

# ORGANIC ACIDS SUPPORT GUIDE

GENOVA  
DIAGNOSTICS



# ◀ ORGANIC ACIDS

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# Organic Acids

## NUTRITIONAL

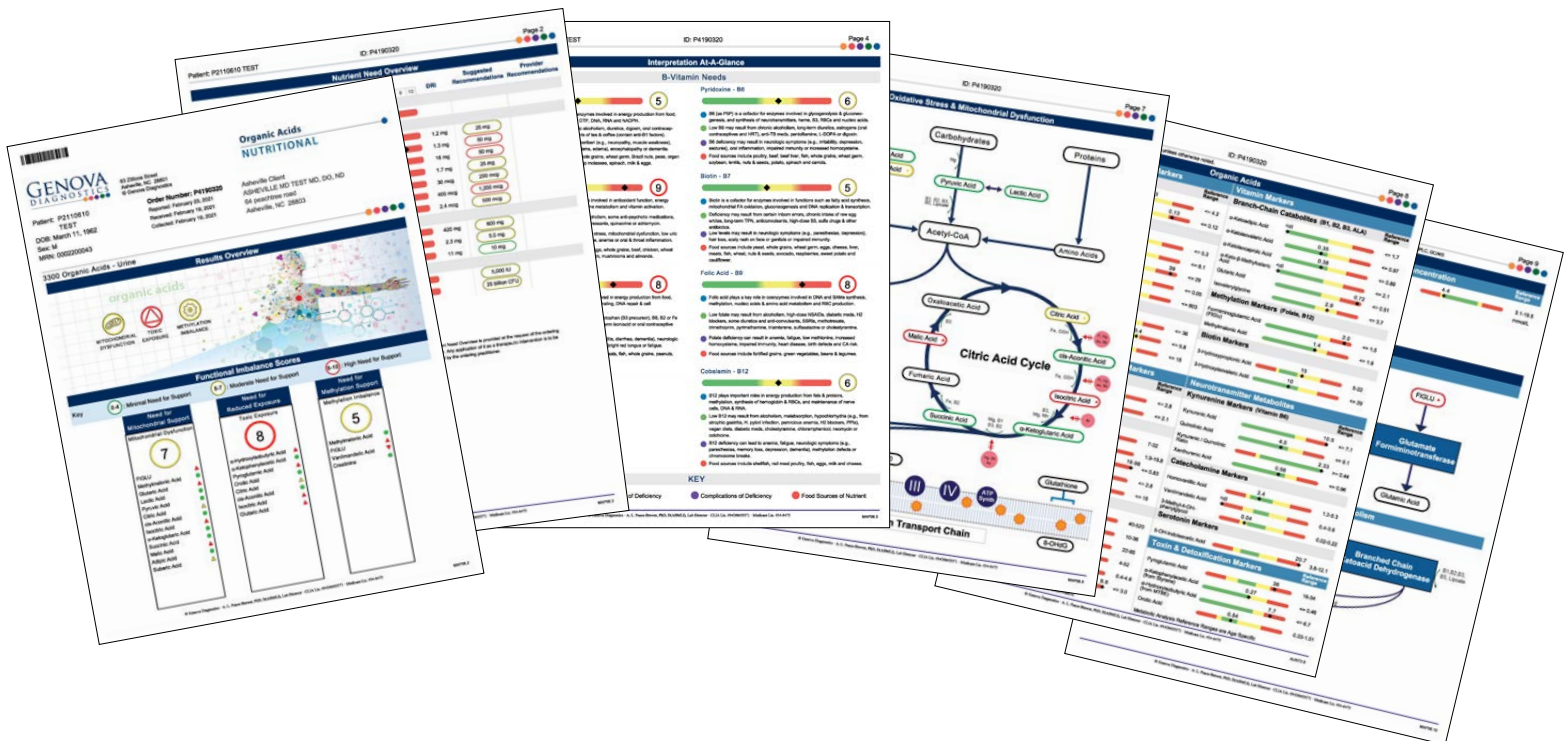
The **Genova Organic Acids** is a functional nutritional assessment of urinary organic acids. Organic acids are a broad class of compounds formed during fundamental metabolic processes in the body. Metabolic reactions produce carboxylic acid compounds derived from the digestion of dietary protein, fat, 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?

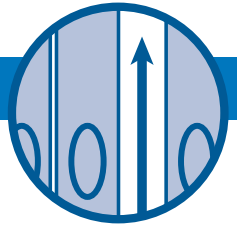
The quantitative measurement of specific organic acids in the urine offers a functional assessment of nutrient status. Enzymes that are responsible for metabolizing organic acids are vitamin and mineral dependent. With this, elevations in organic acids can speak to a functional need for these nutrients on a cellular and biochemical level, even despite normal serum levels.<sup>1-7</sup> 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.<sup>8,9</sup> 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.<sup>10</sup>

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.<sup>11</sup> IAA can be formed from several common gut microbes such as *Clostridia* species, *Escherichia coli*, and *Saccharomyces* species.<sup>12-14</sup>

##### 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.<sup>15-17</sup> 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.<sup>18</sup>

#### 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.<sup>9</sup> Dietary polyphenols may also contribute to PAA elevation.<sup>19</sup>

##### 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.<sup>9</sup> Dietary polyphenols may also contribute to PAA elevation.<sup>19</sup>

##### Clinical Associations:

There is a clinical correlation between decreased urinary PAA and depressive symptoms.<sup>20-22</sup>

## 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.<sup>9,12</sup>

#### 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.<sup>23-26</sup>

#### 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.<sup>27-29</sup>

Much like IAA and PAA, there is an inverse correlation between these markers and depressive symptoms.<sup>20-22</sup>

### 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.<sup>30</sup>

#### 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.<sup>31,32</sup> 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.<sup>33</sup>

## YEAST/FUNGAL DYSBIOSIS MARKERS

### D-arabinitol

**D-arabinitol** is a sugar alcohol produced specifically by *Candida* spp.<sup>34,35</sup> The majority of the published literature shows a correlation between serum or urinary D-arabinitol levels and systemic invasive candidiasis in immunocompromised individuals.<sup>35</sup> 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.<sup>36</sup> 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.

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.<sup>25,26,37-40,42-61</sup>

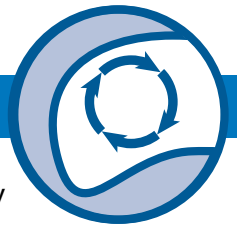
Urinary Metabolite	Common Dietary Sources
Indoleacetic acid	High tryptophan intake, green/black tea
Phenylacetic acid	Wine/grapes
Dihydroxyphenylpropionic acid	Whole grains, chocolate, coffee, green/black tea, olives/olive oil, citrus fruits (animal studies)
3-Hydroxyphenylacetic acid & 4-hydroxyphenylacetic 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

