
# OMX™ – Organic Metabolomics

Quick Reference Guide

## Enhanced Insights into Patient Metabolic Status

OMX™ uses a systems-biology approach and assesses biomarkers that go beyond the traditional lists of metabolites. The test enables practitioners to see a patient's larger health picture by deciphering and connecting perturbations of key metabolic pathways and analytes, allowing for truly personalized therapeutic support.

Diagnostic Solutions laboratory

RESEARCH. TECHNOLOGY. RESULTS.

# What is Metabolomics Testing?

Metabolomics evaluates patterns of metabolites related to core biological systems, offering insight into baseline status and identification of biochemical dysfunctions that could be of concern.

***The test includes organic acids, amino acids, and other small molecules.*** The markers identify levels of substrates and flow of pathways, which together help establish functional status in 6 key areas of health. These areas are **Metabolic Processing, Amino Acid and Protein Metabolism**, **Nutrition**, **Stress & Mood**, **Toxic Impacts**, and **Microbial Metabolism**.

Metabolites are impacted by many factors such as diet, nutrient status, genetics, gut microbiome, exercise, physiologic demands, immune reactions, state of disease, etc.

## How Can OMX Help to Translate Metabolomics Research into Clinical Care?

Metabolomics research has generally compared sick or disease populations to healthy controls, and identified the analytes, pathways, or categories that were most impacted. Emerging research is beginning to characterize core metabolic signatures and increase the understanding of biochemical relationships within different conditions or diseases. The OMX metabolomics test allows clinicians to identify markers or pathways that research has identified as most associated with specific disease or core impairments.

*"Through the measure of thousands of small-molecule metabolites in diverse biological systems, metabolomics now offers the potential for new insights into the factors that contribute to complex human diseases such as cardiovascular disease. Targeted metabolomics methods have already identified new molecular markers and metabolomic signatures of cardiovascular disease risk."*

— S. Cheng, Scientific Statement From the American Heart Association (2017)

# Contents



# 1 – Metabolic Processing

The breakdown products of macronutrients are funneled into the Krebs cycle.

## Glycolysis

The final product of glycolysis, the breakdown of glucose, is pyruvic acid in aerobic settings and lactic acid in anaerobic conditions. Carbohydrates – via glycolysis → glucose → pyruvic acid → acetyl-CoA.

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| **Glucose** | • Glucose identifies processing of overall diet. Small amounts of glucose may be found in the urine of healthy individuals.<br>» Elevated urinary glucose levels should be investigated further.<br>• Researchers found that those with a high waist-to-hip ratio (WHR), but no history of diabetes, had significantly lower urine glucose excretion.<br>• Metabolism of glucose – glycolysis – is heavily dependent on magnesium. |
| **Pyruvic Acid** (Pyruvate) | • Increased pyruvic acid has been associated with increased glucose uptake.<br>• Increased pyruvic acid may indicate an insufficiency of nutrient cofactors (B1, B2, B3, B5, Lipoic Acid), resulting in upstream accumulation.<br>• Impaired pyruvate enzymes are associated with metabolic conditions, such as insulin resistance, obesity, and type 2 diabetes mellitus.<br>• Pyruvic acid may be increased for 2–4 hours after continuous or high-intensity interval exercise.<br>• When amino acids are used for energy in the muscle, pyruvic acid is used as an amino group acceptor (via alanine transaminase) and becomes alanine, which goes to the liver where pyruvic acid is turned into glucose (gluconeogenesis), and the nitrogen enters the urea cycle. |
| **Lactic Acid** (Lactate) | • Lactic acid is produced endogenously under anaerobic conditions.<br>• Main route of lactic acid disposal is conversion to pyruvic acid or excretion via urine.<br>• Higher urine lactic acid levels have been associated with diabetes, fasting glucose, HOMA-IR, IBD, chronic kidney disease, Fanconi syndrome, and age-related macular degeneration.<br>» Both L- and D-lactic acids were elevated in diabetes<br>• Nutrient deficiencies of B1, CoQ10, and/or lipoic acid, have been associated with elevated lactic acid levels in both urine and blood.<br>• Limited research noting a higher decline of T4 was associated with a low lactic acid, alanine and glycine. |
| **D-lactic Acid** | • Only elevated is of concern. D-lactic acid is generally produced in minimal quantities by human cells. It comes from three sources, 1. from human methylglyoxal (MGO) pathway (assumed to be the sole source of blood D-lactate in healthy people), 2. production by gut bacteria (mostly in patients with short bowel syndrome (SBS)), and 3. ingestion of preformed D-lactate.<br>• The source of D-lactic acid is dependent on the situation. MGO is a precursor of glycation of proteins and DNA, resulting in advanced glycation end products (AGEs), which is associated with increased oxidative stress. MGO is predominantly detoxified by the glyoxalase system (requires glutathione), with the majority going to D-lactate. |

## Glycolysis – *continued from previous page...*

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| **Alanine** | • In a review of 46 studies higher plasma alanine was a potential predictor of insulin resistance and diabetes.<br>• In a review of baseline urine markers and conventional metabolic assessments, with a 5-year follow up, elevated baseline urine alanine was found to correlate with elevated levels of A1C (effect size >.8) and insulin resistance, independent of weight.<br>• Plasma alanine and asparagine were reduced in B6 deficiencies in animal studies.<br>• Plasma alanine and glutamic acid both positively correlated with depression.<br>• Branched-chain amino acids (BCAA) are the primary nitrogen source for glutamine and alanine synthesis; BCAA are associated with metabolic disease.<br>• Blood alanine was lower in IBD compared to controls. |

## Krebs Cycle

Carbohydrates (from glycolysis), fats (from beta-oxidation,) and protein (via amino acids) all enter the Krebs cycle to generate energy (ATP). Level of protein intake can impact overall flow of the cycle.

