted percentage of fatty acids taken up into the erythrocyte wall. The total omega-3 percentage is a combined total weight percentage. It is calculated by adding up each of the measured omega-3s. Higher total percentages of omega-3 fatty acids are anti-inflammatory, cardioprotective, and considered beneficial.

It should be noted that when dealing with percentages, the amount of each fatty acid can influence the others. For example, fish oil supplementation may increase the overall omega-3 percentage. By default, this may then lower the omega-6 percentage.

Omega-6 (n-6) Fatty Acids

Throughout evolution, the dietary intake of omega-3 and omega-6 polyunsaturated fatty acids has proportionately changed from a ratio of 1:1 to 20:1 or more, in favor of omega-6 fatty acids. This shift in Western diets toward omega-6s coincides with an epidemic of obesity, metabolic dysfunction, and many other significant clinical implications.[68-70]

The main concern with omega-6 fatty acids revolves around one of the downstream metabolites: arachidonic acid (AA). AA is a precursor for the inflammatory cascade. The potential pro-inflammatory nature of omega-6s, as well as their susceptibility to oxidation, can lead to many clinically deleterious effects. They also compete with the omega-3 cascade for use of the elongase and desaturase enzymes.

It should be emphasized that not all omega-6 fatty acids are concerning. In fact, some have important physiologic effects and can be anti-inflammatory. Omega-6 fatty acids act as structural cell membrane components and as precursors to eicosanoids. These eicosanoids modulate renal and pulmonary function, vascular tone, and the inflammatory response.[71]

Linoleic Acid

Linoleic acid (LA) is the only essential omega-6 fatty acid and must be obtained from the diet. From LA, other omega-6s can be created using elongase and desaturase enzymes. LA contains 18 carbons, with 2 double bonds, the first of which is at the 6th carbon position (18:2n6). LA is found in nuts and vegetable oils (corn, soybean, canola, sunflower, etc.) as well as most meats.[72] When the double bonds of LA are arranged differently, the term conjugated LA (CLA) is used. Although technically CLA can be termed a trans-fat, a natural type of CLA can be obtained in the dietary intake of meat and milk from ruminant animals. There are many isomers of CLA – some beneficial and others are not as well defined.[73]

There is some controversy regarding how much LA is needed from the diet for adequacy. Although LA is needed to synthesize downstream fatty acids, it may lead to increased inflammatory fatty acid production. Several studies show that LA lowers blood cholesterol levels and improves all-cause mortality However, their current role in atherosclerosis and cardiometabolic disease are being revisited. There is difficulty in differentiating the biological effects of LA from arachidonic acid in health and disease. In fact, it has been shown that LA is the most abundant fatty acid found in LDL and is one of the first fatty acids to oxidize. Studies are showing that LA promotes oxidative stress, oxidized LDL, and may be a major dietary cause of cardiovascular disease, especially when consumed via industrial vegetable oils.[9,74-80]

High Levels

Elevations are seen with high dietary fat intake (especially vegetable oils), or with supplementation of CLA. The delta-6-desaturase enzyme converts LA to downstream fatty acids. Lack of vitamin and mineral cofactors, or a SNP in the enzyme may slow its ability to covert and elevate LA levels. Additionally, there is competition with the omega-3 fatty acids for use of this enzyme which may contribute to elevated levels depending on availability.[37]

High levels of LA are associated with obesity, inflammatory conditions such as IBD, various cancers, cardiovascular disease, altered cognition, and brain development.[79,81-85]

Low Levels

Linoleic acid deficiency is rare, especially give current dietary trends which include excess vegetable oils. However, lack or decreased intake of foods containing LA can contribute to lower levels. Additionally, a SNP in delta-6-desaturase may potentially alter the enzyme function and promote downstream metabolism.[86]

Essential linoleic acid deficiencies have been mainly associated with skin conditions and impaired growth and development. Low levels of LA may contribute to impaired wound healing since it has been found to modulate a cellular response in wound healing by increasing the migration and functions of inflammatory and endothelial cells, and by inducing angiogenesis at the wound site.[87-89]

Gamma Linolenic Acid

γ-linolenic acid (GLA) is an omega-6 fatty acid containing 18 carbons and 3 double bonds (18:3n6). It is synthesized from LA by adding a double bond using the delta-6-desaturase enzyme. This enzymatic reaction is very slow and further impaired in vitamin and mineral deficiencies such as zinc and cobalt. Stress, smoking, alcohol, and systemic inflammatory conditions can also slow this conversion.[90]

Since the synthesis of GLA is not efficient, dietary intake of organ meats may be considered to raise GLA levels. Also, many people supplement with GLA-containing products such as borage oil, black currant, and evening primrose. Primrose and borage oil supplementation have been studied as an effective treatment for many conditions such as rheumatoid arthritis, dermatitis, and diabetic neuropathy. They have been shown to decrease inflammation, improve bone health, regulate lipid metabolism, and have beneficial effects on the skin. But, whether it's the GLA component that is beneficial or GLA's downstream fatty acid metabolite is difficult to determine.[91]

The clinical importance of GLA is in its rapid conversion to its downstream fatty acid dihomo-gamma-linolenic acid (DGLA) which is anti-inflammatory. GLA itself, however, does have physiologic importance. It has been shown to exert some tumoricidal activity in various cancers and to inhibit metastases.[90] GLA has been studied for its clinical importance in neurovascular deficits in diabetes and has been shown to normalize nerve conduction velocity and endoneurial blood flow.[92]

There is some concern regarding GLA supplementation leading to rapid conversion through DGLA to arachidonic acid. Supplementing the omega-3s EPA or DHA may help to mitigate the effects since there is enzymatic competition for the delta-5-desaturase enzyme. This enzyme is responsible for both AA production and EPA metabolism.[93]

High Levels

Elevations are seen with supplementation of borage oil, primrose, and black currant. Additionally, the conversion to DGLA requires the elongase enzyme. Lack of vitamin and mineral cofactors, enzymatic SNPs, or competition for use of the enzyme by omega-3 fatty acids may contribute to elevated GLA. It should also be emphasized that smoking, alcohol, and systemic inflammation can slow the elongase enzyme and conversion to DGLA.[37,90]

As noted above, GLA has important clinical implications. The issues of safety have been investigated and GLA appears to be nontoxic. Limited cases of soft stools, belching, and abdominal bloating have been reported. Long-term human studies show that up to 2.8 g/d are well tolerated. However, the possibility exists that GLA will be metabolized through to DGLA and then increase arachidonic acid causing inflammation.[92] The addition of EPA or DHA may help to mitigate these effects.

Low Levels

Decreased intake of the essential LA can result in low levels. Also decreased conversion by the delta-6-desaturase enzyme can result in low levels of GLA due to lack of vitamin and mineral cofactors or SNPs in the enzyme. The competition for use of delta-6-desaturase by the omega-3s should also be considered.[37]

Due to the important clinical implications of GLA and subsequent DGLA formation as outlined, supplementation with evening primrose, borage oil, and black currant may be beneficial.

Dihomo-gamma Linolenic Acid

Dihomo-gamma-linolenic acid (DGLA) is a 20-carbon omega-6 with 3 double bonds (20:3n6) derived from the essential linolenic acid. LA is metabolized to GLA, which is rapidly elongated to DGLA. There are only trace amounts of DGLA found in organ meats, otherwise it must be synthesized from GLA. The inability to convert precursor fatty acids to DGLA is associated with various pathologic and physiologic conditions such as aging, diabetes, alcoholism, atopic dermatitis, rheumatoid arthritis, cancer, and cardiovascular disease.[94,95]

DGLA is a precursor to prostaglandin PGE1, which inhibits platelet aggregation and inflammation, produces vasodilation, inhibits cholesterol biosynthesis and thrombus formation, regulates immune responses and reduces blood pressure. It is also involved in inhibiting the formation of pro-inflammatory compounds from AA. PGE1 can also inhibit growth and differentiation of cancer cells. Although the mechanism of DGLA in cancer has not yet been identified, the potential benefits are being studied.[94,96]

DGLA-enriched oils and fermented DGLA oil supplements are being developed with excellent safety profiles and studied in a variety of clinical conditions.[94,97]

High Levels

Supplementation with DGLA or GLA, as well as high dietary intake of the essential LA, can lead to higher DGLA levels. Lack of vitamin and mineral cofactors, or SNPs in the enzyme which converts DGLA downstream to arachidonic acid, may also contribute to elevations.[37]

Higher DGLA levels are mainly beneficial due to its anti-inflammatory role. Although there is some concern regarding DGLA being converted to its pro-inflammatory metabolite, arachidonic acid, the conversion is generally limited. The reason for this limitation is that inflammatory arachidonic acid-derived lipid mediators (eicosanoids) are made via several pathways two of which are cyclooxygenase (COX) and lipoxygenase (LOX). The synthesis of AA eicosanoids is dependent on DGLA since DGLA competes with AA for COX and LOX. When DGLA is in excess, it inhibits the synthesis of AA-derived eicosanoids due to its higher affinity for the COX and LOX enzymes.[98,99]

High levels of DGLA are associated with elevated body mass index, waist circumference, body fat percentage, and other obesity-related parameters. It should be noted that some of these clinical associations are related to increased overall intake of omega-6 fatty acids. But insulin itself can downregulate the enzyme delta-5-desaturase which synthesizes AA from DGLA. Therefore, obesity and insulin resistance can affect delta-5-desaturase resulting in higher DGLA levels.[100,101]

Low Levels

Decreased intake of the essential LA, or inefficient metabolism of the omega-6 fatty acids can lead to decreased production of DGLA. Lack of vitamin or mineral cofactors, or SNPs in the elongase and desaturase enzymes can contribute to lower DGLA levels either from production to DGLA or increased metabolism to AA. It should also be emphasized that smoking, alcohol, and systemic inflammation can slow the elongase enzyme and conversion to DGLA.[37,90]

Due to the anti-inflammatory and beneficial effects of DGLA, low levels have significant clinical associations such as diabetes, alcoholism, atopic dermatitis, rheumatoid arthritis, cancer, and cardiovascular disease.[94,95] Decreased levels are associated with increased total mortality in patients with acute cardiac events and decompensated heart failure.[102]

Arachidonic Acid

Arachidonic acid (AA) is a 20-carbon polyunsaturated n-6 fatty acid with 4 double bonds (20:4n6). Its double bonds contribute to cell membrane fluidity and predispose it to oxygenation. This can lead to several important metabolites which ensure a properly functioning immune system as well as regulate inflammation, brain activity, and other signaling cascades.

AA's metabolites are called eicosanoids which are signaling molecules. They can be produced via cyclooxygenases, lipoxygenase, cytochrome P450, and oxygen species-triggered reactions. These pathways yield molecules like prostaglandins, isoprostanes, thromboxane, leukotrienes, lipoxins, and epoxyeicosatrienoic acids.

AA can be obtained in the diet from eggs, fish, and animal meats and fats – or produced directly from DGLA using the delta-5-desaturase enzyme. Although often vilified, adequate AA intake is needed to achieve an equilibrium between its inflammatory and resolution effects to support a healthy immune system. It is also fortified in infant formulas due to its importance in growth and development.[103-105]

AA plays a crucial role in regulating innate immunity and inflammation resolution. When tissues become inflamed or infected, AA metabolites (eicosanoids) amplify those inflammatory signals to recruit leukocytes, cytokines, and immune cells to aid in pathogen resistance and clearance. Following the initial inflammatory signaling, these metabolites then balance those signals by producing resolving metabolites for host protection.[103]

High Levels

Dietary intake of animal meats, fats, and eggs contribute to elevated levels. AA can also be produced from DGLA using the delta-5-desaturase enzyme, therefore high intake of omega-6 fatty acids or DGLA supplementation should be considered as a cause of elevations.