### 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.<sup>37-41</sup>

#### 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.

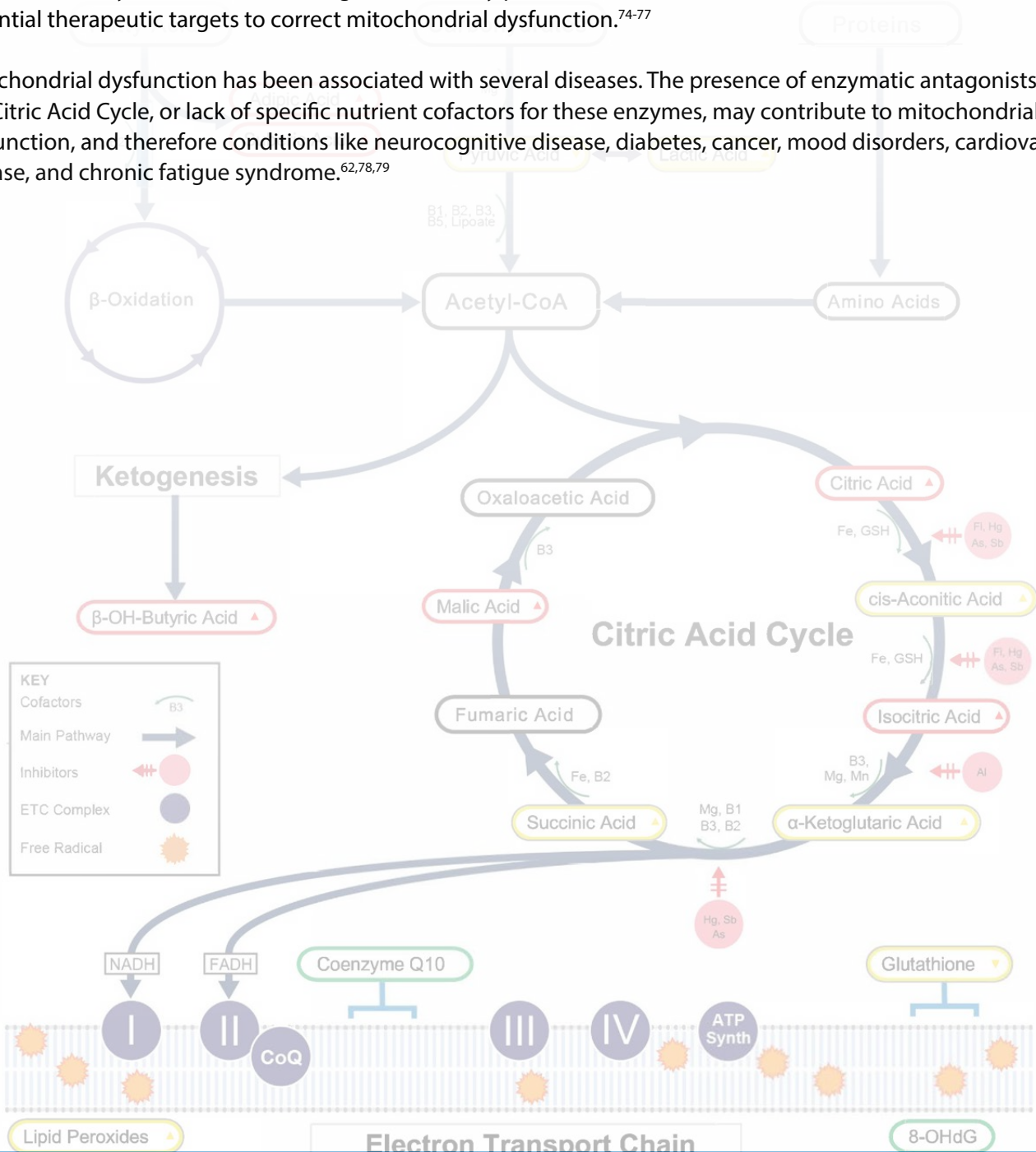


The cellular energy and mitochondrial metabolite markers reflect the body's ability to process dietary macronutrients to feed the Citric Acid Cycle and subsequent energy production. Abnormalities throughout the Citric Acid Cycle, as well as in fatty acid oxidation, glycolysis, and protein metabolism 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.<sup>62-72</sup> Toxic exposures and metals including, but not limited to, mercury, arsenic, and lead can interfere with mitochondrial function.<sup>62,63,73</sup>

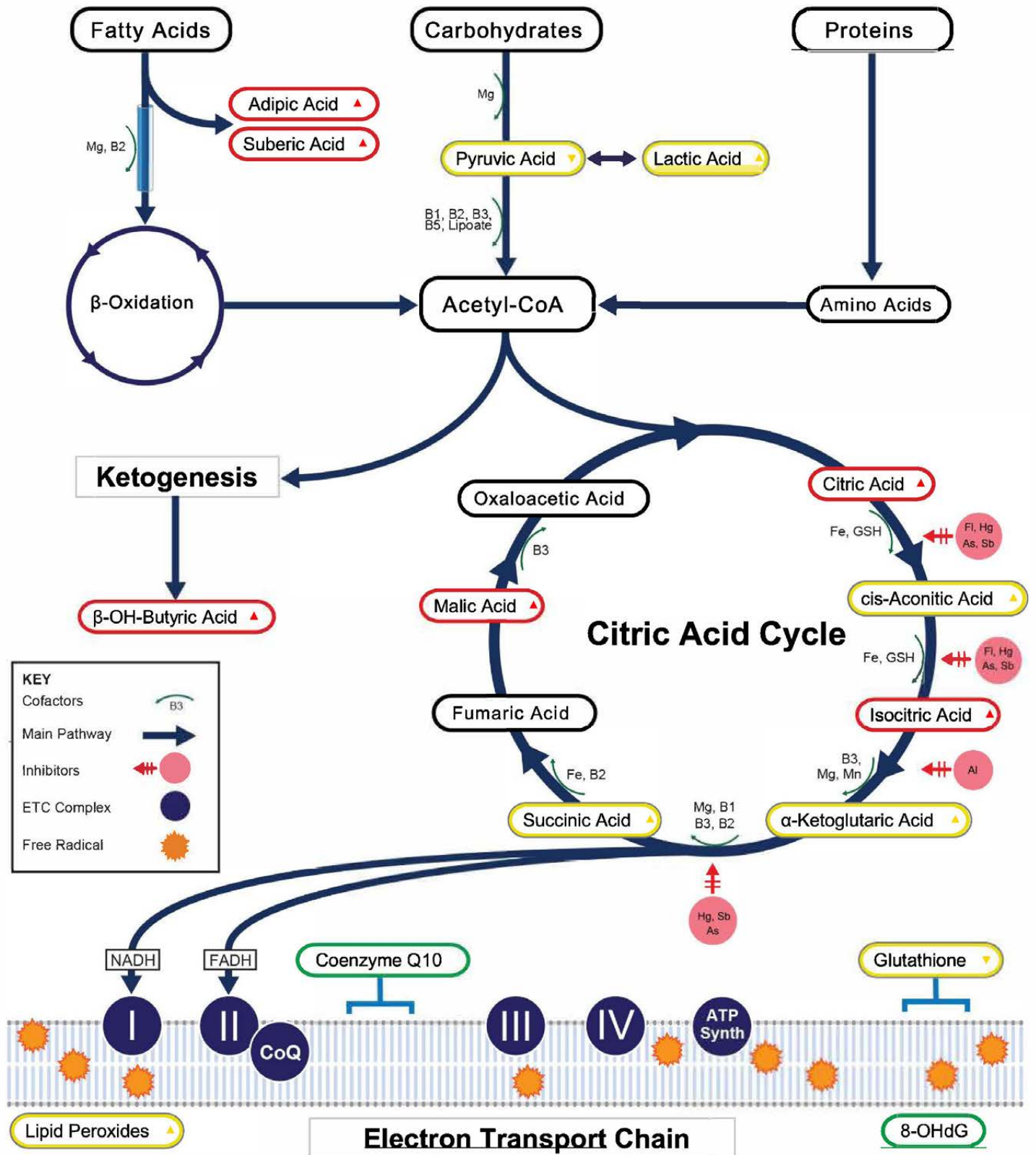
Abnormal urinary excretion of these organic acids may provide a window into various clinical conditions, as well as potential therapeutic targets to correct mitochondrial dysfunction.<sup>74-77</sup>

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.<sup>62,78,79</sup>





### 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.<sup>80</sup> 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.<sup>81,82</sup> Vitamin B<sub>2</sub> and magnesium play a role in optimizing beta-oxidation.<sup>83-88</sup>

#### High Levels:

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

#### 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.

<sup>80,90,91</sup>

## 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.<sup>92</sup> Magnesium is an important cofactor for a number of glycolytic enzymes necessary to produce pyruvate.<sup>93</sup> 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<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>7</sub>, magnesium, and lipoate are needed to support the pyruvate dehydrogenase and pyruvate carboxylase enzymes.<sup>62,64,94-97</sup> 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.<sup>98,99</sup> Pyruvate elevations can also be seen with a high intake of carbohydrates, as well as rare genetic forms of pyruvate dehydrogenase deficiency.<sup>92</sup>

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.<sup>100-104</sup> 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.<sup>92,105,106</sup> 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.<sup>93,107</sup>

#### Clinical Associations:

Pyruvate metabolism abnormalities play important roles in cancer, heart failure, and neurodegeneration.<sup>92</sup>

## **α-hydroxybutyric Acid**

**α-hydroxybutyric acid (2-hydroxybutyric 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.<sup>108,109</sup> 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.<sup>110</sup>