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| **Citric Acid** (Citrate) | • Diet has a significant impact on citric acid levels:<br>» Increased acid load due to diets high in animal-based proteins, carbonated drinks, and in severe carbohydrate restriction can lead to mild metabolic acidosis, hypercalciuria, and reduced citric-acid excretion.<br>» Plant-based diets are associated with increased citric acid. Alkalinization of urine through consumption of citrus foods, alkaline mineral water, fruits and vegetables, or citrate supplements (such as mag-citrate) increase citric acid levels.<br>• Low urine citric acid has been associated with insulin resistance, metabolic acidosis, bone-density, hypokalemia, the development of kidney stones, kidney disease, and chronic kidney disease, and immune-mediated inflammatory diseases, including rheumatoid arthritis, psoriasis, psoriatic arthritis, systemic lupus erythematosus, Crohn's disease, and ulcerative colitis. |
| ***cis*-Aconitic Acid**<br>**Isocitric Acid** | • The aconitase enzyme is sensitive to inflammation, and cis-aconitic acid and isocitric acid may be elevated in inflammation.<br>• *cis*-aconitic acid, along with ketones and suberic acid, were elevated in schizophrenia patients, compared to controls.<br>• In chronic kidney disease, urinary excretion of cis-aconitic acid and isocitric acid were reduced by 40–60%. |
| **α-Ketoglutaric Acid** (Alpha-Ketoglutarate or AKG) | • Check levels and intake of amino acids that flow into the Krebs cycle at AKG: arginine, histidine, and glutamine.<br>• Insufficient B-complex vitamins, lipoic acid, and magnesium can lead to elevations of α-ketoglutaric acid.<br>• Higher α-ketoglutaric acid levels are seen in B1 deficiency; *α-ketoglutarate dehydrogenase,* as well as *pyruvate* and *branched-chain ketoacid dehydrogenases* may all be impacted.<br>• Reduced in chronic kidney disease by 40–68%. |

## Krebs Cycle – *continued from previous page...*

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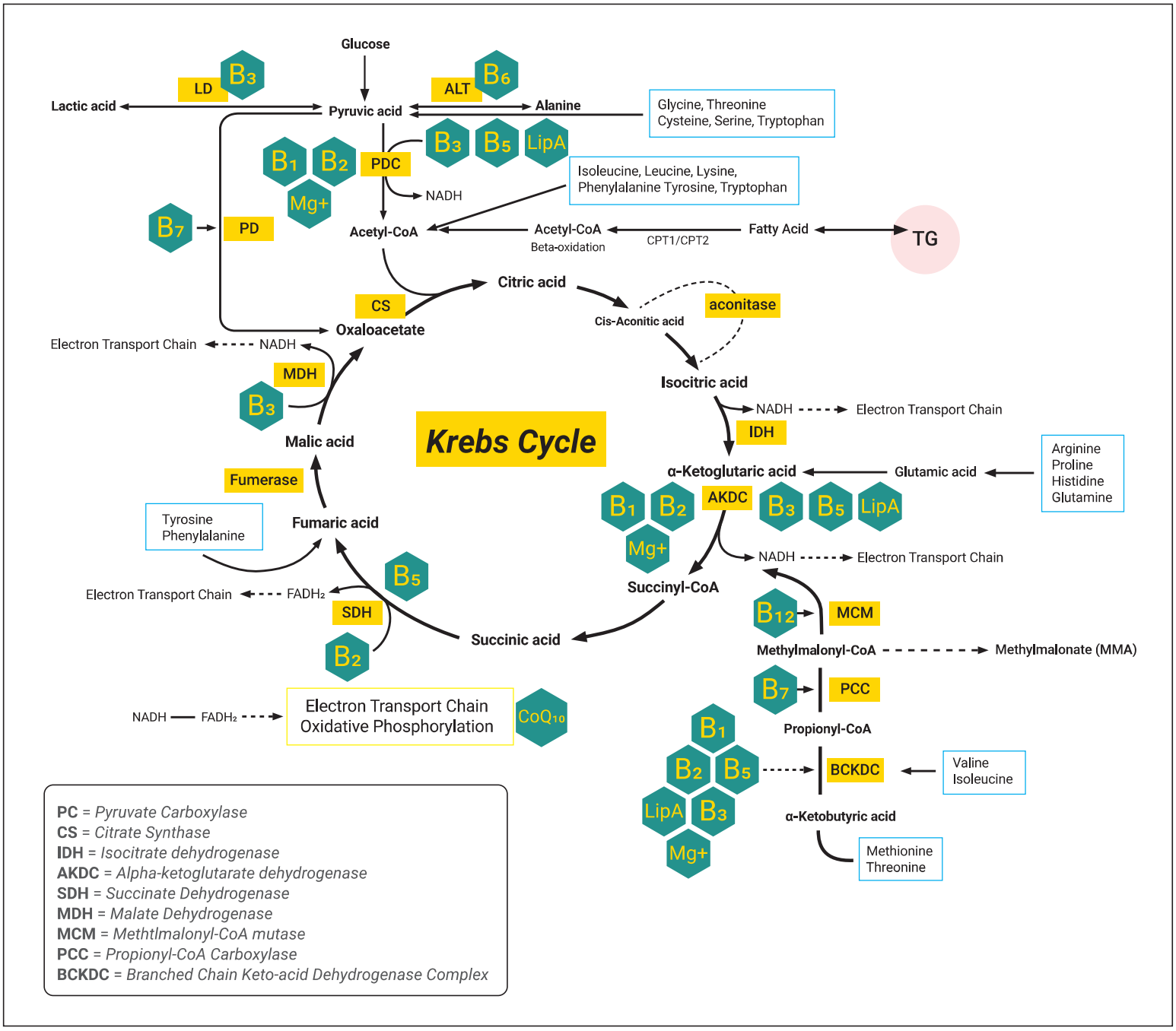
| **Succinic Acid** (Succinate) | • Check levels and intake of amino acids that flow into the Krebs cycle at succinyl CoA: valine, isoleucine, methionine, and threonine.<br>• Insufficient nutrients B2, B6, CoQ10, and magnesium could lead to elevations of succinic acid; antioxidants may lower levels<br>• In a review of irritable bowel disease, both ulcerative colitis and Crohn's disease patients had low urine succinic acid.<br>• In chronic kidney disease, succinic acid was reduced by 40–68%. |
| **Fumaric Acid** (Fumarate) | • Check levels and intake of amino acids that flow into the Krebs cycle: tyrosine and phenylalanine.<br>• Evaluate B-complex status.<br>• In chronic kidney disease in those with type 2 diabetes, higher urinary fumaric acid and malic acid predicted progression of disease. |
| **Malic Acid** (Malate) | • Evaluate dietary sources of malic acid; It is the primary acid in apricot, black and blue berries, cherries, grapes, peaches, pears, and plums and some wines contain malic acid.<br>• Evaluate B-complex status.<br>• In chronic kidney disease in those with type 2 diabetes, higher urinary fumaric acid and malic acid predicted progression of disease. |

## Figure 1 - Krebs Cycle

*The Krebs cycle (also known as the citric acid cycle or the tricarboxylic acid cycle) is the main source of energy for cells.*




**PC** = *Pyruvate Carboxylase*
**CS** = *Citrate Synthase*
**IDH** = *Isocitrate dehydrogenase*
**AKDC** = *Alpha-ketoglutarate dehydrogenase*
**SDH** = *Succinate Dehydrogenase*
**MDH** = *Malate Dehydrogenase*
**MCM** = *Methtlmalonyl-CoA mutase*
**PCC** = *Propionyl-CoA Carboxylase*
**BCKDC** = *Branched Chain Keto-acid Dehydrogenase Complex*

## Fatty Acids and Beta-oxidation

Fatty acids undergo beta-oxidation via *acyl-CoA dehydrogenase* to enter the Krebs cycle to be used as energy.