AA is then metabolized to docosatetraenoic acid using the elongase enzyme. Lack of vitamin and mineral cofactors, or a SNP in elongase, may slow the enzyme and contribute to elevations. It should also be noted that omega-3 and omega-6 fatty acids compete for use of the elongase and desaturase enzymes.

Because of its role in the inflammatory cascade and ability to induce oxidative stress, AA is a relevant factor in the pathogenesis of cardiovascular and metabolic diseases such as diabetes mellitus, non-alcoholic fatty liver disease, atherosclerosis, peripheral vascular disease, and hypertension. Neuroinflammation and brain excitotoxicity is also regulated by an AA cascade. Elevations are associated with Alzheimer's disease and mood disorders. There is also a substantial correlation between COX-catalyzed AA peroxidation and cancer development (prostate, colon, and breast).[106-109]

Low Levels

Reduced intake of animal meats and fats, or low dietary intake of omega-6 fatty acids in general, can result in lower levels of AA. Lack of vitamin and mineral cofactors for the desaturase and elongase enzymes upstream in omega-6 metabolism might contribute to lower levels.[37]

Because of important immune and inflammatory signaling which requires AA, and its role in cell membrane phospholipid metabolism, lower levels of AA do have clinical significance. Psychiatric disorders such as schizophrenia, and neurologic disorders like tardive dyskinesia, show depletion of AA in RBC membranes. Improving AA levels decreased symptoms in some patients.[110]

Monitoring levels and ensuring adequate dietary intake of AA is important in pregnant women, infants, children, and the elderly due to its importance for the development and optimization of the nervous system, skeletal muscle, and the immune system.[111]

Docosatetraenoic Acid (Adrenic Acid)

Docosatetraenoic acid (DTA) is a very long chain omega-6 fatty acid with 22 carbons and 4 double bonds (22:4n6). It is synthesized by adding 2 carbons atoms to the backbone of arachidonic acid using the elongase enzyme. It is sometimes referred to by its common name adrenic acid and is one of the most abundant fatty acids in the early human brain and the adrenal gland.[112]

DTA has not been well studied, though it has recently been shown to have important physiologic functions. It is now believed to be a pro-resolving mediator in inflammation by blocking neutrophilic metabolites and dampening the inflammation response. For example, in osteoarthritis DTA enhances phagocytosis by macrophages which clears products of cartilage breakdown in the joint space. Supplementation of DTA is being studied as a promising intervention in osteoarthritis to dampen inflammation and prevent structural damage.[113]

Much like AA (its precursor) DTA/adrenic acid is an important component of infant development. DTA is the third most abundant PUFA in the brain and it is necessary for neural tissue development.[114]

DTA is also prevalent in the vasculature. It is metabolized to biologically active prostaglandins and epoxyeicosatrienoic acids (EETs) which activate smooth muscle channels causing relaxation and vasodilation.[115]

There is some literature to also support DTA/adrenic acid's role in inducing oxidative stress and cell death through modulating superoxide dismutase enzymes.[116]

High Levels

Elevations of DTA/adrenic acid are seen in diets rich in omega-6s and arachidonic acid (animal meat/fats and eggs).

The clinical significance of adrenic acid is still being studied. Its importance in fetal development, osteoarthritis, and vasodilation have been documented, though some of the research is in animal studies. It has also been found to be elevated in patients with nonalcoholic fatty liver (NAFLD) and nonalcoholic steatohepatitis (NASH).[116]

Because its precursor is AA, elevations due to high AA intake have deleterious associations as outlined above in the AA section.

Low Levels

Diets low in omega-6 fatty acids and arachidonic acid would result in lower levels of DTA/adrenic acid. The clinical significance of low levels may be relevant in infant and fetal development as previously described.

Eicosadienoic Acid

Eicosadienoic acid (EDA) is a rare, omega-6 fatty acid with a 20-carbon backbone and two double bonds (20:2n6). It is mainly formed through the downstream metabolism of omega-6s by elongating LA. EDA can be metabolized to form DGLA and AA. Literature is sparse regarding its role in the inflammatory cascade though it is known to modulate the metabolism of other PUFAs and to alter the responsiveness of macrophages to stimulate inflammation.[117]

High levels

Elevations may be seen with high intake of LA and omega-6 fatty acid-rich foods. The clinical significance of elevations is presumed due to its role in the inflammatory cascade, though in and of itself, EDA hasn't yet been studied epidemiologically for disease associations.

Low Levels

Lower levels may be due to decreased dietary intake of omega-6 foods or decreased downstream metabolism of LA and other omega-6s. There is no known clinical significance of decreased levels.

Percentage Omega-6s

When assessing fatty acids in RBCs, Genova measures a weighted percentage of fatty acids taken up into the erythrocyte wall. The total omega-6 percentage is a combined total weight percentage calculated by adding together each of the measured omega-6s. Because some omega-6 fatty acids are less beneficial than others, each fatty acid abnormality should be addressed. However, in general, assessing the total omega-6 percentage as it relates to the omega-3 percentage is helpful. A more balanced ratio may decrease risk of many chronic diseases.[118]

It should be noted that when dealing with percentages, the amount of each fatty acid can influence the others. For example, fish oil supplementation may increase the overall omega-3 percentage, which may ultimately lower the omega-6 percentage.

Omega-9 (n-9) Fatty Acids

Monounsaturated fatty acids (MUFAs) contain one double bond within their carbon backbone structure. The placement of that bond is responsible for its nomenclature. These MUFAs have a double bond at the 9th carbon; therefore, they are omega-9 fatty acids. The double bond plays a role in increasing cell membrane fluidity.

Omega-9 fatty acids are not considered essential since the body can synthesize them, though they have many food sources. Olive oil is the most common source of n-9, and they are also found in various nuts and seeds. The overall health benefits to n-9s have been extensively studied as it relates to lowering inflammation, being cardioprotective, and important for brain health.[119-121]

Oleic Acid

Oleic acid (OA) has an 18-carbon backbone with one double bond at the 9th position (18:1n9). Oleic acid's main dietary source is olive oil, and it is also available as a supplement. OA can also be synthesized in the body by adding a double bond to stearic acid using the enzyme delta-9-desaturase.

Oleic acid is important in cell membrane fluidity and has attracted a lot of positive attention due the amount of olive oil found in the 'Mediterranean diet.' OA's anti-inflammatory and immunomodulatory effects have been extensively studied and found to be beneficial in many conditions such as cancer, neurodegenerative disorders, inflammation, autoimmunity, cardiovascular disease, diabetes, wound healing, and infection. There is also literature to suggest that OA may be a selective biomarker of isolated impaired glucose tolerance regardless of fasting glucose.[122-128]

OA can lower blood lipids- mainly total cholesterol, LDL-cholesterol, and triglycerides. It also dampens the inflammatory response within the vascular endothelium.[129-131]

High Levels

Elevations can be seen in diets high in olive oil, nuts, and seeds – or in patients supplementing with OA. In general, higher levels are beneficial and no adverse clinical associations are seen.

Low Levels

Decreased dietary intake of OA-rich foods, (olive/safflower/sunflower oils) will lower levels. Because stearic acid is its precursor, low levels of stearic acid may result in low OA. Lack of vitamin and mineral cofactors for delta-9-desaturase, or a SNP in the enzyme, may result in lower production of oleic acid.[27,132]

Nervonic Acid

Nervonic acid (NA) is an omega-9 MUFA with a 24-carbon backbone and one double bond (24:1n9). It is a very important fatty acid in the white matter of the brain and is responsible for nerve cell myelin biosynthesis. There are small amounts of NA in cooking fats, vegetable oils and borage oil. It can also be synthesized in the body by elongating oleic acid (which is essentially desaturated stearic acid).

NA is essential for the growth and maintenance of the brain and peripheral nervous tissue enriched with sphingomyelin.[133]

High Levels

Increased dietary intake of cooking fats, vegetable oil, or borage oil can elevate NA levels.

NA is elevated in clinical conditions marked by impaired white matter or altered desaturation enzyme activity such as major depressive disorder and Alzheimer's disease.[133,134]

Due to its effects on overall lipid metabolism, higher levels of NA are also associated with improved metabolic parameters including blood glucose, insulin, and glucose tolerance. In animal studies, it has been shown that NA might play a role in treating obesity and obesity-related complications.[135-137]

Low Levels

Decreased intake of dietary sources and low levels of oleic acid may result in low NA. Lack of vitamin and mineral cofactors, or a SNP in the delta-9-desaturase enzyme, may also contribute to lower levels.

Clinically, low levels of NA results in a decreased ability to maintain or develop myelin in the brain. In fact, low levels may predict psychosis in schizophrenia. Supplementation with omega-3 fatty acids may offset risks conferred by low levels of NA in certain conditions.[133,138]

Multiple sclerosis (MS) is thought to be an autoimmune reaction to myelin. A defect in the biosynthesis of NA causing lower NA levels may lead to breakdown of myelin which triggers the onset of the autoimmune response. Use of NA as nutritional support in MS is being studied.[139]

Low levels of NA have been found to be an independent predictor of mortality in cardiovascular disease and chronic kidney disease.[119,140]

Percentage Omega-9s

When assessing fatty acids in RBCs, Genova measures a weighted percentage of fatty acids taken up into the erythrocyte wall. The total omega-9 percentage is a combined total weight percentage calculated by adding up each of the measured omega-9s. In general, because the omega-9 fatty acids are beneficial, higher levels are preferred; though identifying root cause of elevations or deficiencies is important.

It should be noted that when dealing with percentages, the amount of each fatty acid can influence the others. For example, fish oil supplementation may increase the overall omega-3 percentage. By default, this may then lower the omega-6 percentage.

Saturated Fatty Acids

Saturated fatty acids (SFAs) are so named because they contain no double bonds among the carbon backbone skeleton; it is saturated with hydrogen atoms. This configuration contributes to a lack of cell membrane fluidity and difficulty for the body to convert them directly as energy. SFAs are not essential nutrients and are obtained mainly through dietary intake of animal fats and processed foods. Some SFAs can be synthesized in the body from carbohydrates via de novo lipogenesis. Attempts to lower SFA levels by removing dietary sources should also include a strategy of limiting carbohydrates.[141]

Compared to unsaturated fatty acids, SFAs have a higher heat index and better oxidative quality, making them ideal as a cooking oil at high temperatures.[142]

SFAs alter overall lipid metabolism and elevate cholesterol levels. They are also involved in the inflammatory cascade which is implicated in dietary SFA disease risk. However, certain SFAs may play an important role in hormone production, gene transcription, cellular membrane structure, and protein signaling.[143-145]

Palmitic Acid

Palmitic acid (PA) is a 16-carbon saturated fatty acid (16:0) and the most common fatty acid in the human body. It can be obtained via diet or synthesized from carbohydrates, other fatty acids, and amino acids. As the name suggests, it is a major component of palm oil, but can also be found in meat, dairy, cocoa butter, coconut oil, and olive oil. Palm oil and palmitic acid are also found in many products ranging from skincare products, margarine, cereals, and baked goods.[146]

Dietary intake of PA is counterbalanced by de novo lipogenesis depending on the physiologic needs of a specific tissue, or nutritional factors. Regardless of PA intake, the body makes it as needed. Excess PA is converted to palmitoleic acid via delta-9-desaturase or elongation to stearic acid. Homeostasis of PA levels is tightly controlled.