### **High levels:**

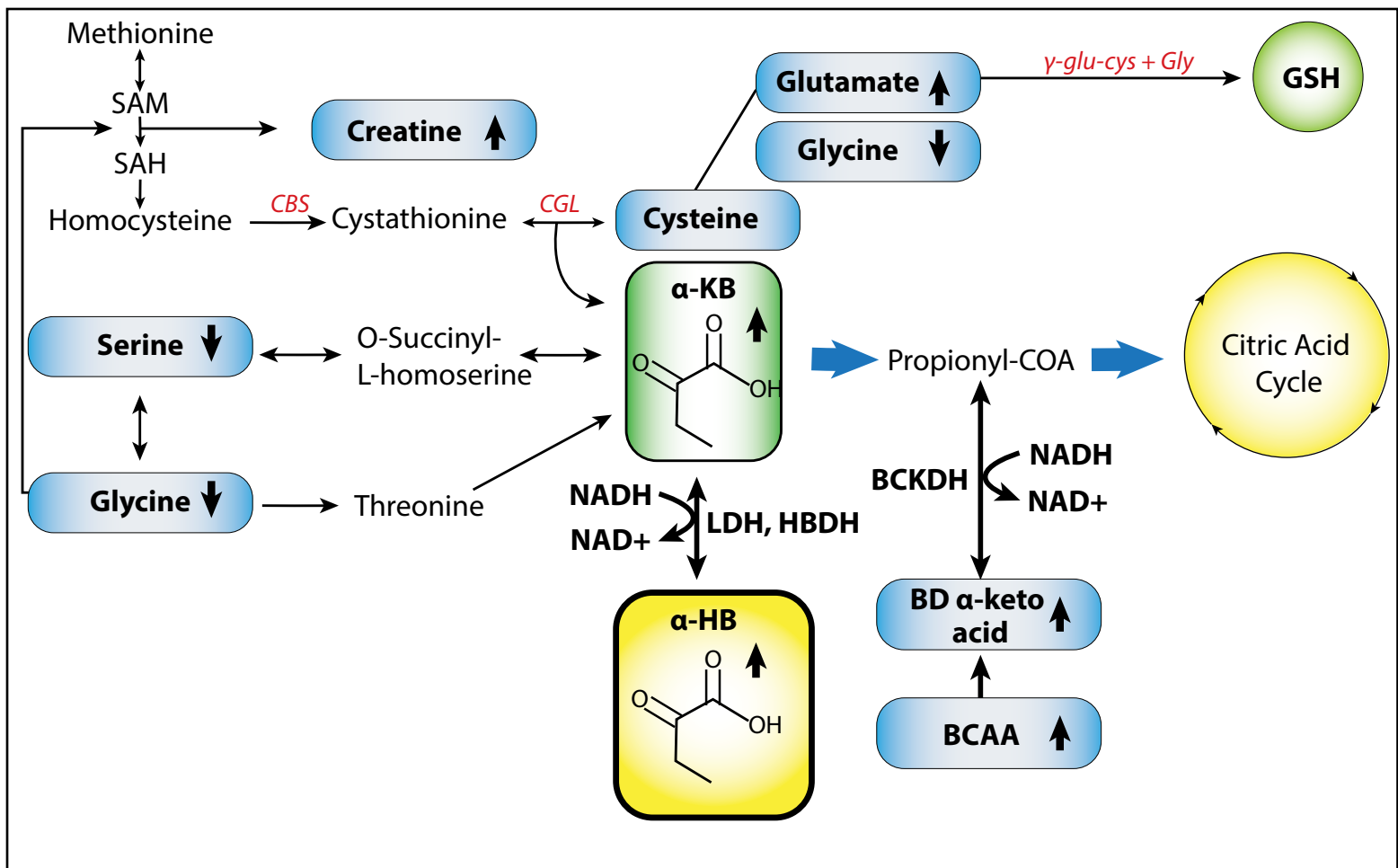
Higher circulating levels of 2-HB are associated with insulin resistance and prediabetes.<sup>108,109</sup>

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.<sup>111</sup>

### **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.<sup>112-114</sup>

## **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.<sup>118</sup>

### **High Levels:**

Iron deficiencies and overload at the systemic or cellular levels can negatively impact the aconitase enzyme and overall mitochondrial health and function.<sup>119</sup> 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.<sup>120</sup> Various toxins may influence mitochondrial enzymes and contribute to mitochondrial dysfunction, such as fluoride, aluminum, mercury, arsenic, and tin.<sup>121-124</sup>

### **Low Levels:**

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

## **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.<sup>115</sup> 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.<sup>116</sup>

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.<sup>117</sup>

### **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<sup>125</sup> 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.<sup>125</sup> 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<sub>3</sub>, zinc, magnesium, and manganese. Higher levels are seen in mitochondrial oxidative phosphorylation disorders and mitochondrial dysfunction.<sup>126</sup> Genetic abnormalities with the enzyme itself can also limit conversion of alpha-ketoglutarate, causing elevations.<sup>127</sup>

### Low Levels:

Low levels of  $\alpha$ -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.<sup>127</sup>

Succinic acid requires the enzyme succinate dehydrogenase to become fumarate. This enzyme is iron-based and requires vitamin B<sub>2</sub> to support flavin adenine dinucleotide (FAD) as a redox coenzyme.<sup>128</sup> 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.<sup>129</sup>

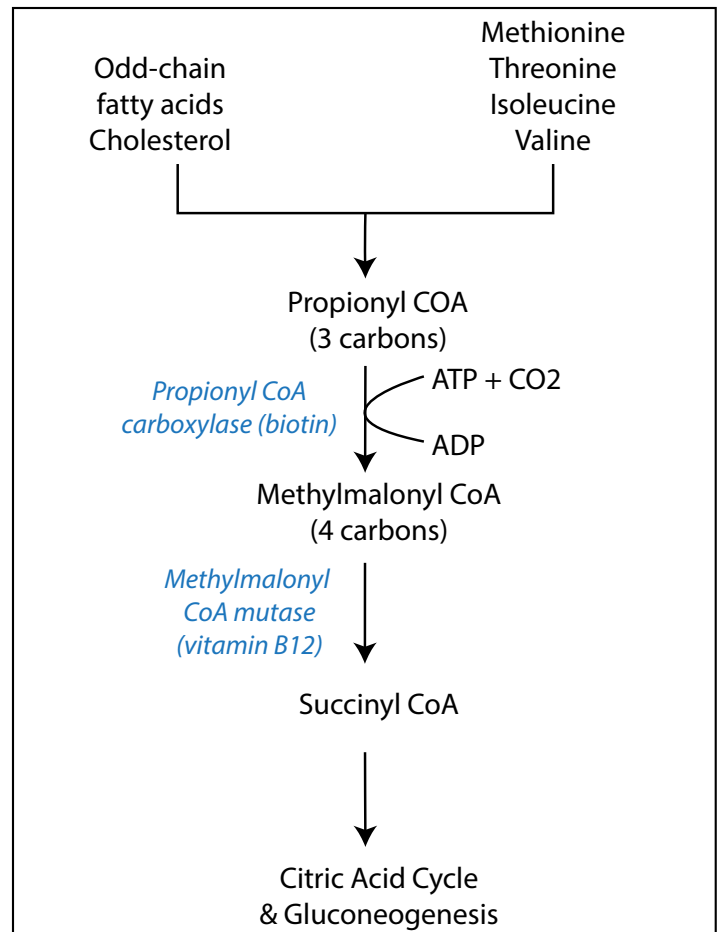
### High Levels:

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.<sup>130</sup>

Several studies indicate that elevations in both succinate and fumarate play a role in oncogenesis by causing DNA damage and hypermethylation.<sup>131</sup>

### 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<sub>12</sub> 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.<sup>132</sup>



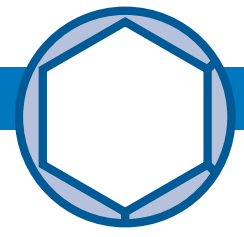
### Malic Acid

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.<sup>133</sup> Riboflavin is an important cofactor for this enzyme and overall mitochondrial energy production and cellular function.<sup>134</sup>