B2 is a cofactor. Fat breakdown – via beta-oxidation → fatty acids → acetyl-CoA.

Increased percent fat intake can increase fatty acid markers. Carnitine is needed for LCFA to enter the cell: Low levels of methionine or lysine, a vitamin C deficiency or kidney dialysis, can lead to carnitine shortages.

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| **Adipic Acid (C6)**<br>**Pimelic Acid (C7)**<br>**Suberic Acid (C8)**<br>**Sebacic Acid (C10)**<br><br>Dicarboxylic Acids | • Hepatic ω-oxidation is generally a minor fatty acid pathway, though it can increase in diabetes, starvation, genetic fatty acid oxidation disorders, chronic alcohol consumption, and consumption of medium-chain triglycerides.<br>• People with metabolic syndrome or diabetes had significantly elevated adipic acid, suberic acid, lactic acid, and fumaric acid.<br>• Ketosis is sometimes accompanied by excessive excretion of adipic and suberic acid.<br>• Lower C7 (pimelic acid) and C8 (suberic acid), and higher C4 (succinic acid) and C5 (glutaric acid) were associated with more efficient energy balance and better oxidative stress handling in Alzheimer's disease research.<br>» Suberic acid was elevated in schizophrenia patients.<br>• Acyl-CoA dehydrogenation enzymes are dependent on riboflavin (B2).<br>• Evaluate for high-adipate foods: beets, sugar cane, baked items, jams, jellies. |
| **Hexanoylglycine (C6)**<br>**Suberylglycine (C10)**<br>**3-Phenylpropionylglycine (C11)**<br><br>Medium-Chain Acylglycines | • Hexanoylglycine (C6), suberylglycine (C10,) and 3-phenylpropionylglycine (C11) are generally minor metabolites of fatty acid metabolism.<br>• Medium-chain acyl-CoA dehydrogenase deficiency (MCAD) is the most common fatty-acid beta-oxidation disorder, with an elevation in hexanoyl glycine, proprionoglycine, and suberylglycine, occurring in 1:20,000 in the Northern European Caucasian population.<br>» MCT supplementation can increase levels<br>• Acyl-CoA dehydrogenation enzymes are dependent on riboflavin (B2); consider supplementation of B2, as well as B6, magnesium, and carnitine.<br>» Increased Glutaric acid is associated with secondary carnitine deficiency |
| **Ethylmalonic Acid (C5)**<br>**Methylsuccinic Acid (C5)**<br><br>Branched-Chain, Dicarboxylic Acids | • A deficiency of short-chain acyl-CoA dehydrogenase (SCAD) can result in increased excretion of ethylmalonic acid, methylsuccinic acid, or butyric acid. SCAD deficiency is often asymptomatic, except in times of stress. If treatment is indicated, a diet high in carbohydrates and low in fat, (primarily low in medium-chain triglycerides); carnitine supplementation may be considered.<br>• Women with gestational diabetes had higher levels of pyruvic and ethylmalonic acids (and lower levels of adipic acid).<br>• Acyl-CoA dehydrogenation enzymes are dependent on riboflavin. Consider supplementation of B2, as well as B6, magnesium, and carnitine. Riboflavin (B2) has been shown to be effective for decreasing ethylmalonic acid. |

## Ketones

The presence of ketones implies the body is using fat for fuel, commonly seen during fasting, a high-fat or ketogenic diet, or prolonged exercise. Ketones are produced in the liver from fatty acid oxidation from adipose tissue.

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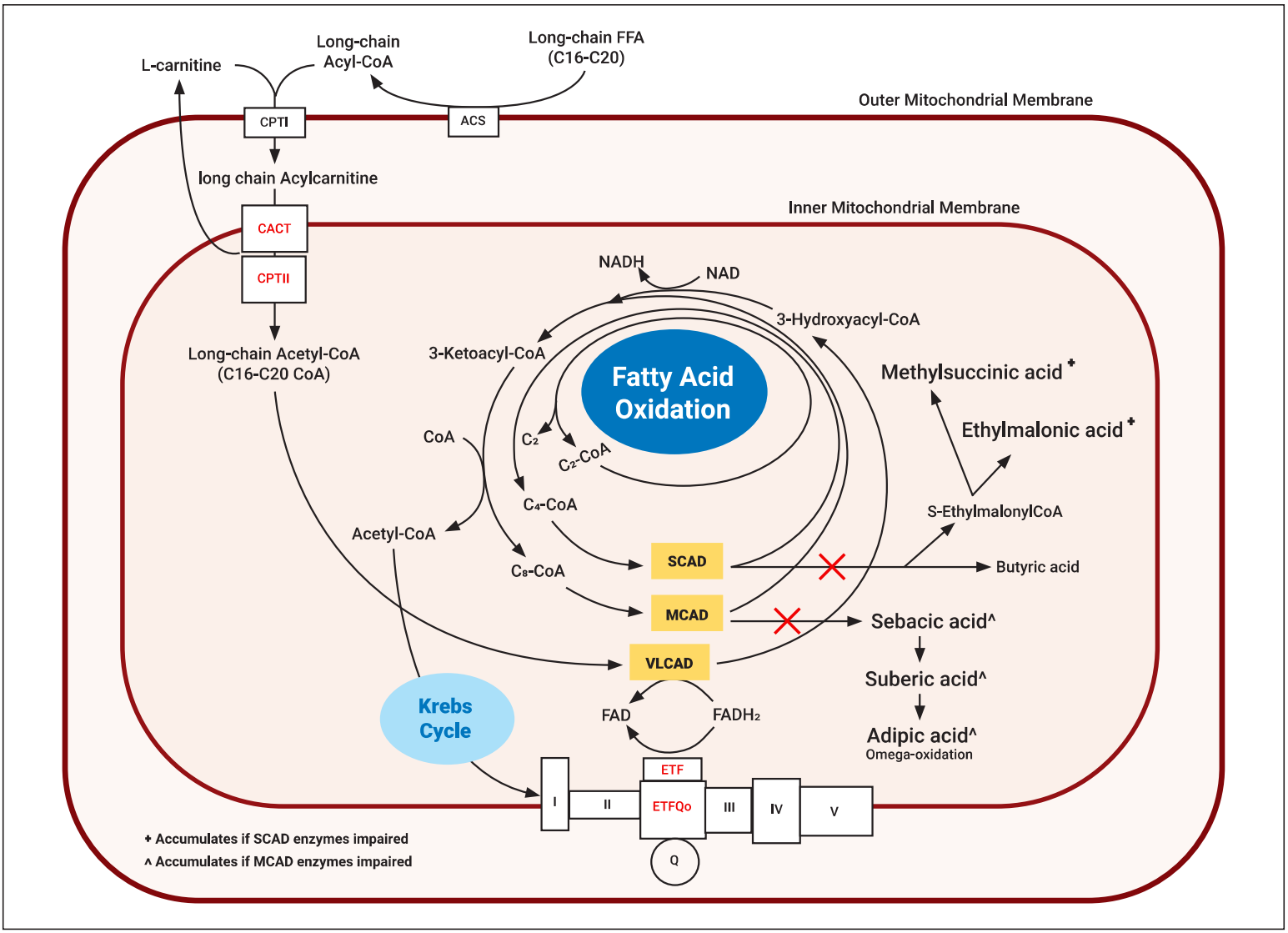
| **β-Hydroxybutyric Acid**<br>(3-Hydroxybutyrate)<br>(β-Hydroxybutyrate) | • β-hydroxybutyric acid is a ketone and a byproduct of fatty acid metabolism and makes up ~70% of ketones produced in liver mitochondria.<br>• Non-diet causes include diabetes, corticosteroid or growth hormone deficiency, excess alcohol or salicylates, and several inborn errors.<br>• In a study of premenopausal women, urine ketones reflected serum ketones.<br>• B3 is a nutrient cofactor for *beta-hydroxybutyrate dehydrogenase*.<br>• Blood levels of β-hydroxybutyric acid in a well-designed ketogenic diet are generally < 5.0 mmol/L (depending on degree of keto-adaptation) vs. a level of 15–25 mmol/L in pathologic states of ketosis. |