PA can be oxidized for energy production. It is also used structurally in cell membranes and cell adhesion molecules, as well as being a component of lung surfactant.[146]

High Levels

Elevations in PA are seen in high dietary intake of saturated fats, proteins, and carbohydrates. Excessive intake of carbohydrates and a sedentary lifestyle can disrupt the PA homeostatic balance resulting in dyslipidemia, hyperglycemia, fat accumulation, lipotoxicity, altered immune responses, and stimulation of the inflammatory cascade. The disruption of this balance (implicated in atherosclerosis, neurodegenerative diseases, and cancer), is related to an uncontrolled endogenous biosynthesis regardless of dietary intake. Excess PA induces apoptosis through mitochondrial dysfunction and endoplasmic reticulum stress.[146-149] Higher levels are correlated with the incidence of type 2 diabetes, cardiovascular disease, and cancer risk.[147]

Low Levels

Decreased intake of saturated fat and PA may contribute to lower levels, however there is tight regulation of PA levels and the body will use carbohydrates, other fatty acids, and amino acids to maintain those levels. The liver plays a strong role in regulating the body concentration of PA using desaturase and elongase enzymes. Lack of nutrient cofactors, or SNPs in these enzymes, may interrupt this balance.[146]

Stearic Acid

Stearic acid (SA) is a saturated fatty acid with an 18-carbon backbone (18:0). Although it is mainly abundant in animal fat, cocoa butter and shea butter are also very high in SA. It is also commonly used in detergents, soaps, cosmetics, shampoos, and shaving cream.[150] Additionally, it can be synthesized in the body from palmitic acid.

SA is not a strong substrate to make triglycerides compared to other saturated fatty acids and it generates a lower lipemic response.[151] As compared to other saturated fats, SA doesn't raise plasma LDL cholesterol. This may be due to absorption of SA and the amount of energy metabolized from it. In fact, SA may have some beneficial effects in regulating mitochondrial morphology and function, though these mechanisms are still being studied.[152]

The American Heart Association recognizes that a diet low in trans fats from industrial food sources and low in saturated fatty acids is optimal for cardiovascular health. SA is being studied as a solid-fat alternative to trans fatty acids in baking goods and shortenings since it is trans-free, oxidatively stable, and doesn't raise LDL cholesterol. (Unsaturated fats are not suitable for solid fat applications but are suitable for liquid fat applications, like frying.) However, the safety of using SA substitutes in industrial products is still being studied and debated.[153-155]

High Levels

SA levels may be high with dietary intake, or through absorption of commercial products containing it. Because it can also be synthesized from palmitic acid, a diet generally rich in saturated fats can contribute to higher levels.

Saturated fatty acid levels are associated with many cardiometabolic conditions. SA alone seems not to exert the same detrimental effects as is seen with other saturated fatty acids, though these are often contained in foods alongside each other. SA itself can elevate serum lipoprotein(a), though to a lesser extent than trans fats.[155]

Low Levels

Decreased intake of foods containing saturated fatty acids, or SA specifically, can contribute to low levels. Because it can also be made from palmitic acid, lack of vitamin or mineral cofactors for the elongase enzyme, or an enzymatic SNP, might also be implicated in lower levels. Clinically, lower levels have not been extensively studied in relation to disease.

Arachidic, Behenic, Tricosanoic, and Lignoceric Acids

Very-Long-Chain Saturated Fatty Acids (VLSFAs)

Very long saturated fatty acids (VLSFAs) are defined as having 20 carbons or more with no double bonds. (It should be noted that the amount of carbons needed to define VLSFAs varies in literature as 20-22 or more).[156-159]

These saturated fatty acids can be obtained in the diet, synthesized from precursor fatty acids (such as stearic acid and palmitic acid), or created de novo in mitochondrial microsomes.[160]

Very long saturated fatty acids exhibit distinct beneficial functions compared to other saturated fatty acids. For example, they can influence liver homeostasis, retinal function, skin barrier, and may have anti-inflammatory effects. As important constituents of sphingolipids such as ceramides and sphingomyelins, levels can also be influenced by genetic factors related to sphingolipid synthesis.[158,161,162]

VLSFAs are too long to be metabolized in the mitochondria and require peroxisomes for metabolism. Certain peroxisomal disorders (adrenoleukodystrophy, Zellweger syndrome) can be associated with high VLSFA levels.[163]

- **Arachidic acid** is very long, 20-carbon backbone saturated fatty acid (20:0). It is found in various nuts, soybeans, peanut oil, corn oil, and cocoa butter. In addition to dietary sources, it can be synthesized by the hydrogenation of the omega-6 fatty acid arachidonic acid or the elongation of stearic acid.[158,164,165]
- **Behenic acid** is a VLSFA which contains 22 carbons (22:0). Its name is derived from Ben oil (behen oil) from the Moringa oleifera tree. Commercially, products containing Moringa oil have high amounts of behenic acid in them such as hair conditioners, topical moisturizers, and other cosmetic oils. It can also be obtained through the diet in canola (rapeseed) oil and peanut oil. Using the elongase enzyme, it can be synthesized from arachidic acid.
- **Tricosanoic acid** is an 23-carbon, odd-chain saturated fat (23:0) synthesized initially from propionic acid and can be derived in the diet from sesame, sunflower, and hempseed oils.[166]
- **Lignoceric acid** has 24 carbons and no double bonds (24:0). It can be formed from behenic acid using the elongase enzyme. It is found in peanuts, nut and seed oils. It can also be found in wood tar. Lignoceric acid is one of many fatty acids which compose brain tissue and myelin.

High Levels

Intake of foods containing these VLSFAs or use of products containing them can contribute to higher levels. Increased intake of precursor fatty acids, or SNPs in the elongase enzyme, may alter levels.

Additionally, as an odd-chain fatty acid, tricosanoic acid elevations can be seen with functional deficiency of vitamin B_{12} since it is required for the conversion of propionate for oxidation. Tricosanoic acid can be high in microbiome dysbiosis with increased production of the short chain fatty acid propionate (its precursor).
The health implications of elevated VLSFAs levels are evolving, though they are generally found to be beneficial in health and aging. Several meta-analyses suggest a beneficial association of very long chained saturated fatty acids with cardiovascular health outcomes as well as lower risks of type 2 diabetes, atrial fibrillation, heart failure, and coronary disease. These VLSFAs may also be important in neural development and cognition. The mechanisms of these very long chained saturated fatty acids are not fully known. Because VLSFAs are components of ceremides involved in apoptosis, there is strong evidence that VLSFAs are protective against apoptosis and cell death.[156,161,167-172]

Low Levels

Decreased dietary intake of these saturated fatty acids, or avoidance of products containing them, can result in low levels. Some VLSFAs can be synthesized from other fatty acids. Therefore, decreased levels of precursors, lack of vitamin and mineral cofactors, or SNPs in the elongase enzyme may also contribute to low levels.

Specific deficiency in VLSFAs is not well studied. Though, due to their importance in brain development and their associations with improved health outcomes, as outlined above, research is evolving.

Tricosanoic, Pentadecanoic, and Margaric Acids

Odd-Chain Saturated Fatty Acids (OCS-FAs)

Most research in fatty acid metabolism has focused on even-chain fatty acids since they represent >99% of total human lipid concentration. For years, it had been concluded that odd chain saturated fatty acids (OCS-FAs) were of little significance and used only as internal standards in laboratory methodology. However, there is now a realization that they are, in fact, relevant and important physiologically.[173]

OCS-FAs mainly originate from dairy fat since microbiome fermentation in ruminant animals is a primary source of production. The human body can also synthesize them by elongating propionic acid, a short chain fatty acid formed in the microbiome. New research is showing they may also be formed by shortening VLCFAs by removing carbon molecules using α-oxidation. Metabolism of OCS-FAs is a bit different than even-numbered chained fatty acids. Both odd and even chain fatty acids undergo oxidation, though OCS-FAs produce a molecule of propionyl-CoA and a molecule of acetyl-CoA instead of two acetyl-CoAs. Propionyl-CoA requires a vitamin B_{12}-dependent enzyme to be converted into succinyl-CoA and used in the citric acid cycle. It should be noted that the microbiome is not the only source for the OCS-FA precursor propionate. Endogenous propionate can be produced by the degradation of some amino acids, which can then lead to OCS-FA production. [173-175]

Several epidemiologic studies show a positive association between OCS-FA and reduced risk for inflammation, cardiometabolic disease, multiple sclerosis, and nonalcoholic steatohepatitis. They are also being studied as adjuvant therapies in cancer due to their cell signaling properties which induce targeted apoptosis. Additionally, it has been found that OCS-FAs increase membrane fluidity more than PUFAs, and they are being studied as a form of treatment for Alzheimer's disease.[173,176-178]

- **Pentadecanoic acid** is a 15-carbon saturated fatty acid (15:0). Its major dietary source is the butterfat in cow's milk. It can also be synthesized from propionate via the mechanisms outlined above.
- **Margaric acid** is also known as heptadecanoic acid. It is a 17-carbon saturated fatty acid (17:0). Food sources mainly include milk and dairy products, though it can be endogenously made as noted.
- **Tricosanoic acid** is a saturated fatty acid which contains 23 carbons (23:0). It can be found in milk and dairy products, as well as some wild mushroom species. It can also be endogenously made.

High Levels

High dietary intake of dairy products can increase levels. Because propionate is a precursor for OCS-FAs, high fiber intake can induce the microbiome to produce propionate to be converted to propionyl-CoA. Because propionyl-CoA competes with acetyl-CoA, fiber intake can increase OCS-FAs levels at the expense of other saturated fatty acids. Some studies suggest that OCS-FA levels may act as a biomarker for dietary fiber intake.[179]

Due to the broad health benefits of OCS-FAs, questions are being raised as to whether they should be considered essential nutrients.[178]

Low Levels

Decreased dietary intake of dairy products and fiber may contribute to low levels. As noted above, literature is evolving as to their health benefits, and lower levels have been associated with risk for cardiometabolic diseases, inflammation, Alzheimer's disease, multiple sclerosis, and nonalcoholic steatohepatitis.

Percentage Saturated Fats

When assessing fatty acids in RBCs, Genova measures a weighted percentage of fatty acids taken up into the erythrocyte wall. The total saturated fatty acid percentage is a combined total weight percentage calculated by adding up each of the measured saturated fatty acids. It should be noted that when dealing with percentages, the amount of each fatty acid can influence the others. For example, fish oil supplementation may increase the overall omega-3 percentage, which then lowers the omega-6 percentage. Because some saturated fatty acids are beneficial, it is important to look at the levels of those specifically as well.

Omega-7 (n-7) Monounsaturated Fatty Acids

Monounsaturated fatty acids (MUFAs) have just one double bond throughout their carbon chain. The position of the double bond within that carbon chain distinguishes it from others and is responsible for the naming convention. If the double bond is at the seventh carbon, it is known as an omega-7 monounsaturated fatty acid.