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<sub>3</sub> 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.<sup>135</sup>

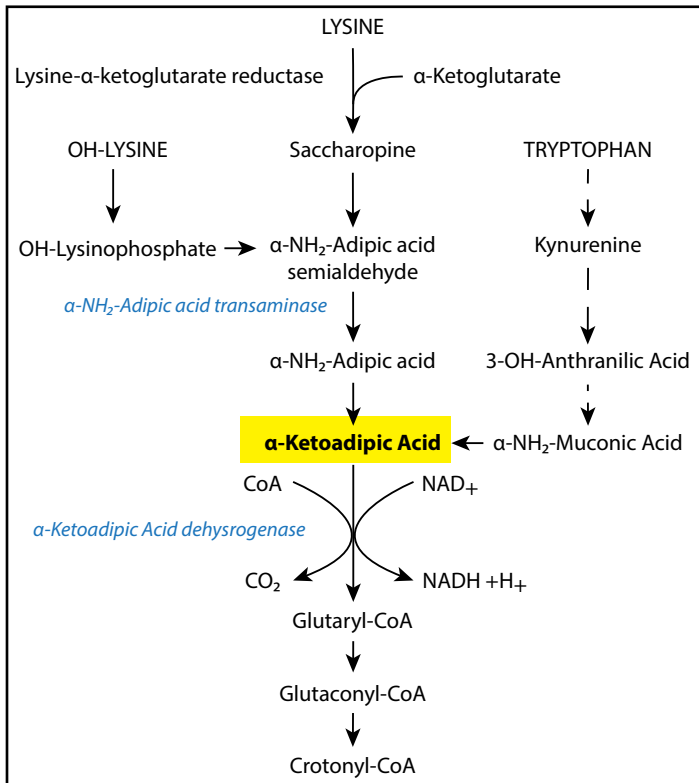


There are groups of organic acids commonly used to assess the status of specific B-vitamins. By measuring organic acids that are known to rely on specific nutrients for enzymatic metabolism, clinicians can gain insight into functional vitamin needs.

## BRANCHED-CHAIN CATABOLITES

### $\alpha$ -Ketoadipic Acid

**$\alpha$ -Ketoadipic Acid (AKAA; 2-Oxoadipic acid, 2-Ketoadipic acid)** is an organic acid formed from  $\alpha$ -aminoadipic acid (which originates with lysine) and also from  $\alpha$ -aminomuconic acid (derived from tryptophan).<sup>136</sup> AKAA metabolizes to form glutaryl-CoA via oxidative decarboxylation. The cofactors needed in this step are Coenzyme A, NAD, thiamine pyrophosphate (vitamin B<sub>1</sub>), lipoic acid, and vitamin B<sub>2</sub>.<sup>189</sup>



#### High Levels:

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

### $\alpha$ -Ketoisovaleric Acid, $\alpha$ -Ketoisocaproic Acid

#### $\alpha$ -Keto- $\beta$ -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  $\alpha$ -ketoacids, which are then released from muscles back into the blood to be further metabolized, mainly in the liver.<sup>137</sup> BCAA act as substrates for protein synthesis, energy production, neurotransmitter production, glucose metabolism, immune response, and many other beneficial metabolic processes.<sup>137</sup>

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

These  $\alpha$ -ketoacids then require an enzyme complex called branched-chain  $\alpha$ -keto acid dehydrogenase (BCKD) for further metabolism.<sup>138</sup> This enzyme complex requires multiple vitamin cofactors, such as vitamin B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, and lipoic acid.<sup>72,139-141</sup>

#### High Levels:

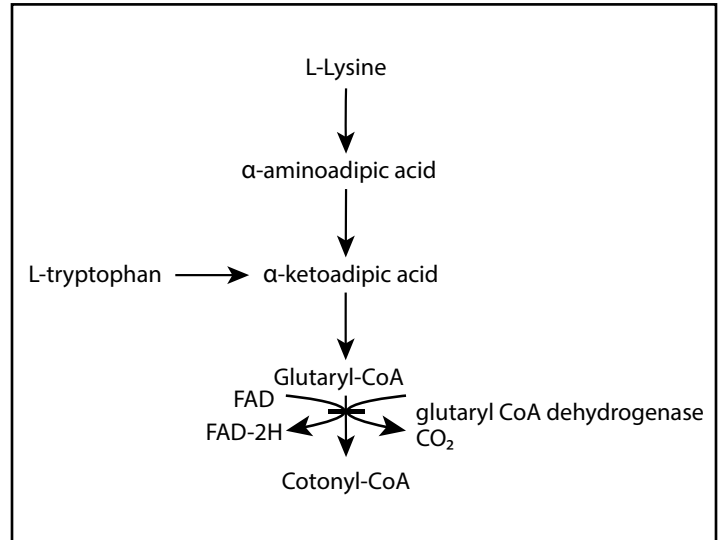
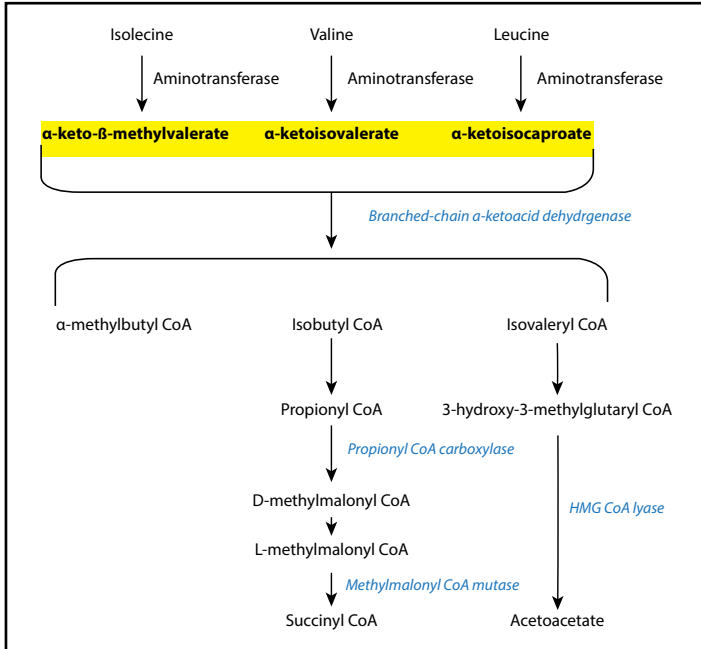
Urinary elevations of these ketoacids can be the result of functional need for the vitamin cofactors to support BCKD.<sup>72,141</sup>

A genetic defect of the  $\alpha$ -keto acid dehydrogenase enzyme complex is responsible for maple syrup urine disease, which results in very elevated levels of AKIC, AKIV, AKMB.<sup>137</sup>

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.<sup>142,143</sup>

## 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<sub>2</sub>) as a cofactor.



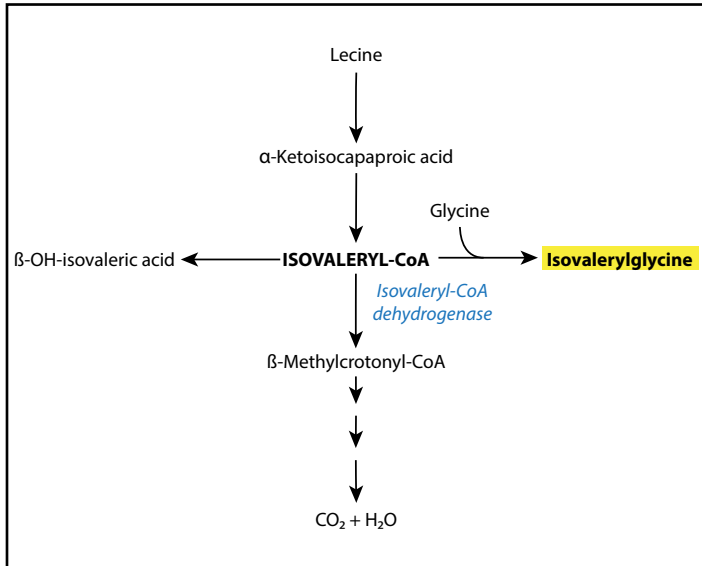
### High Levels:

Elevations of urinary glutaric acid may reflect enzymatic insufficiency requiring vitamin B<sub>2</sub> 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.<sup>144,145</sup> In these cases, riboflavin, carnitine, and CoQ10 have been used therapeutically.<sup>145-147</sup>

## Isovalerylglycine

**Isovalerylglycine** is produced from leucine catabolism. It is further metabolized via isovaleryl-CoA dehydrogenase. This enzyme requires vitamin B<sub>2</sub> as a cofactor.<sup>148,149</sup>



### High Levels:

Acyl-CoA dehydrogenase enzymes are not only involved in branched-chain amino acid metabolism, but also beta-oxidation of fatty acids.<sup>89</sup> 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.<sup>150,151</sup>

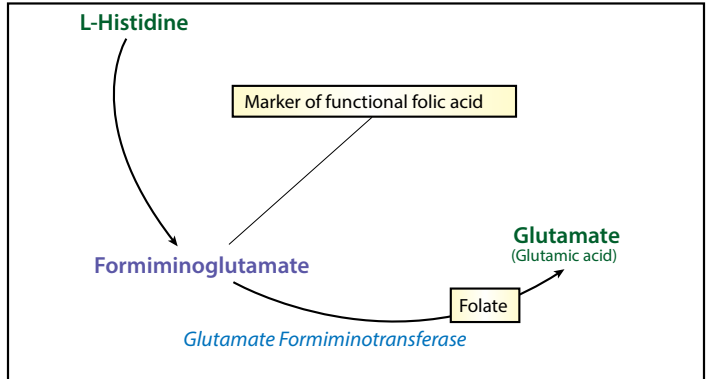
Carnitine, glycine, vitamin B<sub>2</sub>, and antioxidants have been used therapeutically to treat abnormal levels of isovalerylglycine.<sup>152-154</sup>

There is an association between elevated isovalerylglycine and anorexia nervosa. The mechanism is believed to be due to poor thyroid conversion of vitamin B<sub>2</sub> into active FAD, which normalized in some patients after a refeeding program.<sup>155</sup>

## 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.<sup>156</sup>



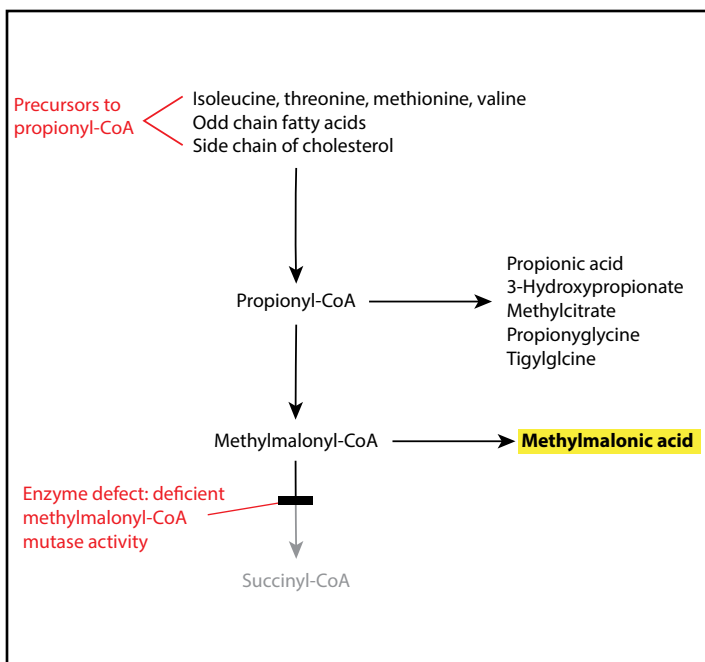
### High Levels:

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

There are multiple clinical associations with elevated urinary FIGlu, including acute and chronic alcohol use, pregnancy, and oral contraceptive use.<sup>159-162</sup>

## 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.<sup>163</sup> 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<sub>2</sub> dependent. In B<sub>12</sub> deficiency, methylmalonyl-CoA is hydrolyzed to methylmalonic acid.<sup>164</sup>



### High Levels:

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

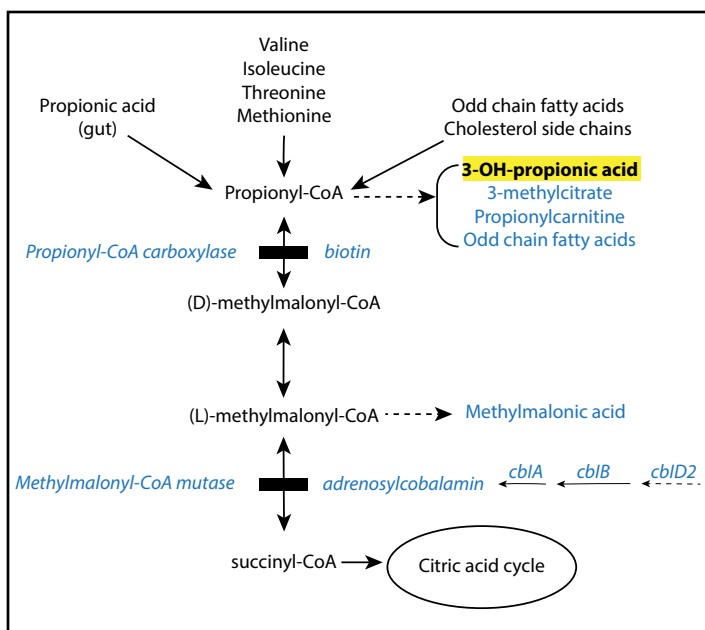
Methylmalonic acid, as a functional biomarker, is considered a more sensitive index of B<sub>12</sub> status when compared to serum B<sub>12</sub>.<sup>165-170</sup> Urinary MMA correlates with serum MMA, making the simple urine test a useful screening tool for B<sub>12</sub> deficiency in at-risk populations,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

Vitamin B<sub>12</sub> therapy lowers MMA. Monitoring this metabolite may help prevent the consequences of B<sub>12</sub> deficiency, such as cognitive decline and neuropathy.<sup>169,172,173</sup>

## 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.<sup>174</sup> 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.<sup>175</sup>

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.<sup>176</sup>

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

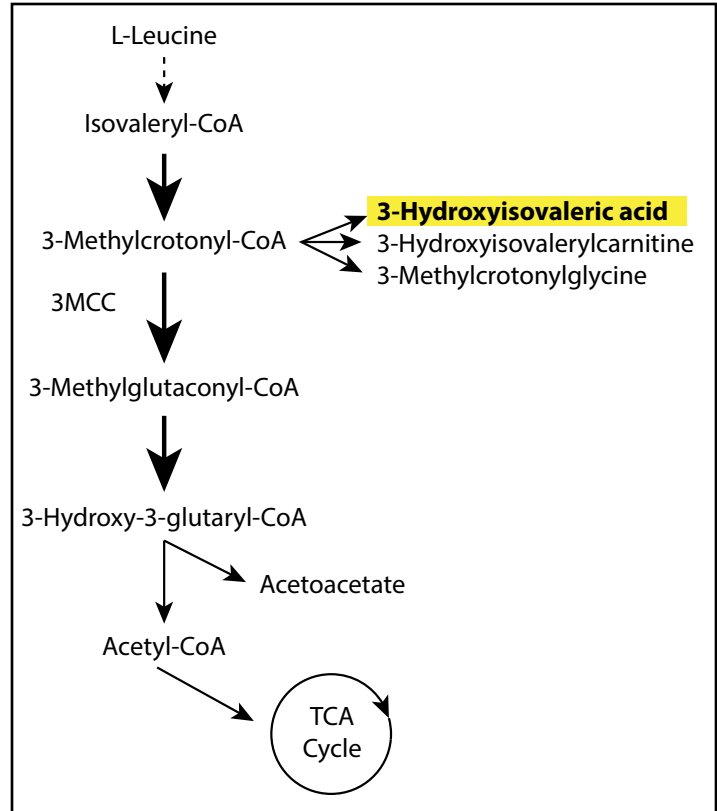


### Low Levels:

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.<sup>178</sup> In fact, low protein diets and antibiotics are used acutely to treat inborn errors of metabolism which cause propionic acidemia.<sup>179</sup>

### 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.<sup>175</sup>

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



These organic acid compounds are down-stream metabolites of neurotransmitter synthesis and degradation.<sup>73</sup> 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.<sup>183,184</sup>

## 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<sup>+</sup>, 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.<sup>185</sup>

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.<sup>186-188</sup> The correlation to levels of urinary excretion needs further study.