## Figure 2 - Fatty Acid Oxidation

*Fatty acid oxidation breaks down fatty acids from the diet or adipose tissue 2 carbons at a time to provide acetyl-CoA for the Krebs cycle, or is synthesized to ketones when fasting. Omega oxidation is an alternative pathway for medium-chain fatty acids.*



# 2 – Amino Acid & Protein Metabolism

Plasma amino acids are the preferred specimen, representing intake over the last 1–2 weeks and a general steady state. Urine primarily represents intake over the last 24–48 hours. Higher levels are noted with higher protein/calorie intake, or impaired breakdown. Decreased levels seen with decreased intake, increased uptake, body losses (urine, sweat, etc.), or can be related to insulin and glucagon activity. Proteins – via catabolism → amino acids → pyruvic acid or acetyl-CoA or alpha-ketoglutaric acid or succinyl-CoA or fumaric acid.

## Phenylalanine Metabolism

Produces catecholamines, thyroid hormones, melatonin.

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| **Phenylalanine**<br>Final products include: DOPA, dopamine, norepinephrine, epinephrine, thyroid hormones, melanin, in TCA cycle, or 4-hydroxyphenylacetic acid. | • Low protein or calorie intake, or inflammation can lead to lower levels. A higher protein intake or supplementation results in higher levels.<br>• Nutrient cofactors of phenylalanine catabolism include tetrahydrobiopterin (BH4), non-heme iron, vitamins B6 & B3, copper, niacin, vitamin C, magnesium, SAMe.<br>• Elevated levels of phenylalanine compete with other large neutral amino acids.<br>• Higher phenylalanine levels have been found in type 2 diabetes, and with increased BMI, schizophrenia, and rheumatoid arthritis.<br>• Lower plasma phenylalanine levels seen in depression and associated with lower neurotransmitter levels.<br>• Inflammation can impair the function of *phenylalanine hydroxylase,* leading to elevated phenylalanine levels and reduced tyrosine levels. In animal studies cortisol increased the activity of *phenylalanine hydroxylase.*<br>• Elevated phenylalanine in PKU has been shown to inhibit *HMG-CoA reductase* and can result in lower CoQ10 and cholesterol. |
| **Phenylacetic Acid**<br>(Phenylacetate) | • Phenylacetic acid is a metabolite of excess phenylalanine.<br>• Phenylacetic acid may also be of bacterial origin, and has been positively associated with Crohn's disease, and negatively with *F. prausnitzii.*<br>• Phenylethylamine from food is quickly metabolized into phenylacetic acid by *monoamine oxidase* to prevent significant concentrations from reaching the brain. Individuals with an impaired conversion may have symptoms of headache if supplementing with phenylalanine.<br>• High levels of phenylacetic acid have been found in nephritis, hepatitis, and phenylketonuria (PKU).<br>• Lower levels of phenylacetic acid found in depression and in children with obesity. |
| **Tyrosine**<br>Produced from phenylalanine via *phenylalanine hydroxylase* + BH4 | • A higher protein intake or supplementation results in increased levels. Low protein intake or inflammation can lead to lower levels.<br>• Nutrient cofactors of tyrosine pathways include BH4, non-heme iron, vitamins B6 & B3, copper, niacin, vitamin C, magnesium, SAMe.<br>• Elevated tyrosine associated with a higher risk of type 2 diabetes and gestational diabetes, and higher BMI.<br>• Tyrosine-supplementation effects on cognition vary: unfavorable effects were noted on working-memory performance in older adults. Higher tyrosine was related to better cognitive skills in younger adults.<br>• Urine and blood tyrosine were noted to be lower in depression. |