Clinically, some literature suggests that a high-MUFA diet may be preferable to low-fat diets as it relates to cardiovascular disease. MUFA diets do not appear to increase triacylglycerol concentrations nor do they lower HDL levels. MUFAs have also been shown to decrease the oxidative susceptibility of LDL cholesterol. A high-MUFA diet can decrease platelet aggregation, increase fibrinolysis, and increase bleeding time which may protect against thrombogenesis.[180]

Palmitoleic Acid

Palmitoleic acid (POA) is a monounsaturated omega-7 fatty acid (16:1n7). The main dietary sources of palmitoleic acid include dairy products, avocado oils, oily fish, and macadamia nuts. Macadamia nuts contain the cis- isomer of POA, while dairy products mainly contain the trans- isomer. Like many fatty acids, POA can also be endogenously made from the breakdown of triglycerides, the desaturation of palmitic acid, or de novo synthesis from carbohydrates.[181]

POA is an important signaling lipokine, produced mainly by white adipose tissue, that regulates important metabolic processes such as skeletal muscle glucose disposal, insulin sensitivity, and hepatic lipid deposition. It is also a modulator of adipocyte lipolysis, however, studies are mixed as to POA's specific role in obesity.[181,182]

Epidemiologic studies show that circulating POA levels are involved in cholesterol metabolism and hemostasis, though the results are mixed as to their specific cardiovascular outcomes.[181]

High Levels

Elevations of POA can be seen with dietary intake of dairy products or macadamia nuts. Surplus dietary carbohydrates and high intake of its precursor palmitic acid might also result in higher POA.

As noted above, POA has many beneficial physiologic effects. In epidemiologic studies, higher intake of the trans-isomer of POA from dairy has been associated with lower levels of inflammation, improved insulin sensitivity, and decreased risk of diabetes. Alternatively, high POA levels have also been associated with various forms of cancers. There is a theory that endogenous production of palmitoleic acid may be an underlying cause of cell proliferation and survival in cancer progression. However, this needs more investigation.[183]

Low Levels

Decreased intake of POA-containing foods, or palmitic acid can lower POA levels. Also, since POA can be made in the desaturation of palmitic acid, lack of vitamin and mineral cofactors, or a SNP in that enzyme, may contribute to lower levels.

Clinical associations of low POA levels are mixed as previously discussed.

Vaccenic Acid

Vaccenic acid (VA) is a monounsaturated omega-7 fatty acid (18:1n7). VA is a naturally occurring trans-fat unlike those produced industrially. The trans-configuration occurs around carbon 11, therefore VA is sometimes denoted as trans11-18:1n7. Ruminant animals produce vaccenic acid in a fermentation process in their microbiome. The dairy products (cheese, milk, butter) or meat obtained from these animals contain VA. There is also a cis-configuration of vaccenic acid created by de novo lipogenesis. VA can then be converted to an isomer of conjugated linoleic acid (CLA) using a desaturase enzyme. CLA has been associated with anti-inflammatory activity and affects lipid metabolism.[184]

VA, as a cis-isomer, has demonstrated associations with lower insulin resistance and decreased risk of diabetes. The trans-isomer has also been shown to be beneficial as it relates to insulin secretion and resistance. Both isomers have been studied in vitro (and in animal studies) and may suppress adhesion molecules in the vascular endothelium. Therefore, VA isomers are being studied as possible prophylaxis in patients with risk of atherosclerosis. However, human intervention studies are limited.[24,185-188]

High Levels

Elevations in VA are seen in high dietary intake of meat and dairy products from ruminant animals. Overall, VA may not adversely affect health as compared to industrial trans fats, though studies are ongoing.

Low Levels

Decreased dietary intake of dairy and meat from ruminant animals may result in lower levels of VA. Because it can also be endogenously produced and further metabolized into CLA, lack of precursor fatty acids, or rapid metabolism by desaturation, may result in lower levels.

Trans fatty acids (TFAs) is a general term for unsaturated fatty acids with at least one double bond in the trans configuration. Dietary TFAs are primarily obtained in the diet from partially hydrogenated vegetable oils. Hydrogenation of oils has been used in the food industry to prolong shelf life of certain foods as well as to create semi-solid fats more suitable for cooking. In addition to artificial trans fats, vaccenic acid is a trans fatty acid naturally obtained from ruminant animal products. There is evidence that this difference in food sources of trans fatty acids contribute to differing biological effects with different clinical consequences.[189]

Industrial trans fats have been extensively studied and shown to have significant adverse effects on the cardiovascular system. TFAs also contribute to obesity, cancer, inflammation, and endoplasmic reticulum stress.[189-192]

Elaidic Acid

Elaidic acid (EA) is an 18-carbon chained fatty acid with one double bond in the trans formation at the 9th carbon (18:1n9t). It is the trans isomer of oleic acid. EA is the principal and most abundant trans fatty acid in the Western diet. It is found in partially hydrogenated vegetable oil and margarine. There are trace amounts of EA in the meat and dairy products from ruminant animals.

EA has been shown to induce oxidative stress and alter mitochondrial signaling. It is quickly incorporated into triglycerides and cholesterol esters. Once incorporated into plasma membranes, it activates nuclear factor-kB to induce adhesion molecules and become proinflammatory leading to endothelial dysfunction.[193,194]

Intake of trans fats, specifically EA, has been implicated in cancer, cardiovascular disease, insulin resistance, neurotoxicity, obesity and many inflammatory conditions.[193,195-198]

High Levels

Dietary intake of industrial hydrogenated oils and margarine, fried foods, baked goods, donuts, crackers, etc., can elevate levels. Due to the many deleterious health effects of EA as noted above, the recommendation is to limit intake of EA and trans fat.

Low Levels

Low intake of processed foods and hydrogenated oils lead to lower levels of EA. Given the health implications, low levels are preferred.

Delta-6-Desaturase Activity

Dihomo-γ-linolenic acid (DGLA) is an important anti-inflammatory n-6 fatty acid. Because it needs to be synthesized from precursor fatty acids, conversion steps in fatty acid metabolism must be optimal. Two enzymatic steps are required to synthesize DGLA from the essential LA – namely the use of the enzymes delta-6-desaturase and elongase. Although there are several vitamin and mineral cofactors required for each enzyme, the inability to convert LA to DGLA has been proposed as a functional biomarker of zinc status. Zinc not only directly affects desaturase activity, but can influence fatty acid absorption, oxidation, and incorporation into RBCs.[199,200]

The inability to convert precursor fatty acids to DGLA is associated with various pathologic and physiologic conditions such as aging, diabetes, alcoholism, atopic dermatitis, rheumatoid arthritis, cancer, and cardiovascular disease.[94,95]

High levels (Impaired activity)

Elevations may indicate impaired delta-6-desaturase activity. Literature points to zinc insufficiency as an important cause. Other considerations include lack of other vitamin and mineral cofactors, or SNPs in the delta-6-desaturase and elongase enzymes. Many clinicians supplement with evening primrose, borage, and black currant to bypass the delta-6-desaturase enzyme, though cofactors for elongase should be optimized as well. Additionally, keep in mind that there is competition between the omega-3 and omega-6 fatty acids for these enzymes. Therefore, supplementation with fish oils/omega-3 fatty acids can compete with omega-6 fatty acid metabolism.

Low Levels (Upregulated activity)

Anything that may increase DGLA might result in a lower LA:DGLA ratio. Patients who supplement with evening primrose, borage, and black currant may have elevated DGLA which may lower the ratio. Assessing the levels of linoleic acid is also warranted. If LA levels are normal, a higher DGLA may not be of concern. If LA is low, ensure essential fatty acid adequacy. A SNP in either the desaturase or elongase enzymes could alter the enzymatic conversions as well.

Fatty acid research is rapidly evolving due to their association with health and disease. However, conventional laboratories and published researchers use differing matrices to measure them, and differing reference ranges. To mitigate this, many use relative ratios to gain a better understanding of disease correlation. Because cardiovascular disease and fatty acid imbalances have been widely studied, several fatty acid ratios have been established as a way to assess risk.[201-204]

Omega-6s/Omega-3s Ratio

There has been a significant change in the balance of n-6s to n-3s with the evolution of the Western diet. Close to a 1:1 balance existed throughout history. However, rapid dietary changes and food industry advances have altered this to now be vastly in favor of n-6s by upwards of 20:1. This change correlates with many chronic diseases such as cardiovascular disease, cancer, metabolic syndrome, obesity, mood disorders, autoimmunity, and neurogenerative disease.[68,202,203,205,206]

Dietary interventions which favor omega-3, in lieu of omega-6s, is recommended with elevations in this ratio to achieve a closer balance between the two.

Arachidonic acid/Eicosapentaenoic acid (AA/EPA) Ratio

EPA (n3) and AA (n6) both compete for use of the delta-5-desaturase enzyme to be synthesized. Increased dietary intake of animal fats alters fatty acid metabolism in favor of inflammation. There are many chronic diseases associated with elevations of this ratio including cardiovascular disease, mood disorders, and cancer.[207-212]

Increasing dietary intake of fish oils, or omega-3 fatty acid containing foods such as flax, chia, oily fish, or walnuts, can shift delta-5-desaturase activity toward the metabolism of the more beneficial n-3 metabolites. Decreasing intake of animal fats is also recommended.

Omega-3 Index

The omega-3 index is defined as the RBC percentage sum of EPA+DHA, both of which are important anti-inflammatory omega-3 fatty acids. This index was first proposed in 2004 as a cardiovascular risk factor by Dr. Willian S. Harris and Dr. Clemons von Schacky as a way of assessing risk for coronary artery disease and related death. Since then, it has been repeatedly verified as an important cardiovascular biomarker, and studied in other diseases including obesity, mood disorder, and insulin resistance.[213-219]

A reasonable target for the omega-3 index is >8% to decrease disease risk. Drs. Harris and von Schacky stratified risk zones as high risk (<4%), intermediate risk (4-8%), and low risk (>8%). These percentages have been continually verified in outcome studies and risk assessment.[213,220,221]

Dietary intervention to increase the omega-3 index should include oily fish, flax, walnut, and chia. Fish oil supplementation can also be considered.