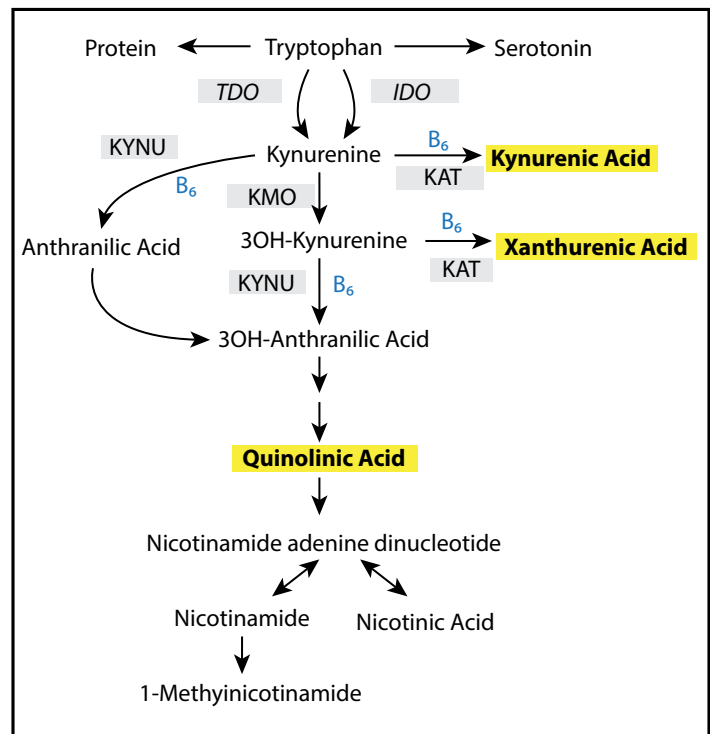
Quinolinic acid, in and of itself, can be inflammatory and neurotoxic.

#### High Levels:

The kynurenine pathway is particularly sensitive to vitamin B<sub>6</sub> deficiency, which can elevate urinary kynurenic acid (and xanthurenic acid).<sup>189-191</sup> Vitamin B<sub>2</sub> is also an important vitamin cofactor in the enzymatic conversion reactions within the pathway.<sup>192</sup> Because a major-end product of this pathway is also NAD<sup>+</sup>, elevations in kynurenic and quinolinic acid may also reflect vitamin B<sub>3</sub> 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<sub>6</sub> insufficiency.<sup>193</sup>

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.<sup>194-198</sup>



### 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.<sup>199,200</sup>

## 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<sub>6</sub>, elevations of xanthurenic acid can reflect a functional need for vitamin B<sub>6</sub>.<sup>201</sup> Kynurenine pathway metabolites may also become elevated when there are needs for vitamin B<sub>3</sub>.<sup>202,203</sup>

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.<sup>5,204</sup>

Abnormalities in the kynurenine pathway have been associated with many clinical conditions including immune suppression, cancer, and inflammatory conditions.<sup>201</sup>

Administration of vitamin B<sub>6</sub> can decrease xanthurenic acid excretion.<sup>205,206</sup>

## 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.<sup>207</sup>

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

Dopamine can be metabolized to homovanillic acid using both monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).<sup>207</sup> MAO requires a vitamin B<sub>2</sub> (FAD) cofactor, while the COMT enzyme requires SAM, magnesium, and vitamin B<sub>6</sub>.<sup>210,211</sup>

### 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.<sup>212</sup> Dietary flavanols, such as tomatoes, onions, and tea are also known to elevate urinary HVA.<sup>213</sup>

Like VMA, urinary HVA is elevated in conditions such as neuroblastoma and neural crest tumors.<sup>214,215</sup> 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.<sup>216-221</sup>

### 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.<sup>222,223</sup>

## Vanilmandelic Acid

**Vanilmandelic acid (VMA)** is formed in the liver by the oxidation of 3-methoxy-4-hydroxyphenylglycol.<sup>224</sup> As a downstream metabolite of tyrosine-derived catecholamines, levels of VMA can reflect the overall synthesis and metabolism of catecholamines.<sup>225</sup> 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.<sup>226</sup>

### High Levels:

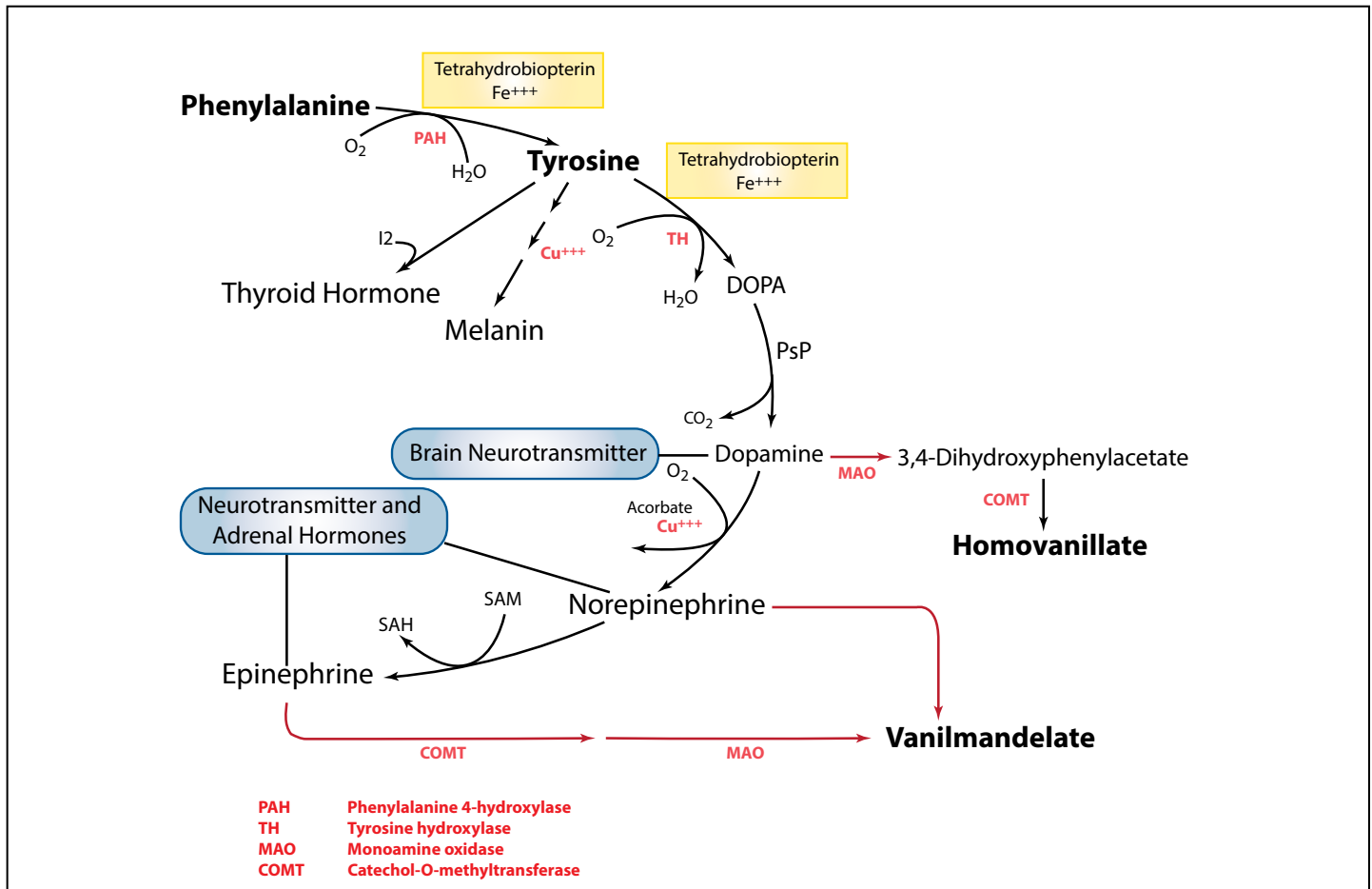
Centrally-acting medications, such as antidepressants and stimulants used for ADHD can elevate overall catecholamines and therefore urinary metabolites.<sup>227,228</sup> Urinary levels have been shown to correlate with generalized anxiety disorder.<sup>229</sup> VMA is sometimes used in the work up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