## Phenylalanine Metabolism – *continued from previous page...*

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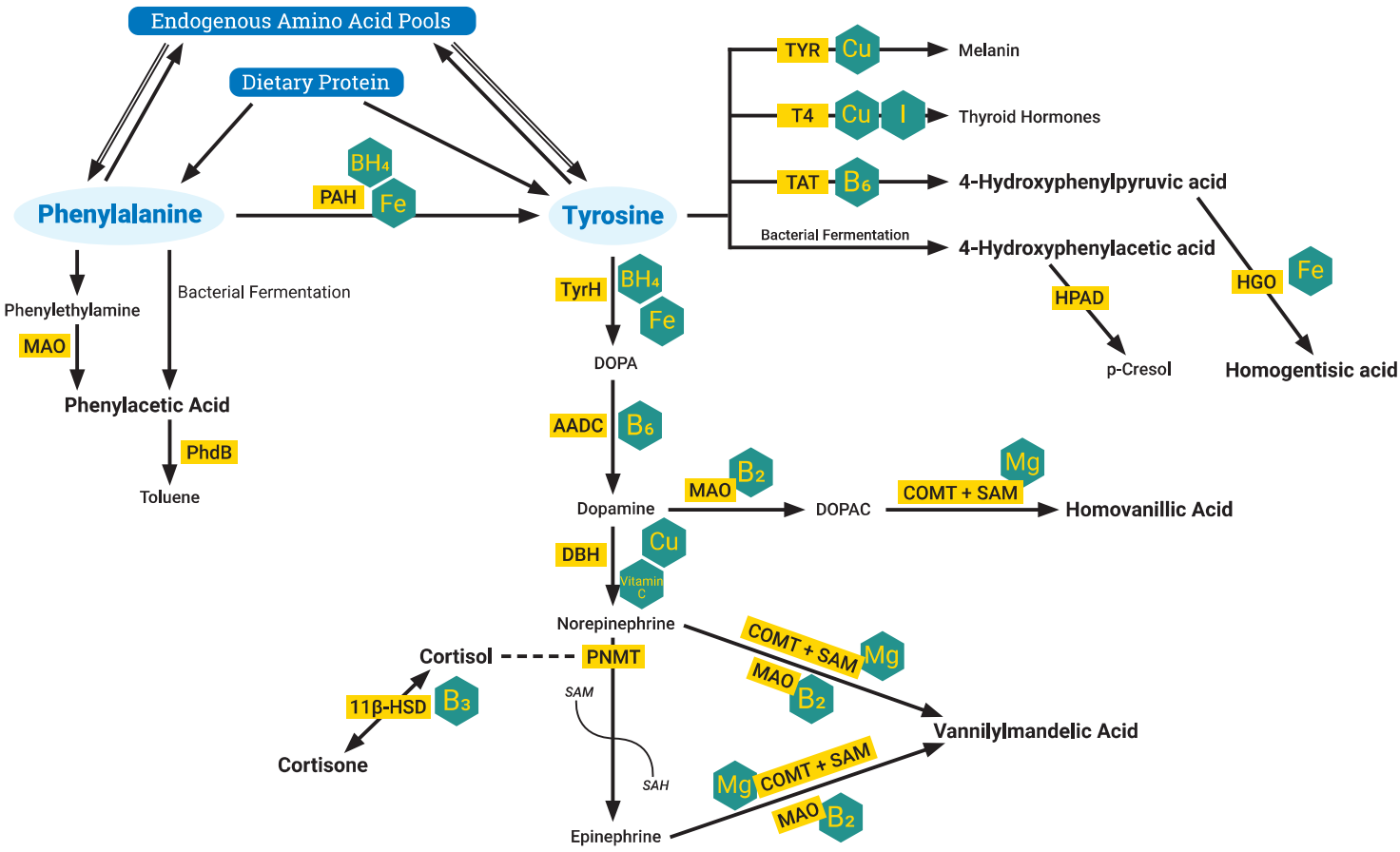
| **Homovanillic Acid**<br>**HVA** (Homovanillate) | • The major catabolic product of dopamine is homovanillic acid.<br>• Elevated homovanillic acid has been reported in children with autism, obstructive sleep disorder, neuroblastomas, with a higher intake of polyphenols and polysaccharides, high olive intake, and vitiligo disease.<br>• Lower homovanillic acid has been associated with early glomerular lesions in diabetic kidney disease, and more favorable outcomes in ischemic stroke/transitory ischemic attacks, and in cancer patients. Higher polychlorinated biphenyl (PCB) exposure is associated with lower HVA and increased severity of depressive symptoms. |
| **Vanillylmandelic Acid**<br>**VMA** (Vanillylmandelate) | • VMA is the major urinary excretion product of catecholamines, epinephrine, and norepinephrine.<br>• Extremely elevated levels are seen in neuroblastoma, pheochromocytoma, and some brain tumors.<br>• Moderate increases have been noted in severe anxiety and stress, olive intake, and vitiligo disease activity (from release of catecholamines from autonomic nerve endings of melanocytes).<br>• Lower VMA reported in ischemic stroke and transitory ischemic attacks, associating lower VMA and HVA with more favorable outcomes.<br>• Higher polychlorinated biphenyl (PCB) exposure associated with lower VMA and increased severity of depressive symptoms.<br>• Lower stress and anxiety associated with lower VMA and 5-HIA. |
| **4-Hydroxyphenylpyruvic Acid**<br>(4-Hydroxyphenylpyruvate)<br>(4-HPPA) | • 4-hydroxyphenylpyruvic acid is an intermediate in the breakdown of phenylalanine.<br>• 4-hydroxyphenylpyruvic acid is converted to homogentisate; a blockage at this step results in increased homogentisate, which can be diagnostic of alkaptonuria. If the pathway is not blocked, 4-HPPA ends up in the Krebs cycle converted into fumaric acid. |
| **Homogentisic Acid**<br>(Homogentisate) | • Homogentisic acid when oxidized becomes alkapton. Alkaptonuria is a rare disorder of tyrosine metabolism. Research has established normal levels of a healthy population, which may give insight into pathway dysfunction.<br>• Homogentisic acid can be an osteo-toxin and a renal toxin.<br>• Diets low in phenylalanine and tyrosine have been advocated in combination with ascorbic acid to decrease urinary homogentisic acid. |

## Figure 3 - Phenylalanine Pathway

*Phenylalanine is an essential, aromatic amino acid, and a precursor to tyrosine, catecholamines, tyramine, and thyroid hormones. Catecholamines include dopamine, norepinephrine, and epinephrine. Gut bacteria can also impact its breakdown. Complete metabolism of phenylalanine requires several nutrient cofactors including, tetrahydrobiopterin (BH4), iron, niacin, vitamin B6, copper, and vitamin C. The carbon skeleton of degraded tyrosine can enter the Krebs cycle at fumaric acid.*




**11β-HSD** = *11β-Hydroxysteroid dehydrogenase*
**AADC** = *Aromatic L-amino acid decarboxylase*
**COMT** = *Catechol-O-methyltransferase*
**DBH** = *Dopamine beta-hydroxylase*
**HGO** = *Homogentisate 1,2-dioxygenase*
**HPAD** = *p-Hydroxyphenylacetate Decarboxylase*
**MAO** = *MonoAmine Oxidases*
**T4** = *Thyroxine*
**TAT** = *Tyrosine Aminotransferase*
**TyrH** = *Tyrosine Hydroxylase*
**TYR** = *Tyrosinase*
**PAH** = *Phenylalanine Hydroxylase*
**PhdB** = *Phenylacetate Decarboxylase*
**PNMT** = *Phenylethanolamine N-methyltransferase*

## Branched-Chain Amino Acid Metabolism (BCAA)

BCAA's are nitrogen donors, facilitate glucose uptake by liver and skeletal muscle, and enhance glycogen synthesis.