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TOXIC AND NUTRIENT ELEMENTS

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Aluminum (Al)	Found in virtually all food and food additives, water, air, and soil. Also found in antacids, antiperspirants, cosmetics, astringents, cans, pots, pans, siding, roofing, and foil.[1,2]	Calcium deficiency, citric acid, and low gut pH causes increased Al absorption.[1,3,4] Low iron intake increases Al absorption (rat study).[5] Selenium may be protective against Al.[4] Al reduces phosphorus and fluoride absorption. Al disrupts lipid membrane fluidity, altering Fe, magnesium, and calcium homeostasis, causing oxidative stress.[6]	Accumulates in bone, liver, kidney, and spleen.[5] Causes mitochondrial dysfunction due to Krebs cycle enzyme activity disturbance and electron transport chain alterations.[6] Alters the enzymes in the glutamate system, which may be one of the causes of aluminum-induced neurotoxicity.[7] Parathyroid hormone levels and osteoclast activity are disrupted by Al.[8] Al can also slow calcification of new bone and deposits in the bone matrix in place of calcium.[5] Disrupts normal iron homeostasis and iron-dependent cellular metabolism.[5]	Anemia, CNS functional, sensory and cognitive alterations, and bone abnormalities like osteodystrophy.[2,5]
Antimony (Sb)	Found naturally in the environment, air, soil, water. Found in lead storage batteries, solder, sheet and pipe metal, pewter, bearings and castings, paints, ceramics, fireworks, plastic enamels, metal and glass. Sometimes used medically to treat parasites.[9]	Unknown	Highest accumulation in the lungs, GI tract, RBC's, liver, kidney, bone, spleen, and thyroid. It is excreted in urine and feces, and partially in bile after conjugation with glutathione. Trivalent antimony is predominantly excreted in feces while pentavalent antimony in urine.[10,11] Binds to sulfhydryl groups with subsequent inhibition of enzymes involved in cellular respiration and carbohydrate/protein metabolism.[12]	Lung and skin irritation, cardiac and EKG alterations, GI symptoms such as nausea, vomiting, ulceration. In animal studies, antimony can decrease serum glucose levels.[9,11]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Arsenic (As)	Found in water, air, soil, cigarettes, and cosmetics. Food grown in contaminated water sources, such as rice and vegetables, or fish, are a common source.[13] Major sources of occupational exposure is the manufacture of pesticides, herbicides, and agricultural products.[14] 90% of all arsenic produced is used as a preservative for wood to prevent rotting and decay. Copper chromated arsenate (CCA), also known as pressure-treated wood, was phased out for residential use in 2003, but wood treated prior could still be in existing structures. CCA-treated wood is still used in industrial applications. Organic arsenic found in seafood is relatively nontoxic, while the inorganic forms are toxic.[15]	Folate, SAM, vitamin B12, and choline are needed to optimize methylation. Arsenic is metabolized by a series of methylation reactions to dimethylarsenic and methylarsonic acids. There are some highly reactive intermediates in its trivalent form which are toxic.[16] Magnesium may have protective effects against As toxicity, and Zinc may increase As excretion.[4] Vitamin C, α-tocopherol, flavonoids, polyphenols, and selenium have been shown to decrease arsenic-induced toxicity.[16]	The absorption rate of arsenic in the GI tract is 90%. (Though arsenic compounds of low solubility are not absorbed as efficiently.) As binds to RBC's and deposits in the liver, kidneys, muscle, bone, hair, skin, and nails. It is excreted mainly through urine, with 50-80% excreted within three days.[14,17] Most of the arsenic in blood is rapidly cleared within hours. It is not possible to distinguish organic from inorganic arsenic in urine or blood.[18] Impairs cellular respiration by inhibiting mitochondrial enzymes and the uncoupling of oxidative phosphorylation. Reacts with protein sulfhydryl groups, producing inhibition in the oxidation of pyruvate and beta-oxidation of fatty acids. Inhibits DNA repair and induces chromosomal aberrations.[19]	Increased risk of cancer (skin, lung, bladder, liver, prostate). Associated with neurobehavioral changes, memory, intellectual function abnormalities, diabetes, cardiovascular disease, reproductive effects, skin hyperpigmentation, peripheral neuropathy, respiratory irritation, nausea, and hematologic effects.[14,17]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Barium (Ba)	Radiologic testing contrast, paint, bricks, ceramics, glass and rubber. Air, water, and food. Fish and aquatic organisms can accumulate barium.[20]	Barium toxicity can induce severe hypokalemia.[21,22]	Barium is excreted mainly in feces and urine within 1-2 weeks. Can deposit in bones and teeth.[20] Barium is a competitive potassium channel antagonist blocking the passive efflux of intracellular potassium. Barium toxicity can cause hypokalemia.[23]	Barium compounds that do not dissolve well in water, such as barium sulfate, are generally not harmful. Water soluble forms can cause cardiac dysrhythmias, GI disturbances, muscular weakness, vomiting. Electrolyte abnormalities can induce cardiac dysrhythmia, muscle cramping/paralysis, and vomiting. Nephropathy is also possible based on direct renal toxicity.[20]
Bismuth (Bi)	Used in alloys, electronics, batteries, crystal ware, cosmetics, flame retardants, and in antimicrobial therapy (H. pylori), antiseptic dressings, paraffin paste.[24,25] Bismuth medical therapies exhibit high therapeutic effects and little side effects, though over-dosage can cause toxicity.[26]	Unknown	Very limited absorption in the GI tract. When absorbed, it binds mainly to transferrin and lactoferrin, interacts with enzymes due to a high affinity to cysteine residues, blocking the active site.[26] Can accumulate in the kidney, lung, spleen, liver, brain, and muscles, while being eliminated in urine and feces via bile and intestinal secretions.[27]	Nephropathy, GI complaints, encephalopathy, difficulty walking/standing, memory deterioration, behavioral change, insomnia, muscle cramping.[26,28] Decreased appetite, weakness, gingivitis, dermatitis, and diarrhea have also been seen clinically with chronic bismuth toxicity.[14]
Cadmium (Cd)	Found in food such as shellfish, leafy vegetables, rice, cereals, cocoa butter, dried seaweed, and legumes.[29] Also present in nickel cadmium batteries, cigarette smoke (including second-hand smoke), insecticides,	Iron deficiency is associated with higher cadmium burden and absorption of cadmium may increase during very early stages of iron deficiency.[29] Zinc deficiency is associated with an increase in Cd, as a result of the antagonistic relationship between the elements.[30]	Cd accumulates in the liver and kidneys and has a long half-life (17-30 years). The renal and skeletal systems are the main targets of Cd toxicity.[5] Urinary cadmium reflects integrated exposure over time and body burden. Urinary levels do not rise significantly after acute exposure. Elevated blood cadmium levels confirm recent acute exposure.[31-33]	Renal tubular toxicity, decreased bone density with increased bone turnover and fractures.[29] Chronic inhalation exposure is associated with emphysema. Acute oral ingestion leads to abdominal pain, nausea, vomiting, muscle cramps, and GI tract erosions.[19]