### 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.<sup>238</sup>

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.<sup>239,240</sup>



### 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.<sup>241</sup>

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

#### 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.<sup>246,247</sup>

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

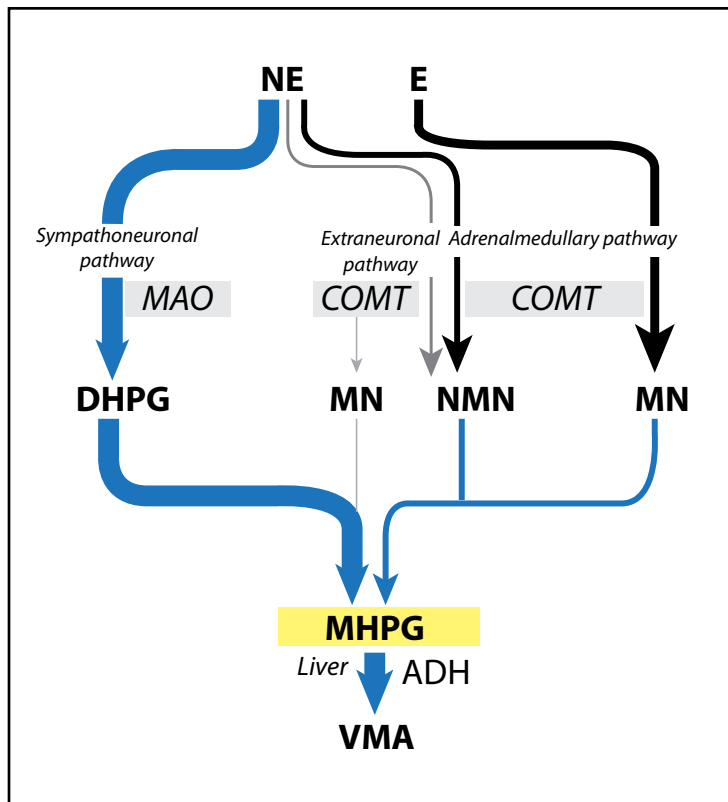
Sleep deprivation can act as a stimulus to the peripheral sympathetic nervous system, which can influence central nervous noradrenergic neurotransmitter levels and elevate MHPG.<sup>251</sup> As a central nervous system metabolite, levels can correlate with central catecholaminergic disturbances, as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

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.<sup>255</sup>

#### 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.<sup>256-258</sup>



## 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.<sup>259</sup>

#### **High Levels:**

Elevations, as well as low levels of urinary 5-HIAA, can reflect underlying intestinal microbial balance.<sup>260</sup> Serotonin produced by intestinal enterochromaffin cells is necessary for GI motility.<sup>261</sup> Because of this, antidepressants such as tricyclics and serotonin selective reuptake inhibitors have been used in treating IBS.<sup>262</sup> 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.<sup>259</sup>

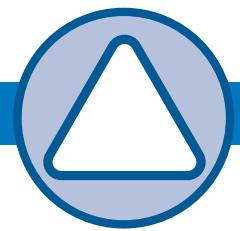
The excretion of 5-HIAA seems to vary among individuals who supplement with 5-hydroxytryptophan (5HTP).<sup>259</sup> Carcinoid tumors are well-differentiated neuroendocrine tumors derived from the enterochromaffin cells in the GI tract and lung. These tumors secrete vasoactive peptides, especially serotonin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

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.<sup>259,264-267</sup> 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.<sup>259,267,268</sup>

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.<sup>269</sup> Low levels of urinary 5-HIAA have been observed in cardiovascular disease, metabolic syndrome, IBS patients, and those with mood disorders and migraines.<sup>270-272</sup>



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/>

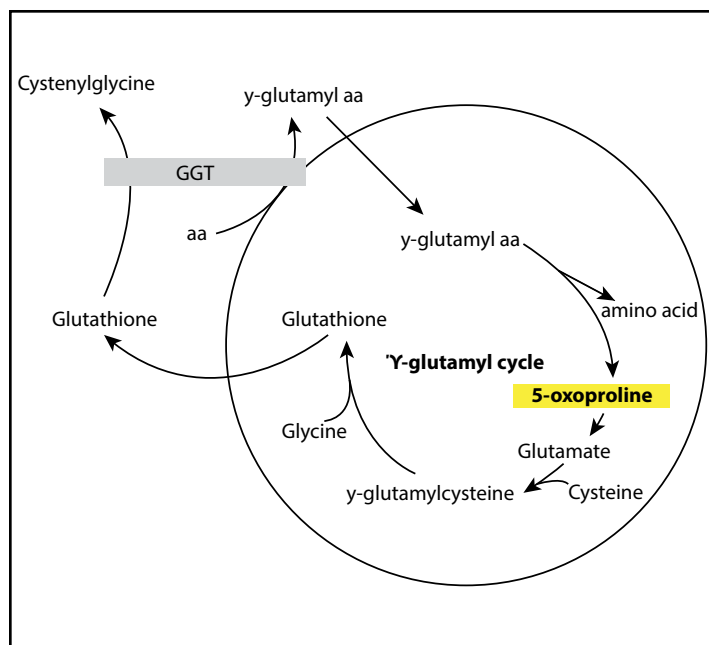
Agency for Toxic Substances and Disease Registry: <https://www.atsdr.cdc.gov/>

## 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.<sup>273,274</sup> This enzymatic reformation requires cofactors such as ATP and magnesium.<sup>275</sup>



## 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.<sup>276,277</sup>

Oxidative stress, in general, can upregulate the detoxification pathways and result in elevated pyroglutamic aciduria.<sup>278,279</sup> 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.<sup>280-282</sup>

Deficiency in glutathione synthetase has also been described in literature as presenting with pyroglutamic aciduria.<sup>283</sup>

## 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.

## **$\alpha$ -Ketophenylacetic acid (from Styrene)**

**$\alpha$ -Ketophenylacetic Acid**, also known as **phenylglyoxylic 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.<sup>284</sup> The biologic half-life of styrene in humans is fairly short and corresponds with the disappearance of PGA from the urine.<sup>285,286</sup>

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.<sup>287</sup> 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.<sup>288</sup>

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

## **$\alpha$ -Hydroxyisobutyric Acid (from MTBE)**

**$\alpha$ -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.<sup>292</sup> Urinary  $\alpha$ -hydroxyisobutyric acid is a marker of recent MTBE exposure.<sup>293,294</sup>

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.<sup>295</sup> There are also clinical associations with autism, DNA oxidative damage, and methylation defects.<sup>296-299</sup> Studies on cancer, reproductive abnormalities, nonalcoholic fatty liver, and neurotoxicity have been either negative or inconclusive thus far.<sup>300-302</sup>



## 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.<sup>303</sup>

It is formed from aspartic acid and carbamoyl phosphate.<sup>304</sup> 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.<sup>305</sup>

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.<sup>306</sup> 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.<sup>303</sup> Hyperammonemia is characteristic of all urea cycle disorders; orotic acid is only elevated in a few.<sup>303</sup>