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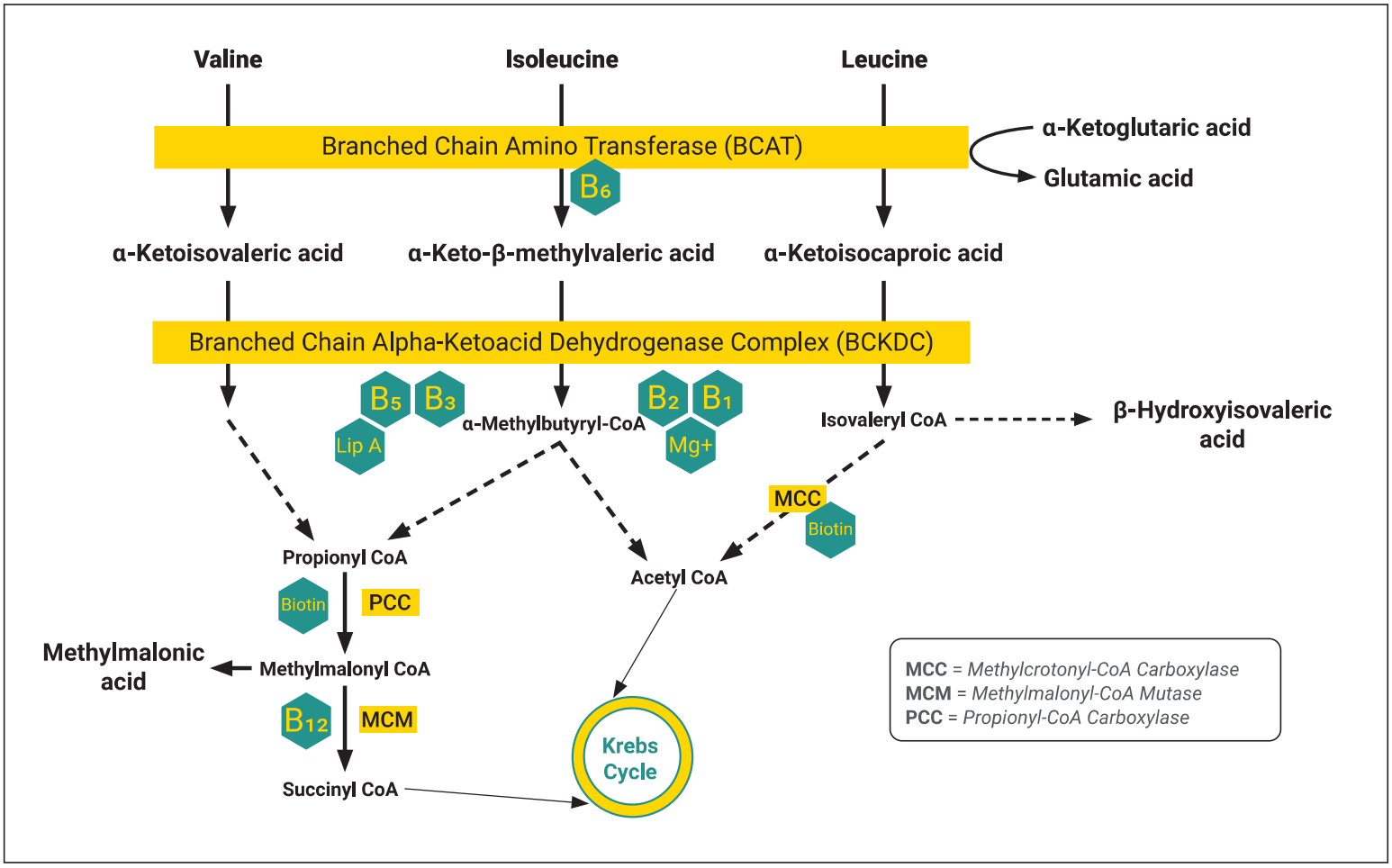
| **Total BCAA**<br>*Branched-chain amino acid transferase* (BCAT) + B6 | • BCAAs are key nitrogen donors in the form of glutamic acid, glutamine, and alanine.<br>• Elevated total BCAAs have been associated with obesity, weight loss, insulin resistance, and NAFLD. Elevated plasma BCAAs were associated with an increased risk of hypertension, cardiovascular disease.<br>• BCAAs are higher in a "Western" diet. Check B6 need.<br>• Lower levels seen in liver cirrhosis and urea cycle disorders. Decreased amino acids are seen with decreased protein and calorie intake; increased tissue uptake, and body losses (urine, sweat, etc.). |
| **BCAA: Valine** | • BMI was positively associated with urine 2-hydroxyisobutyrate, isoleucine, valine, tryptophan, and tyrosine.<br>• Plasma valine, lysine, and tyrosine positively associated with gestational diabetes mellitus and insulin activity.<br>• Elevated urine levels have been associated in higher colorectal cancer. |
| **BCAA: Isoleucine** | • BMI was positively associated with urine 2-hydroxyisobutyrate, isoleucine, valine, tryptophan, and tyrosine.<br>• Elevated urine levels were associated with higher colorectal cancer. |
| **BCAA: Leucine**<br>• Activator of mTOR<br>• 3-hydroxymethylglutaric acid (HMG) is an "off-product" intermediate in leucine degradation. | • Has an anabolic effect on cell signaling and protein synthesis; an activator of the mammalian target of rapamycin (mTOR).<br>• Elevated urine levels have been associated with higher colorectal cancer rates and a possible biomarker of rheumatoid arthritis (along with phenylalanine).<br>• Leucine supplementation has been shown to increase plasma ammonia concentrations. |
| **Alpha-Ketoisovaleric Acid**<br>**Alpha-Keto-Beta Methylvaleric Acid**<br>**Alpha-Ketoisocaproic Acid**<br>Branched-Chain Keto Acids (BCKA) | • Each of the BCAA is catabolized by a dehydrogenase enzyme forming branched-chain keto acids (BDKA), or 2-oxo acids. The dehydrogenase enzyme is heavily dependent on B-complex vitamins, the lack of which may decrease pathway function, leading to an elevation of the BCKA.<br>• Early research found a vitamin B1 (thiamin)-responsive form of MSUD.<br>• Higher urinary BCKA were found to decrease with B-complex vitamins supplementation. Evaluate intake of B-complex, primarily thiamin (B1).<br>• Evaluate dietary intake or supplementation with branched-chain amino acids. |

## Figure 4 - Branched-Chain Amino Acid Pathway

*Branched-Chain Amino Acids (BCAAs) are required for protein synthesis and are metabolized outside hepatic tissues, unlike most other essential amino acids. They are converted to branched-chain keto acids which require B-complex vitamins. BCAAs have been associated with obesity, weight loss, insulin resistance, and nonalcoholic fatty liver disease (NAFLD). Leucine is an activator of the mammalian target of rapamycin (mTOR), the master regulator of cell growth and proliferation.*



## Tryptophan Metabolism

Involved in serotonin production, NAD+ production and inflammation (KTR and QKR).