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
	fertilizer, motor oil, emissions and exhaust.[19] Drinking water, air, and occupational exposures are also seen.	Dietary cadmium inhibits GI absorption of calcium and interferes with calcium and vitamin D metabolism. Low dietary calcium stimulates synthesis of calcium-binding protein which enhances Cd absorption.[5]		
Cesium (Cs)	Naturally occurring Cs can be found in a stable form (measured on Genova's tests). Radioactive Cs is produced by the fission of uranium in fuel elements, usually near nuclear power plants. These are unstable but eventually become stable through radioactive decay. Some Cs can be found in air, water, and soil (and thereby food) based on location near nuclear plants.[34]	Higher levels of vitamin D ($25(OH)D_3$) have been linked to enhanced absorption of radioactive isotopes like cesium.[4] Cs and potassium compete for uptake and cell membrane potential.	Cs acts like potassium, entering cells and altering electrical charges. It has a higher distribution in the kidneys, skeletal muscle, liver, and RBC's. In RBC's it decreases their ability to release oxygen in tissues. Additionally, the acute effects of Cs on cardiac tissues consists of membrane potential changes due the interaction within the electrical current and channel pore, causing dysrhythmia.[35] It is usually excreted by the kidney, but also in feces.	Stable Cs may not likely cause significant health defects. However, radioactive Cs could cause nausea, vomiting, diarrhea, or acute radiation syndrome, though most exposures are not large enough to cause these effects unless it's a significant industrial or occupational event.[34]
Gallium (Ga)	Used in integrated circuits, LED's, solar cells, laser diodes. It is also used in medicine, where the radioisotopes are used as imaging agents, and stable compounds are used in chemotherapy. Ga can be a antimicrobial agent, and used to treat life-threatening, malignancy-related hypercalcemia.[36] Can be found in ground water near mining, manufacturing and coal combustion plants. Most commonly seen in occupational exposures, while there is less data on consumer electronic exposures.[37]	Ga competes with iron for transferrin binding and inhibits receptor-mediated iron uptake by cells, rendering cells iron-deficient. Iron replacement has been shown to restore hemoglobin production in Ga exposed cells.[38] It was also found to interact with bone metabolism and to lower calcium levels in the blood.[36]	Ga binds to transferrin and interferes with protein synthesis and the heme pathway. It's use in medicine shows that it tends to localize to tumors and cause cell death via interference with iron metabolism.[37] Ga is excreted in the urine, and in rats, renal toxicity was noted with the formation of precipitates of gallium complexed with calcium and phosphate.[2]	In animal studies, toxicity is associated with pulmonary conditions, immunosuppressive effects, and renal toxicity. Direct exposure to Ga has been shown to cause skin rashes, and neurological pain and weakness.[37] Anemia is possible due to its interference with heme pathways.[38]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Gadolinium (Gd)	Used as a nuclear MRI contrast agent (usually in its chelated form). Also used in magnets, compact discs, superconductors, magnets, and fluorescent materials. Can also be found in ground and drinking water.	Gd ions in chelates can be exchanged with cations like zinc, copper, calcium, or iron. Zinc is a major contributor, therefore adequate zinc levels improve Gd excretion.[39,40]	Gd can accumulate in tissue, bone, and brain. Usually removed via kidney.[39] Chelated Gd can dissociate under certain metabolic conditions and inhibit intracellular calcium signaling and disrupt the action of thyroid hormone.[41] Gd targets iron recycling macrophages, induces cellular iron import/export, and labile iron release, which participates in systemic fibrosis.[42]	In spite of past research, recent studies reveal brain deposition of Gd post MRI can occur, but information on adverse health effects in humans is lacking.[43-45] In chronic kidney disease however, there is risk of nephrogenic systemic fibrosis (cutaneous and visceral fibrosis with renal failure).[46]
Lead (Pb)	Found naturally in soil. More often found in fossil fuels, gasoline/exhaust, manufacturing, lead-acid batteries, ammunitions, metal solder and pipes, X-ray shields, paint, glass, pigments, and sheet lead.[19]	Iron and lead share a common transporter; therefore, iron deficiency increases lead absorption.[29] There is some evidence that higher amounts of dietary calcium are associated with lower blood lead levels.[29] Calcium and phosphorus supplementation decreases lead absorption and retention.[4] Selenium has been useful as an adjunct in chelation in lead intoxication.[4] Zinc deficiency enhances lead absorption and lead increases zinc excretion.[5] Zinc supplementation decreases tissue lead accumulation.[4] Vitamin D increases lead absorption.[4]	Mainly taken into the kidney, liver, and other soft tissues such as the heart and brain. However, lead in the skeleton is the major body fraction. The nervous system is a vulnerable target of lead toxicity.[19] Binds to sulfhydryl groups and amide groups of enzymes, diminishing their activity. This enzymatic inhibition can be seen in heme synthesis, neurotransmitter metabolism, and other sodium-dependent processes.[47] Produces reactive oxygen species, and competes with metallic cations for binding sites, altering the transport of cations such as calcium and interferes with calcium-dependent processes.[19] Lead replaces zinc on heme enzymes, and inhibits the enzyme needed to incorporate iron into the hemoglobin molecule by replacing iron. Copper and iron supplementation have been used to counter these heme synthesis effects.[5] Whole blood lead levels estimate recent exposure to lead, but it is also in equilibrium with bone lead stores.[48]	Headache, poor attention span, irritability, memory loss, and weakness are early CNS symptoms of exposure.[19] Reproductive effects, GI diseases, anemia, kidney damage, and adverse effects on vitamin D metabolism are also seen.[19,47] The CDC provides recommendations for follow-up and case management of children based on confirmed whole blood lead levels beginning at levels of 5 mcg/dL.[49] There are guidelines with specific cut-points for adults at risk for occupational lead exposure and for lead-exposed adults in general.[48,50] Urine lead is less validated than blood lead levels as a biomarker of external exposure or predictor of health effects.[48]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Mercury (Hg)	Hg has three forms: Elemental (metallic)- older glass thermometers, fluorescent light bulbs, dental amalgams, folk remedies, combustion, electrical industry (switches, batteries, thermostats), solvents, wood processing Organic (methyl mercury)- seafood, thimerosal (preservative), fungicides Inorganic- skin lightening compounds, industrial exposure, folk medicine, lamps, photography, disinfectants[19,51]	Calcium, magnesium, and selenium, iron, and copper protect against acute toxicity of mercury.[4,5]	A major proportion of absorbed mercury accumulates in the kidneys, neurological tissues, and liver. Molecular mechanisms of toxicity involve oxidative stress. Once in a cell, Hg depletes intracellular antioxidants and therefore causes mitochondrial dysfunction.[19] Mercury releases intracellular calcium, disrupting neuronal transport, alters cell membrane integrity, interrupts microtubule formation, disrupts or inhibits enzymes, inhibits protein and DNA synthesis and impairs immune function.[52] Methyl mercury (organic) appears to be absorbed almost completely in the GI tract, and in this form is higher in the brain compared to other forms.[53] It is excreted fecally and can be measured in blood; urine is not a reliable indicator of organic mercury.[52] Inorganic mercury accumulates in the kidneys. It is excreted in the urine and both inorganic and elemental Hg can be measured in urine and blood.[51,52] Blood and urine mercury levels correlate fairly well with each other, but not with total body burden; blood and urine mercury reflects recent exposure.[54]	GI symptoms, neurotoxicity (headaches, tremor, decreased mental concentration), and nephrotoxicity are common, as well as iron deficiency.[4,19] Inhaling elemental Hg vapors causes acute symptoms including cough, chills, fever, shortness of breath, nausea, vomiting, diarrhea, metallic taste, dysphagia, salivation, weakness, headaches and visual disorders. Chronic inhalation may cause cognitive impairment and personality changes.[52]
Nickel (Ni)	Used in making metal coins and jewelry, valves and heat exchangers, and stainless steel. Also used for nickel plating, color ceramics, cosmetics, tobacco, and batteries. Can be found in the soil, air, and water. There are also nickel-containing foods such as almonds, chick peas, cocoa, tomato, lentils, oats, peanuts, and walnuts.[55]	Iron is a competitive inhibitor of nickel absorption, therefore absorption is enhanced with iron deficiency.[55] Vitamin C works as an antioxidant to counter ROS from nickel, and may also inhibit nickel absorption.[55]	Nickel can affect the lungs via inhalation and if ingested, pass through the GI tract to be excreted in feces. Nickel that is absorbed through the skin or GI tract can either be excreted in urine or deposit anywhere, though mainly the kidneys. In vitro and in vivo studies demonstrate that divalent nickel promotes lipid peroxidation at DNA bases.[55]	Allergic dermatitis/skin rash, asthma/lung inflammation, stomach aches, proteinuria and kidney diseases are seen with exposures. There is some carcinogenic potential.[53]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Niobium (Nb)	Niobium is sometimes found in jewelry, and is used with other alloys, like titanium, to make surgical implants and dental applications.[2] It is also a component of superconducting magnets and nuclear reactor cores.	Unknown	Niobium is poorly absorbed from the GI tract.[2]	It is a moderate eye and skin irritant. Due to poor GI absorption, it has a low order of toxicity. Lethargy and respiratory depression have only been seen with parenteral administration.[2]
Platinum (Pt)	Can be found in soil and river sediments, air, and jewelry. Used as a catalyst in the automotive, chemical, and pharmaceutical industries. It's resistance to oxidation makes it important in the manufacturing of laboratory equipment. It is also used as a chemotherapeutic agent.[2]	Unknown	Platinum binds to DNA and interferes with transcription and replication resulting in apoptosis.[56]	Metallic forms are inert, but the complex salts can produce conjunctivitis, urticaria, dermatitis, and eczema with dermal exposure.[2] Nephrotoxicity and thrombocytopenia are seen with platinum chemotherapeutic agents. Respiratory exposures can produce wheezing and shortness of breath.[14]
Rubidium (Rb)	Soil, rocks, vegetation, water, contrast agent for PET scans, atomic clocks, photoelectric cells, magnetometers, GPS systems, fireworks.[57-61]	Rubidium resembles potassium, and these two elements are metabolically interchangeable.[62]	Rb is rapidly and completely absorbed by the GI tract when ingested and is excreted mainly through the kidneys.[61] Urinary excretion is consistent with a 50-day half-life.[63] Physiologically, rubidium most resembles potassium, and these two elements are metabolically interchangeable.[62] In the myocardium it is an active participant in the NA/K pump.[58] Rubidium and lithium are often studied for CNS dysfunctions including mania and depression, and may work through the NMDA/nitrergic pathways.[64]	Rb chloride was used historically to treat cardiac issues, syphilis, epilepsy and more recently has been studied for depression.[65] Excess rubidium chloride was associated with weight gain, diarrhea, nausea/vomiting, polyuria, confusion, excitement/agitation and dermatitis.[61] In rats, rubidium chloride administration led to hypokalemia.[66]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Thallium (Tl)	Fish, shellfish, plants, cigarettes, soil, air, water, electronic devices, switches and closures for the semiconductor industry, glass for medical procedures.[67]	Some of its toxic effects results from interference with biological functions of potassium.[14]	Thallium is absorbed through the skin and GI tracts. Highest concentrations are found in the kidney. Large amounts are excreted in the urine within 24 hours, then excreted via feces. Tl undergoes enterohepatic circulation.[14] Tl can accumulate in bones, renal medulla, liver and CNS.[68] Thallium has a similar charge and radius as the potassium ion. Some of its toxic effects results from interference with biological functions of potassium. Additionally, it binds to sulfhydryl groups in the mitochondria interfering with oxidative phosphorylation. With these mechanisms, cardiac dysfunction, mitochondrial dysfunction, abnormal protein synthesis and heme synthesis are seen.[14]	GI irritation, paralysis, alopecia, and psychological disturbances are seen.
Thorium (Th)	Rocks, soil, water, plants, ceramics, gas lantern mantles, metals in the aerospace industry and nuclear reactions, fuel for nuclear energy, and mining.[69]	Unknown	Th can damage chromosomes.[69]	Exposure may lead to increased risk of certain cancers including gallbladder, liver, and leukemia, as well as cirrhosis. Inhaled Th (mainly among workers exposed to Th dust) can cause lung damage many years after being exposed.[69]
Tin (Sn)	Found in manufacturing, food packaging, solder, bronzing, dyeing textiles, plastics, PVC pipes, fungicides, toothpaste, perfume, soap, food additives, electronic cigarette aerosol, and dyes. Naturally present in rocks and nearby air, water, and soil.[70,71] Seafood is the primary route of human exposure to organotin compounds.[72] Tin is found in both organic and inorganic forms. Inorganic tin is generally regarded as safe (GRAS) by the FDA as a food additive for human consumption.[73]	Tin disturbs copper, zinc, and iron metabolism.	Very limited GI absorption of inorganic tin orally, with 90% excreted in feces, and therefore non-toxic. When absorbed, it deposits in the liver and kidneys. Organic tin is better absorbed and concentrates in blood, liver, muscles, brain, and heart.[2,14]	Headaches, visual defects, depression, skin and eye irritation – rarely hepatotoxicity and neurotoxicity.[74] Organic tin compounds have been identified as environmental obesogens and urinary tin levels are associated with diabetes.[73,75]

Toxic and Nutrient Elements

ELEMENTS	SOURCES	NUTRIENT INTERACTIONS	PHYSIOLOGIC EFFECTS	CLINICAL SIGNIFICANCE
Tungsten (W)	Found naturally in soil and rocks or airborne emissions from industries using W. Used in high speed and cutting or forming tools (tungsten carbide), welding electrodes, turbine blades, golf clubs, darts, fishing weights, gyroscope wheels, phonograph needles, bullets, armor penetrators, x-ray tubes, light bulbs, ceramic pigments, fire retardant for fabrics, color-resistant dye for fabrics, generally mixed with other metals to make alloys.[76]	W ions antagonize the normal metabolic action of the molybdate ion, therefore molybdenum deficiency promotes W affects.[2]	W is not metabolized in the body and 90% of inhaled W is eliminated in urine after 14 hours.[77] Toxicity of W is enhanced when present in a metal mixture. The synergistic effect promotes oxidative stress and DNA damage.[77]	Exact effects in humans is unknown, however animal models suggest a connection with cancer. A cluster of patients with leukemia in Nevada was shown to have higher urine levels of W, however causation has not been established.[78] Memory and sensory deficits, and lung issues were observed in workers when exposed to hard metal dusts containing 79-95% W, 10% cobalt and other metals.[77]
Uranium (U)	Largely limited to use as a nuclear fuel. Present naturally in air, water, food, and soil. The uranyl ion forms water-soluble compounds and is an important component in body fluids. Three different kinds are defined: natural, enriched, and depleted uranium (DU). The radiological and chemical properties of natural and DU have similar chemotoxicity, though natural is 60% more radiotoxic.[79]	U is reactive. It can combine with and affect the metabolism of lactate, citrate, pyruvate, carbonate, and phosphate, causing mitochondrial damage.[79] It replaces calcium in bone.[80]	On average, only 1-2% of ingested U is absorbed via the GI tract. It rapidly enters the bloodstream and forms a diffusible ionic complex. Once in the bloodstream, it has a very short half-life and approximately 60% is eliminated within 24hrs.[79] The skeleton and kidney are the primary sites of U accumulation. Under alkaline conditions, uranium can be excreted in the urine and feces. Inhalation exposure is toxic to the lungs, though less toxic to distal organs.[79]	The soluble U present in plasma as the uranyl ion complexed with bicarbonate can cause renal toxicity. Nephritis is the main chemically induced effect of U ingestion.[80] Uranyl ion is most concentrated intracellularly in lysosomes, which explains its association with β-microglobulinuria and amino aciduria.[14] Osteopenia, weight loss, hemorrhages in the eyes, legs, and nose have also been seen.[80]

Toxic and Nutrient Elements

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Calcium (Ca)	Dairy products, vegetables, legumes, grains, fish, eggs, dietary supplements, and many other foods which have been fortified with calcium.[81]	Active Ca absorption depends on calcitriol and intestinal vitamin D receptors. Passive diffusion in the intestine relies on luminal: serosal electrochemical gradients.[81] Ca can interfere with the absorption of iron and zinc. Protein intake enhances calcium absorption, but also increases urinary Ca excretion. Alcohol, caffeine, high sodium and phosphorus increase urinary excretion or reduce absorption. Foods with high levels of phytates (whole grain products, wheat bran, beans, seed, nuts, soy isolates) and oxalates (spinach, collard greens, sweet potatoes, beans) bind calcium and interfere with absorption.[82]	Necessary for teeth and skeletal structure, vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion.[81,82]	Hypercalcemia can cause renal insufficiency, vascular and soft tissue calcification, nephrolithiasis, and is most often associated with hyperparathyroidism or malignancy. Inadequate dietary intake does not produce obvious symptoms in the short term, but in the long term may lead to osteoporosis. Hypocalcemia generally results from renal failure and medication use and includes numbness and tingling in the fingers, muscle cramps, convulsions, lethargy, poor appetite and arrhythmias.[82]