## 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.<sup>303,305</sup>

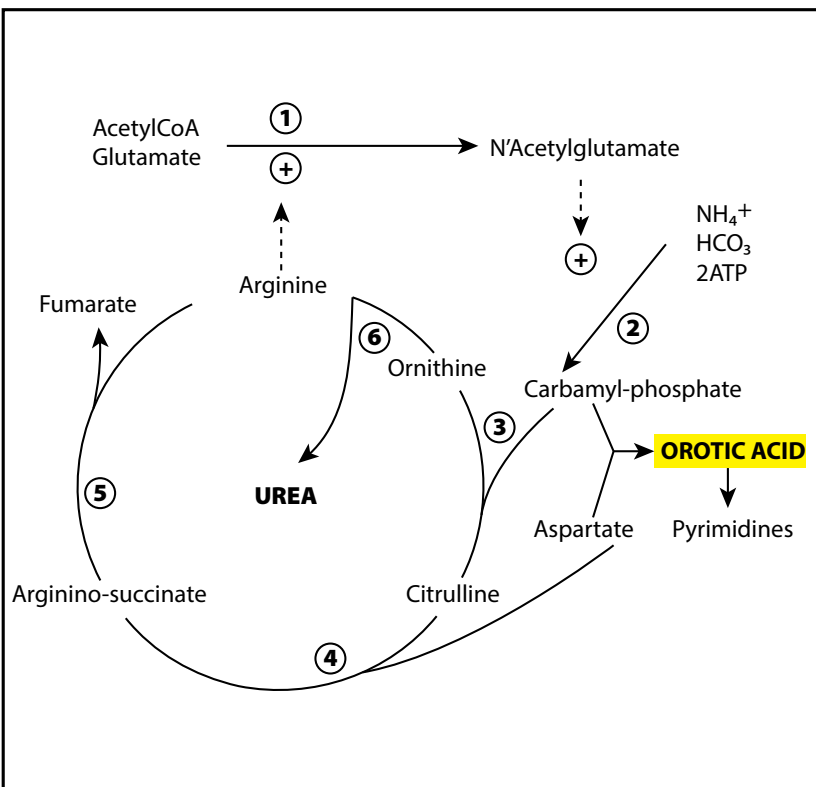
Any hepatotoxin or underlying liver condition can affect ammonia metabolism and increase orotic acid. There are studies that show elevations in orotic acid after drinking alcohol, which then declined with abstinence.<sup>307</sup>

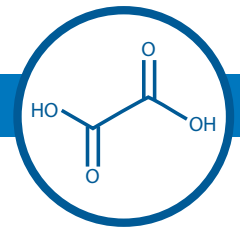
Orotic acid excretion is increased by allopurinol and 6-azauridine seemingly related to action of these drugs on pyrimidine synthesis.<sup>308</sup>

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.<sup>309</sup> Random case studies also show an association between megaloblastic anemia and orotic aciduria as a result of hereditary defects in pyrimidine synthesis.<sup>310</sup>

## Low Levels:

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

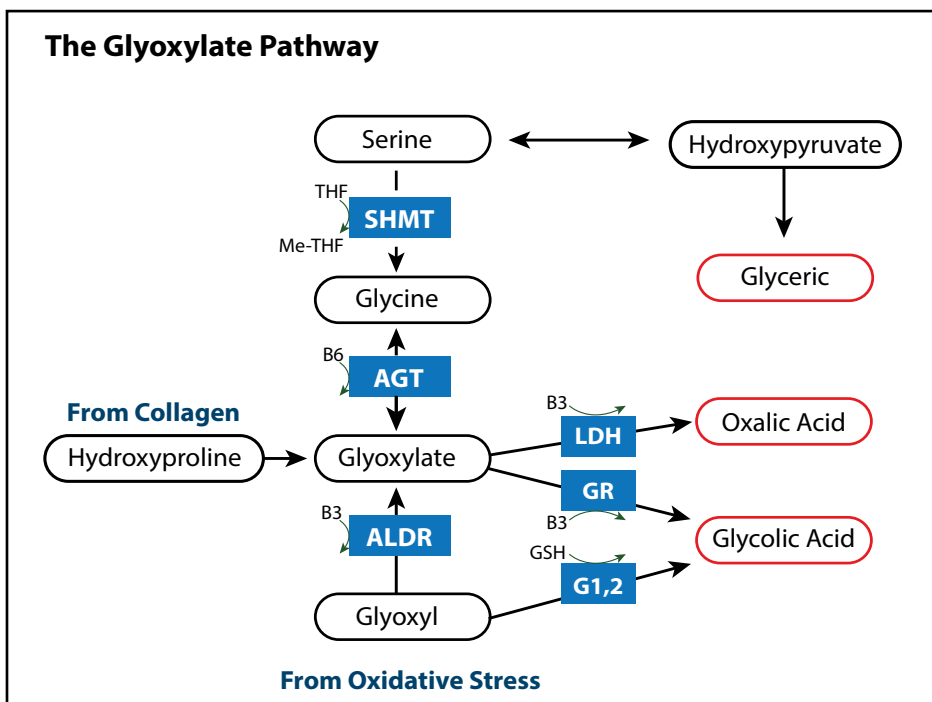




The oxalate markers are a collection of 3 organic acids that are metabolic end-products of the glyoxylate pathway (see diagram). They consist of glyceric acid, glycolic acid, and oxalic acid. As a collection of biomarkers, the oxalate markers may provide insight into abnormal metabolism in the glyoxylate 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.<sup>311</sup> 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.<sup>312</sup>

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.

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.<sup>313</sup> 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.<sup>312</sup>



## Glyceric Acid

**Glyceric acid** is an organic acid that stems from the catabolism of the amino acid serine.<sup>314</sup> 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.<sup>313</sup> While many case studies have linked this disorder with severe developmental abnormalities,<sup>315</sup> there is some debate as to whether glycerate kinase deficiency is the cause or rather a confounding variable.<sup>316</sup>

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.<sup>313</sup>

### 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.<sup>317</sup> 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.<sup>318</sup> 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.<sup>319</sup> This enzyme requires vitamin B<sub>3</sub> 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<sub>3</sub> 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.<sup>320</sup> When this pathway is blocked, due to inborn error, glyoxylic acid ultimately leads to higher production of glycolic acid and oxalic acid.<sup>321</sup>

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.<sup>322</sup>

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.<sup>323</sup> 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.<sup>312</sup> Glyoxal is derived, in part, from oxidative stress in the forms of lipid peroxidation and protein glycation.<sup>324</sup> The majority of this glyoxal is converted into glycolic acid utilizing glutathione as a cofactor.<sup>325</sup>

### 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<sub>6</sub> as a cofactor.<sup>326</sup> The extent to which urinary glycolic acid could be a functional indicator of vitamin B<sub>6</sub> insufficiency has not been studied, however patients with PH1 have shown improvement with B<sub>6</sub> intervention.<sup>327</sup>

Aside from inborn error, higher levels of glycolic acid may be indicative of increased oxidative stress.<sup>312</sup> This is because oxidative stress causes higher levels of glyoxal which is ultimately converted into glycolic acid for excretion utilizing glutathione as a cofactor.<sup>325</sup> 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.<sup>323</sup> 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.<sup>321</sup> 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.<sup>328</sup>

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 foods.

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.<sup>329</sup> 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.<sup>311</sup> 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.<sup>311</sup>

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.<sup>325</sup> With lower glutathione levels, glyoxal is more likely to shunt toward glyoxylate and ultimately could become oxalic acid.<sup>325</sup>

## High Levels

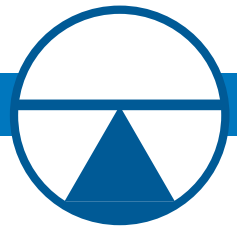
Elevated urinary oxalic acid can be a result of several factors. First, dietary intake of oxalate-rich foods must be considered, especially in the context of dysbiosis and microbiome deficiency. A GI Effects stool test may be warranted to evaluate the concentration of *Oxalobacter formigenes* alongside 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.<sup>323</sup> It is also estimated that 5-20% of urinary oxalic acid excretion stems from collagen turnover in the body.<sup>323</sup>

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,<sup>330</sup> in general the research has shown that vitamin C intake is not associated with urinary oxalic acid or kidney stone risk.<sup>331,332</sup>

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.<sup>333,334</sup> 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.<sup>328</sup> 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<sub>6</sub> as a cofactor (discussed above). Vitamin B<sub>6</sub> therapy has been used in the setting of primary hyperoxaluria with varying degrees of success. Also, intake of vitamin B<sub>6</sub> has been shown to decrease risk of kidney stones in some, but not all, investigations.<sup>331</sup>



**Urinary creatinine** is commonly used as a laboratory standardization when evaluating urinary analytes.<sup>335-337</sup> 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.<sup>338</sup> Hydration status may also play a role in urinary creatinine levels.

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