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| **Tryptophan**<br>Least abundant amino acid.<br><br>Three pathways:<br>• Kynurenine Pathway (primary pathway) – leading to niacin production<br>• Serotonin/Melatonin<br>• Indoles | • Higher urine tryptophan was noted in children with ASD; supplementing with<br>• B vitamins and magnesium lowered urine tryptophan.<br>• Inflammation can lead to higher tryptophan and lower KYN/TRP ratio.<br>• Higher tryptophan associated with higher BMI, glucose, antidepressants, nicotinamide.<br>• Lower serum tryptophan and kynurenine was correlated with higher scores on the Adult ADHD self-report scale.<br>• Low tryptophan levels: can result in lower serotonin production which can impact memory, cognition, sleep, and mood; was noted in depression and anxiety; methionine, phenylalanine, tyrosine levels were also lower.<br>• A diet rich in tryptophan and antioxidants was shown to have a positive impact on mood and cognition. A high intake of large neutral amino acids (like BCAA) can compete with tryptophan absorption.<br>• Modulation of tryptophan metabolism can either aggravate or prevent inflammaging-related diseases; age-related low-grade chronic inflammation known as inflammaging is involved in many age-related diseases. | |
| **5-Hydroxyindoleacetic Acid** (5-HIAA) (5-Hydroxy-indoleacetate)<br><br>The primary breakdown product of serotonin | • 5-HIAA represents serotonin turnover<br>• Lower urinary 5-HIAA is found in IBS, presumably due to increased TNF-alpha levels; authors noted that excessive kynurenine production with concurrent decrease of tryptophan is related to several disease conditions.<br>• Higher urine 5-HIAA has been found in those taking 5-HTP or SSRI's, hyperserotonemia, autism, and in appendicitis.<br>• Banana, pineapple, tomato, kiwi fruit, and walnuts increased 5-HIAA excretion.<br>• Spot-urine levels compared to 24-hr samples were highly correlated | |
| **Kynurenine**<br><br>The primary breakdown product of tryptophan | • Kynurenine blood levels have been found higher in type 2 diabetes, obesity, CVD, ADHD in children, HOMA-IR.<br>• Higher kynurenine increases Treg cell differentiation via the AhR (aryl hydrocarbon receptor) pathway. | • Blood levels were lower in acute ischemic stroke patients, older age, adults with ADHD.<br>• Upregulation of other tryptophan breakdown enzymes KMO *(Kynurenine monooxygenase)* and KYNU *(Kynureninase)* may decrease kynurenine. |
| **Kynurenine/Tryptophan Ratio** (KTR) | • KTR estimates activity of the extrahepatic tryptophan-degrading enzyme, *indoleamine 2,3-dioxygenase (IDO).*<br>• Plasma or urine levels have been correlated with interferon-γ activity and considered a marker of systemic inflammation. KTR has also been noted as a marker for neuroinflammation.<br>» Interferon (IFN)-g and other cytokines activate *IDO*, while NO and excess tryptophan inhibit its activity.<br>• Higher KTR blood levels have been associated with a higher BMI, obesity, inflammation, renal failure & CKD, cancer, AIDS, sepsis, pregnancy, ALS, reduced cognition, CVD. The GSH/GSSG ratio correlated negatively with blood KTR; KTR rises as glutathione is used. Systemic tryptophan and kynurenine levels change upon aging and in age-related diseases.<br>• Upregulation of other tryptophan breakdown enzymes *kynurenine monooxygenase (KMO)* and *kynureninase (KYNU)* may decrease kynurenine, resulting in a decrease in KTR.<br>• Weight loss resulted in a decreased KTR, along with a reduction of tryptophan, kynurenine, and CRP, and an increase in vitamin B6. | |