Toxic and Nutrient Elements

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Chromium (Cr)	Ubiquitous in foods at low concentrations. Derived from processing of food with stainless steel equipment.[83] Also present in tobacco smoke, chrome plating, dyes and pigments, leather tanning, and wood preserving and is deposited into air, water and soil.[84] There is debate as to whether chromium is an essential trace element. Hip prosthesis bearing surfaces are made from cobalt and other materials including chromium.[85] Welding produces fumes that may contain chromium and other metals (Mn, As, Fe, Ni).[86]	Dietary chromium absorption is low. Chromium is bound to the protein transferrin in the bloodstream.[83] Conditions that increase circulating glucose and insulin increase urinary chromium output.[87] Blood distribution of chromium appears to be equally divided between plasma and RBCs, making whole blood chromium the sample type for total Cr measurement. Cr (VI) is more concentrated in the RBCs, while Cr (III) does not enter the RBCs. Therefore, it is possible to distinguish sources and types of exposure to indicate toxic (Cr[VI]) exposure versus benign (Cr[III]) by measuring RBC chromium.[88-90] Chromium rapidly clears from the blood and measurements relate to recent exposure. Urinary Cr excretion reflects absorption over the previous 1-2 days.[89,90]	Often given as a supplement to treat glucose intolerance by improving insulin sensitivity.[83]	Currently, no symptoms of chromium deficiency exist. Compounds containing hexavalent chromium (Cr [VI]) are mutagenic and carcinogenic in large quantities. No adverse effects have been associated with trivalent chromium (Cr[III]), the form in food and supplements.[83] Inhaled chromium may cause irritation to the lining of the nose, nose ulcers, runny nose, and breathing problems including asthma, cough, shortness of breath or wheezing. Dermal contact with chromium may cause skin ulcers, redness and swelling. Ingested chromium (in animals) may cause irritation and ulcers in the stomach and small intestine and anemia.[84] The FDA recommends testing chromium in whole blood in patients with metal on metal hip implants who have symptoms. These symptoms may include localized pain due to damaged bone and/or tissue surrounding the implant and joint.[91]

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Cobalt (Co)	Cobalt is a hard silvery-bluish metal widely dispersed in nature in low concentrations. Diet, environment and supplements are the main sources of cobalt for the general public. The highest Co concentration in foods include chocolate, butter, coffee, fish, nuts, green leafy vegetables and cereals. Vitamin B12 (contains Co) is found in meat and dairy. Cosmetics, jewelry and electronics may be other sources of exposure.	Binds to albumin.[93] Iron deficiency can be associated with increased absorption of Co. The GI absorption of Co is approximately 25%. Once absorbed, Co disseminates to serum, whole blood, liver, kidneys, heart and spleen and to a lesser extent bones, hair, lymph, brain and pancreas.	Cobalt is a necessary element for the formation of vitamin B_{12} (cyanocobalamin).[94] The B_{12} RDI is 2.4 mcg/d and contains 0.1 mcg Co.[92] Elevated cobalt can result in generation of reactive oxygen species (ROS) and lipid peroxidation, interruption of mitochondrial function, alteration of calcium (Ca) and iron (Fe) homeostasis, interactions with body feedback systems triggering erythropoiesis, interruption of thyroid iodine uptake, and induction of genotoxic effects and possible perturbation of DNA repair processes.[92]	Excessive administration produces goiter and reduced thyroid activity. Industrially, exposure can cause a contact dermatitis or occupational asthma. Polycythemia has been observed in some studies.[92,94] Cobalt toxicity from a hip prosthesis is determined by monitoring blood measurements. Symptoms include peripheral neuropathy, sensorineural hearing loss, cognitive decline, visual impairment, hypothyroidism and cardiomyopathy.[85,92]
	Industrially, workers may be exposed to cobalt powders in hard metal production (often combined with tungsten), construction, electronic waste recycling, diamond polishing and paint. Contamination from these industries may affect the general public through water, soil or air.[92] Hip prosthesis bearing surfaces are made from cobalt and other materials including chromium.[85]	The kidneys are responsible for Co excretion.[92]		

Toxic and Nutrient Elements

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Copper (Cu)	Legumes, mushrooms, chocolate, nuts and seeds, shellfish and liver are high in copper (all >2.4 µg/g).[95,96] Food (Cu^{+}), water (Cu^{2+}) and air (via combustion of fossil fuels and agriculture) are sources of copper.[97] Copper pipes and fixtures in household plumbing may allow copper to leach into water.[96]	Cu absorption occurs in the upper small intestine and compared to other elements, has a relatively high absorption rate at 55-75%.[95] Copper levels in the body are homeostatically maintained by copper absorption from the intestine and copper release by the liver into bile to provide protection from copper deficiency and toxicity. Most copper is excreted in bile/feces and a small amount excreted in urine.[96] Urinary copper declines only when dietary copper intake is very low. A 24-hour urinary copper provides a screening for suspected cases of toxicity or copper deficiency anemia.[98,99] Correlation was seen in Wilson's disease using first morning or 24-hour urine.[100] Grains contain phytates that may inhibit copper absorption in the intestines.[98] Intestinal iron absorption is a copper-dependent processes.[95] Iron, vitamin C, zinc, lead poisoning, hemochromatosis, excessive soft drink ingestion, bariatric surgery and Zn-containing denture creams adversely affect Cu bioavailablity.[95] Cadmium exposure may result in increased urine excretion of copper due to possible renal tubular damage.[101] Increased Mo intake may elevate urinary copper excretion.[102] Serum and 24-hour urine copper excretion was similar in long-term copper IUD users as in a control group that did not have an IUD.[103]	Cu is a cofactor for more than 20 enzymes, particularly those involved in cellular respiration and energy metabolism, neurotransmitter and hormone biosynthesis, iron metabolism, gene transcription, melanin formation and antioxidant defense. Copper is also involved in blood coagulation and blood pressure control, myelination, and connective tissue cross-linking.[95,97] Ceruloplasmin (CP) carries the predominance of copper in the blood, so alterations in blood copper likely reflect the amount of circulating CP. Plasma Cu and CP can increase during an acute phase response to infection and inflammation, pregnancy and other hormonal perturbations, some carcinogenic phenotypes, and smoking. Plasma Cu may be elevated in these states while tissue Cu could be low. Low plasma Cu indicates physiological impairment.[95]	Copper deficiency is associated with osteoporosis, hypochromic, microcytic anemia, impaired cholesterol and glucose metabolism, cardiovascular disease, connective tissue abnormalities, CNS disorders, and impaired immune function.[95,98] Reductions in plasma copper and ceruloplasmin (CP) activity are noted in severely copper-deficient individuals.[95] Copper toxicity is rare due to adequate homeostatic control, however an upper tolerable intake level of 10 mg/day has been established. Wilson's disease is an inherited disease that results from decreased biliary Cu excretion due to biliary atresia or biliary cirrhosis.[95] Signs and symptoms include jaundice and abnormal LFTs, ascites, Kayser-Fleischer rings, and neurological and psychiatric symptoms. Copper dyshomeostasis involving either deficiency or excess has been implicated in Alzheimer's disease and cognitive decline.[97]

Toxic and Nutrient Elements

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Lithium (Li)	Cereals, fish, nuts, potatoes, tomatoes, cabbage, mineral water, tap water, nutmeg, coriander seeds, cumin, medication. Naturally found as a trace element in the earth's crust and water sources. Not officially considered a micronutrient, although it is recognized as important.[110] Industrial use includes lithium-based batteries, dessicants.[111]	Li is rapidly absorbed, has a small volume of distribution and is excreted in the urine unchanged (lithium is not metabolized).[112] Pregnancy and increased sodium, xanthines (theophylline and caffeine), nifedipine, and carbonic anhydrase inhibitors (e.g., acetazolamide) increase lithium excretion in the urine.[110]	Li modulates the activity of norepinephrine, serotonin, dopamine, glutamate, GABA, acetylcholine and glycine. It can resynchronize circadian rhythms by modulating the expression of clock genes and HPA axis regulation. Li stimulates the production of neural stem cells and is protective against oxidative stress.[110]	Lithium excess from overmedicating can result in interstitial nephritis, cardiac abnormalities and seizures.[112] It is hypothesized that low Li intake can cause mood worsening and increase impulsiveness and nervousness.[110] Li is prescribed for bipolar disorder and major depressive disorder due to its impact on neurotransmission. It is also prescribed for vascular headaches and neutropenia.[112]
Magnesium (Mg)	Green leafy vegetables, legumes, nuts, seeds, whole grains, medicines (e.g., Milk of Magnesia), Epsom salt. Over the last 60 years, the Mg content in fruits and vegetables has decreased by 20-30%, and 80-90% of Mg is lost during food processing.[113]	The intestine, bone and kidney maintain magnesium homeostasis.[114] Unlike other minerals, Mg can be absorbed along the entire length of the GI tract.[115] Soft drinks, low protein diets, foods containing phytates, polyphenols and oxalic acid, fluoride, antibiotics, and oral contraceptives bind to magnesium and produce insoluble precipitates or complexes, negatively impacting Mg availability and absorption. Caffeine, alcohol and diuretics (e.g., furosemide, bumetanide) increase renal excretion of Mg. Antacids (e.g., omeprazole) affect Mg absorption due to the increase in GI pH.[115]	Mg plays a role in hundreds of enzymatic reactions involved in hormone receptor binding, muscle contraction, neural activity, neurotransmitter release, vasomotor tone, blood glucose control, mitochondrial energy production, and cardiac excitability.[114,116] RBC magnesium is often cited as preferable to serum and plasma levels due to their higher magnesium content (0.5% vs 0.3%). Serum is used to assess hyper- or hypomagnesemia. Urinary Mg may not correlate with Mg status in the body due to the variable degree of renal reabsorption and secretion. Variables affecting this include dietary intake, existing Mg status, mobilization from bone and muscle, hormones (estrogen, parathyroid, calcitonin, glucagon), medications (diuretics, chemotherapy), diabetes. Anywhere between 5% to 70% of filtered Mg may be excreted in the urine. 24-hour urine levels may be more reliable than spot urine.[115] Normal or high urinary excretion is thought to indicate renal Mg wasting, whereas low Mg excretion suggests reduced intestinal absorption.[113]	Low magnesium is associated with hypertension, coronary heart disease, diabetes, osteoporosis, neurological disorders (migraine, depression, epilepsy), asthma, muscle cramps, sleep disorders, fibromyalgia, and chronic fatigue.[113,115] Elevated magnesium is associated with nausea, vomiting, lethargy, headaches, flushing, bradycardia, hypotension and cardiac abnormalities.[113]

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Manganese (Mn)	Whole grains (wheat germ, oats and bran), rice, and nuts (hazelnuts, almonds, and pecans) contain the highest amounts of Mn. Other food sources include chocolate, tea, mussels, clams, legumes, fruit, leafy vegetables (spinach), seeds (flax, sesame, pumpkin, sunflower, and pine nuts) and spices (chili powder, cloves and saffron).[117] Airborne exposure can occur through automobile exhaust, unleaded gasoline and occupational exposure (mining, welding, ferroalloy and steel industry, battery manufacturing). It is also present in fungicides, textile bleaching, manufacture of glass and ceramics, paint, matches and fireworks, leather tanning, hydroquinone, potassium permanganate and other chemical production. Soil manganese concentrations can contaminate well water.[117,118]	Only about 1 to 5% of dietary Mn is absorbed in the gut. Absorption is influenced by intestinal pH, the presence of divalent metal transporter DMT1, other divalent metals competing for absorption (iron, copper, zinc, calcium) and phytic acid.[119] The absorption of Mn is tightly regulated in the gut and therefore toxicity from diet has not been reported.[117] Iron deficiency increases Mn absorption.[119] Supplemental magnesium (200mg/day) may decrease Mn availability by decreasing absorption or increasing excretion.[120] Mn is eliminated mainly via bile.[118]	Required for immune function, regulation of blood sugar and cellular energy, reproduction, digestion, bone growth, blood coagulation, hemostasis, wound healing and antioxidant. Mn is incorporated into metalloproteins, such as superoxide dismutase and others.[117,120]	Mn deficiency is rare and results in impaired growth, poor bone formation and skeletal defects, abnormal glucose tolerance, altered lipid and carbohydrate metabolism, dermatitis, slowed hair/nail growth.[117,118] Diseases reported with low blood Mn concentrations include epilepsy, Mseleni disease, Down's syndrome, osteoporosis and Perthest disease.[118] Individuals with increased susceptibility to manganese toxicity include patients with chronic liver disease, newborns and children, iron-deficient populations, patients on parenteral nutrition, and occupational exposure.[118,120] Mn is neurotoxic and excess levels have been associated with Parkinson's-like symptoms. A blood Mn level may provide the best estimate for brain Mn levels when exposure is recent. Mn toxicity is generally due to environmental or occupational exposures including airborne (inhaled) and drinking water.[117] Periods of occupational exposure of 6 months to 2 years may lead to manganism and the motor and neuropsychiatric symptoms may remain several years after the exposure. Symptoms include dystonia, bradykinesia and rigidity (due to damage to dopaminergic neurons) and gliosis.[86] Additional symptoms include tremors, muscle spasms, tinnitus, hearing loss, ataxia, mania, insomnia, depression, delusions, anorexia, headaches, irritability, lower extremity weakness, changes in mood or short-term memory, altered reaction times and reduced hand-eye coordination.[121]

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Molybdenum (Mo)	Beans (lima, white, red, green, pinto, peas), grains (wheat, oat, rice), nuts, vegetables (asparagus, dark leafy, Brassicas), milk, cheese.[102,122]	Mo absorption is passive in the intestines. Urinary excretion is a direct reflection of dietary Mo intake, not necessarily Mo status.[102] Increased Mo intake may elevate urinary copper excretion.[102]	Four enzymes require Mo as a cofactor: sulfite oxidase, xanthine oxidase, aldehyde oxidase and mitochondrial amidoxime-reducing component (mARC). These enzymes are important in detoxification. Sulfite oxidase converts sulfite to sulfate.[122] Mo has been used clinically to treat Wilson's disease to bind copper and prevent absorption. Because of the copper-chelating property, Mo has been studied as an antitumor therapy as well as its ability to inhibit profibrotic and proinflammatory cytokines for the treatment of arthritis and MS.[122]	Mo deficiency is quite rare and a case report shows an acquired deficiency due to long-term parenteral nutrition.[122] The potential for Mo toxicity is low and may be associated with aching joints, gout-like symptoms, hyperuricosuria, elevated blood Mo, hallucinations and seizures.[122]
Potassium (K)	Fruits and vegetables especially potatoes, apricots (dried), prunes, citrus juices, tomatoes, beet greens, avocadoes, bananas, leafy greens, legumes, yogurt, salt substitutes.[123-126]	Approximately 90% of the daily K intake is excreted in the urine and 10% by the GI tract.[124] Salting foods, then discarding the liquid reduces the potassium content.[127] Approximately 98% of K is found within cells and 2% in the extracellular fluid.[124] A standard metabolic panel includes serum potassium to assess hyper or hypokalemia. RBC potassium indicates intracellular levels. The correlation between dietary K intake and urinary K is high.[125] Increased urinary potassium loss may result in hypokalemia. While the 24-hour urinary collection is considered gold standard for assessing urinary potassium excretion, a spot urine adjusted to creatinine correlates with a 24-hour urine collection.[128] Thiazide diuretics have a common side effect of lowering serum potassium leading to hypokalemia.[127]	Potassium is critical for normal cellular function. All cells possess a sodium-potassium exchanger that pumps Na out of and K into cells, creating a membrane potential. Excitable tissues such as nerve and muscle rely on this gradient. Insulin, catecholamines and aldosterone are responsible for maintaining the regulation of K distribution between the intracellular and extracellular space. Additionally, the kidneys play a role in maintaining K homeostasis.[124] The potassium: sodium intake ratio has decreased from early to modern times and contributes to the negative effect on blood pressure.[123]	Anorexia nervosa, crash diets, alcoholism, excessive sweating, intestinal malabsorption and diarrhea are clinical situations associated with K deficiency. Hypokalemia is characterized by low serum K and can lead to glucose intolerance via impaired insulin secretion, cardiac arrhythmias, and muscle weakness. Mild hypokalemia is characterized by constipation, fatigue, muscle weakness, and malaise.[124-127] Adequate potassium intake is important for heart and bone health, reduces the risk of stroke and coronary heart disease, and is associated with a reduction in recurrent kidney stones. The primary health outcome used to evaluate potassium intakes for dietary guidelines is blood pressure.[123] Hyperkalemia is characterized by elevated serum K and symptoms include paresthesias, fasciculations in the arms and legs, ascending paralysis with eventual flaccid quadriplegia, respiratory failure (rare), ECG changes, ventricular fibrillation and asystole.[124]

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Selenium (Se)	The selenium content of grains and vegetables depends on the Se content of the soil. In meats, Se content is dependent on the diet of the animals. Foods with higher selenium content include Brazil nuts, seafood (especially tuna), chicken, beef, pork, lamb.[129,130] Also present in air, water, soil, metallurgy, airborne coal/oil emissions, dandruff shampoo, paints, photo cells, drums, photocopiers, glass, ceramics, rubber, pharmaceuticals.[129]	Selenium tends to be well absorbed and the bioavailability of Se in the form of selenomethionine is greater than 90%.[130] Sulfur, lead, arsenic, calcium and iron (Fe3+) reduce the absorption of Se.[129] Urine is the main route of excretion and reflects recent dietary intake. Plasma is useful for assessing nutritional selenium status.[129,130]	Selenium is part of selenoproteins that are important for antioxidant defense, thyroid hormone formation, DNA synthesis, and reproduction. Deiodinases in the thyroid incorporate Se and are important for conversion of thyroxine to active T3. Glutathione peroxidase is a selenium-dependent antioxidant enzyme that neutralizes hydrogen peroxide.[129] Some research suggests that plasma glutathione peroxidase levels may be a good indicator of selenium status.[130]	Symptoms of selenium deficiency occur in extreme cases of deprivation and include necrotizing cardiomyopathy, peripheral myopathy, decreased muscle tone, thinning hair, opaque nails, and anemia. The disease associated with selenium deficiency is called Keshan disease. Selenium deficiency may be a cancer promoting factor.[129] Selenium toxicity symptoms include hair and nail brittleness and loss (selenosis), GI disturbances, skin rash, garlic breath odor, fatigue, irritability and nervous system abnormalities.[130]
Strontium (Sr)	Sr is found in fish, grains, leafy vegetables, dairy, soil, water, air, and is also used in the manufacturing of televisions, fireworks, paints, glass, ceramics, fluorescent lights, medicines, magnets.[111,131]	Vitamin D, calcium, and protein reduces the absorption of Sr.[131] Sr is eliminated mainly through urine.[111]	Sr is considered a trace mineral that is similar to calcium, accumulates in bone and is involved in bone metabolism. Sr promotes calcium uptake into the bone and has been used as a prescription drug in the treatment of osteoporosis.[111,132]	Toxic levels may be associated with rickets especially in children.[111] Urinary Sr levels were associated with breast cancer risk.[133]
Sulfur (S)	Protein (specifically amino acids methionine and cysteine as organic sulfur), eggs, meat, fish, dairy, garlic, onion, broccoli and other cruciferous vegetables, supplements (chondroitin sulfate, glucosamine sulfate, MSM, etc.), sulfiting agents (inorganic sulfur) as food additives in processed meats, wine, beer, dried fruits, seafood.[134,135]	Unknown	Sulfur is involved in cartilage synthesis. Sulfation is a major detoxification pathway.[135] The sulfur-containing amino acids cysteine and methionine are not stored in the body. Any dietary excess is oxidized to sulfate, excreted in the urine, or stored in the form of glutathione.[135] Sulfur deficiency is involved in the development of oxidative tissue damage.[136]	Nutritional deprivation of sulfur is associated with cardiovascular disease and stroke.[136]

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Vanadium (V)	Mushrooms, shellfish, black pepper, parsley, dill seed, beer, wine, grains, sweeteners, infant cereals.[102] Fossil fuels, welding, catalysts, steel alloys, batteries, photographic developer, drying agent in paints/varnishes, reducing agent, pesticides, black dyes/inks/pigments in ceramics, printing and textile industries.[137,138]	The absorption of V is <5% and most ingested V is found in the stool.[102] V is transported mainly in the plasma. It is found in large amounts in the blood initially and at trace levels 2 days after exposure.[139] V has a half-life of around 10 days. Body clearance occurs directly via urinary excretion.[138]	Vanadium mimics insulin and has been used as a supplement for diabetic patients. V stimulates cell proliferation and differentiation. The highest concentrations are found in the liver, kidney and bone.[102] V is studied in the treatment of diabetes, cancer, and diseases caused by parasites, viruses and bacteria, and for anti-thrombotic, anti-hypertensive, anti-atherosclerotic, and spermicidal properties.[138] Vanadium-induced cytotoxicity can be mitigated by glutathione, ascorbic acid, or NADH to convert oxidized vanadium (5+) into its reduced (4+) form.[140]	Acute toxicity is rare. V exposure is through ingestion or inhalation. Vanadium may cause abdominal cramps, loose stools, green tongue, fatigue, lethargy, focal neurological issues. Animal studies show renal toxicity with high doses.[102] Urinary vanadium concentrations during pregnancy were associated with preterm delivery and impaired fetal growth. Cardiovascular and respiratory symptoms may be present.[140]
Zinc (Zn)	Red meat, seafood (oysters), whole grains (in the germ and bran portion). As much as 80% of total Zn is lost during the grain milling process.[102] Denture cream and galvanized steel or iron also contain Zn.[141,142]	The majority of Zn is absorbed in the jejunum via a transcellular process and at high Zn intakes paracellular transport may occur. Iron and calcium supplements, phosphorus-containing salts, phytates (grains, legumes), phosphorus-rich proteins (milk casein, nucleic acids), and long-term alcohol consumption may decrease Zn absorption.[102] Zn decreases the concentration of copper.[143] Occupational silica exposure may lead to increased urinary loss of copper and Zn.[144] Increased urinary Zn levels may be seen with muscle catabolism, cirrhosis, long-term alcohol consumption, and use of thiazide diuretics.[102,145-147] A drop in urinary Zn occurs before a decrease in plasma Zn in Zn-	Zinc is important for immune function, cell division, cell growth, wound healing, breakdown of carbohydrates, enhancing insulin action, sense of smell and taste and as an antioxidant. During pregnancy, infancy, and childhood, zinc is a requirement for proper growth and development.[143] There are over 300 active Zn metalloproteins and more than 2000 Zn-dependent transcription factors involved in gene expression of various proteins.[143] Some of the well-known metalloenzymes include RNA polymerases, alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase (ALP).[102] Over 85% of total body Zn is found in skeletal muscle and bone.[102]	Zinc deficiency is associated with lymphopenia, frequent infection, hair loss, diarrhea, poor appetite, problems with taste and smell, slow growth, hypogonadism in males, nighttime vision loss, dermatitis, delayed wound healing, depression, schizophrenia, multiple sclerosis.[102,143] Acrodermatitis enteropathica is a rare inherited condition that results in low zinc.[151] Excess intake of Zn can result in copper or iron deficiency, nausea, vomiting, epigastric pain, lethargy, fatigue and headaches.[102,143]

NUTRIENT	SOURCES	ABSORPTION FACTORS	BIOCHEMICAL ACTIONS	SYMPTOMS OF IMBALANCE
Zinc (Zn)		deficient diets, indicating that urinary Zn responds more rapidly than plasma to dietary changes. Plasma Zn is useful for assessing the exchangeable pool of Zn between tissues, and can be low due to stress, inflammation, infection, low albumin or other metabolic conditions, as well as extreme dietary deficiency. Homeostatic mechanisms are effective in maintaining plasma Zn concentrations for many weeks of even severe dietary Zn restriction.[102,148-150]		

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