## Tryptophan Metabolism – *continued from previous page...*

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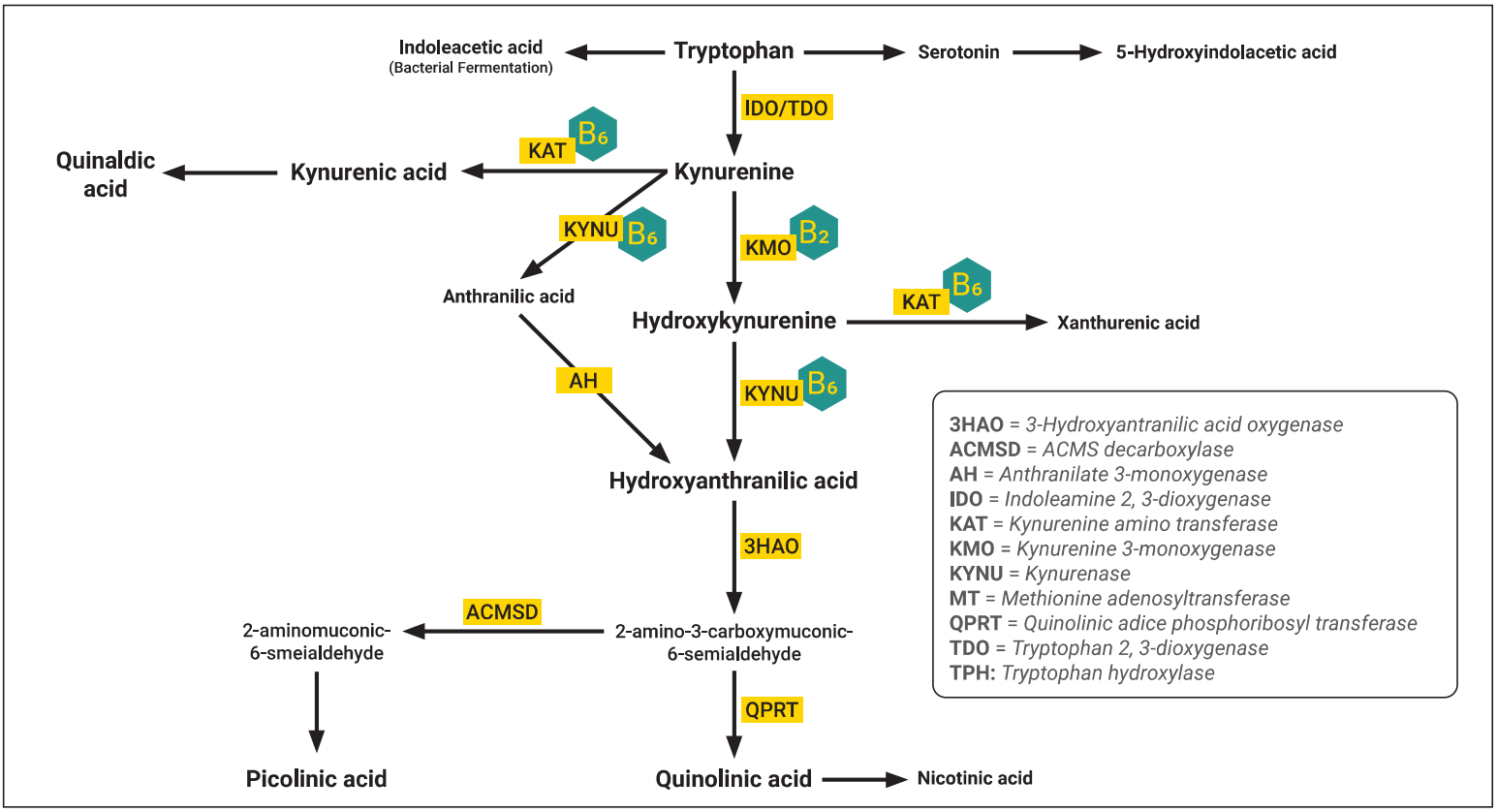
| **Hydroxykynurenine** (HK)<br>3-hydroxykynurenine (3-HK) from the breakdown of kynurenine via *kynurenine monooxygenase (KMO)* | • Hydroxykynurenine is a breakdown product from kynurenine; It is a primary pathway and the preferred substrate for *kynureninase* over anthranilic acid.<br>• The ratio between hydroxykynurenine and xanthurenic acid (HK/XA) in plasma has been considered a functional marker of vitamin B6 status. | |
| **Xanthurenic Acid** (Xanthurenate)<br>From the breakdown of hydroxykynurenine via *kynurenine aminotransferases* (KAT) +B6 | • Elevated xanthurenic acid has been noted with B6 deficiency.<br>• Elevated levels have been noted as more significant in oral contraceptive users in studies using a tryptophan load.<br>• In a mathematical model without a tryptophan load, a moderate vitamin B6 deficiency resulted in a slight increase in xanthurenic acid and a slight decrease in kynurenic acid and anthranilate.<br>• Without a tryptophan load, urine kynurenine and xanthurenic acid both increase in a pronounced B6 deficiency. | • Animal studies found a low urinary excretion ratio of xanthurenic acid/ kynurenic acid as a possible marker of niacin need, proposing that levels may increase with repletion.<br>• Niacin (vitamin B3) is a product of tryptophan degradation. In alcoholic pellagra patients, the tryptophan-niacin pathway is inhibited after the *3-hydroxyanthranilate oxidase* step, which can result in increased kynurenic acid, and decreased xanthurenic acid and quinolinic acid. |
| **Anthranilic Acid** (Anthranilate)<br>From the breakdown of kynurenine via *kynureninase* (KYNU) + B6, a side pathway | • Several clinical studies have reported increased excretion of anthranilic acid and other metabolites in bladder cancer patients.<br>• Anthranilic acid was one of nine markers that positively correlated with proteinuria.<br>• Anthranilic acid comes from the kynurenine pathway, which is B6 dependent; Anthranilic acid activity may be reduced during vitamin B6 restriction.<br>• In a mathematical model without a tryptophan load, a moderate B6 deficiency resulted in slight decreases in kynurenic and anthranilic acids.<br>• Patients with acute intermittent porphyria had significantly increased urinary excretion of kynurenine and anthranilic acid. | |
| **Picolinic Acid** (Picolinate)<br>From the breakdown of hydroxykynurenine via *ACMS decarboxylase* | • Decreased picolinic acid and increased quinolinic acid blood levels noted in suicidal subjects.<br>• A tryptophan metabolite produced through non-enzymatic conversion. | |
| **Kynurenic Acid** (Kynurenate)<br>A breakdown product from kynurenine via *kynurenine aminotransferases (KAT)* | • Elevated kynurenine, kynurenic acid, xanthurenic acid, quinolinic acid, and a high KYN/TRP ratio seen in metabolic syndrome.<br>• In animal studies exercise-induced conversion to kynurenic acid was seen.<br>• Alcoholic B3-deficient patients had increased kynurenic acid, and decreased xanthurenic acid and quinolinic acid.<br>• Urine levels of citric acid, hippuric acid, p-cresol, 2-aminobutyrate, putrescine, and kynurenic acid, were able to discriminate colorectal cancer patients from healthy controls. Kynurenic acid was lower in patients.<br>• In a mathematical model without a tryptophan load, a moderate B6 deficiency resulted in slight decreases in kynurenic. | |

## Tryptophan Metabolism – *continued from previous page...*

| | |
|---|---|
| **Quinolinic Acid** (Quinolinate)<br><br>A breakdown product of hydroxykynurenine via *QPR transferase* | • Excessive quinolinic acid production has been correlated with other neuroinflammatory markers.<br>• Increased xanthurenic acid and quinolinic acid, plus a decrease in kynurenic acid seen in ASD cases.<br>• Phthalates structurally mimic tryptophan metabolites. High phthalate exposure increased urinary concentrations of quinolinic acid.<br>• Elevated levels of kynurenic acid, xanthurenic acid, and quinolinic acid were found in patients with metabolic syndrome.<br>• Decreased picolinic acid and increased quinolinic acid blood levels reported in suicidal subjects.<br>• Niacin (B3) is a product of tryptophan degradation. In alcoholic pellagra patients, kynurenic acid increased, and xanthurenic acid and quinolinic acid decreased. |
| **Quinolinic Acid/ Kynurenic Acid Ratio** (QKR) | • Quinolinic acid and kynurenic acid have opposing neuroactive properties and alterations in their balance may play a role in neurodegenerative and neuropsychiatric diseases.<br>• Breast cancer patients had significantly elevated quinolinic acid at baseline, which decreased after a 12-week resistance exercise program. Healthy subjects had decreases in the quinolinic acid/ kynurenic acid ratio, and patients had an increased quinolinic acid/ kynurenic acid ratio.<br>• Both quinolinic acid and hydroxykynurenine can be excitotoxins, and both kynurenic acid and picolinic acid can be neuroprotective. |

## Figure 5 - Tryptophan Pathway

*Tryptophan is responsible for serotonin, melatonin and niacin production.*



## Methionine Metabolism

Involved in methylation and glutathione production.

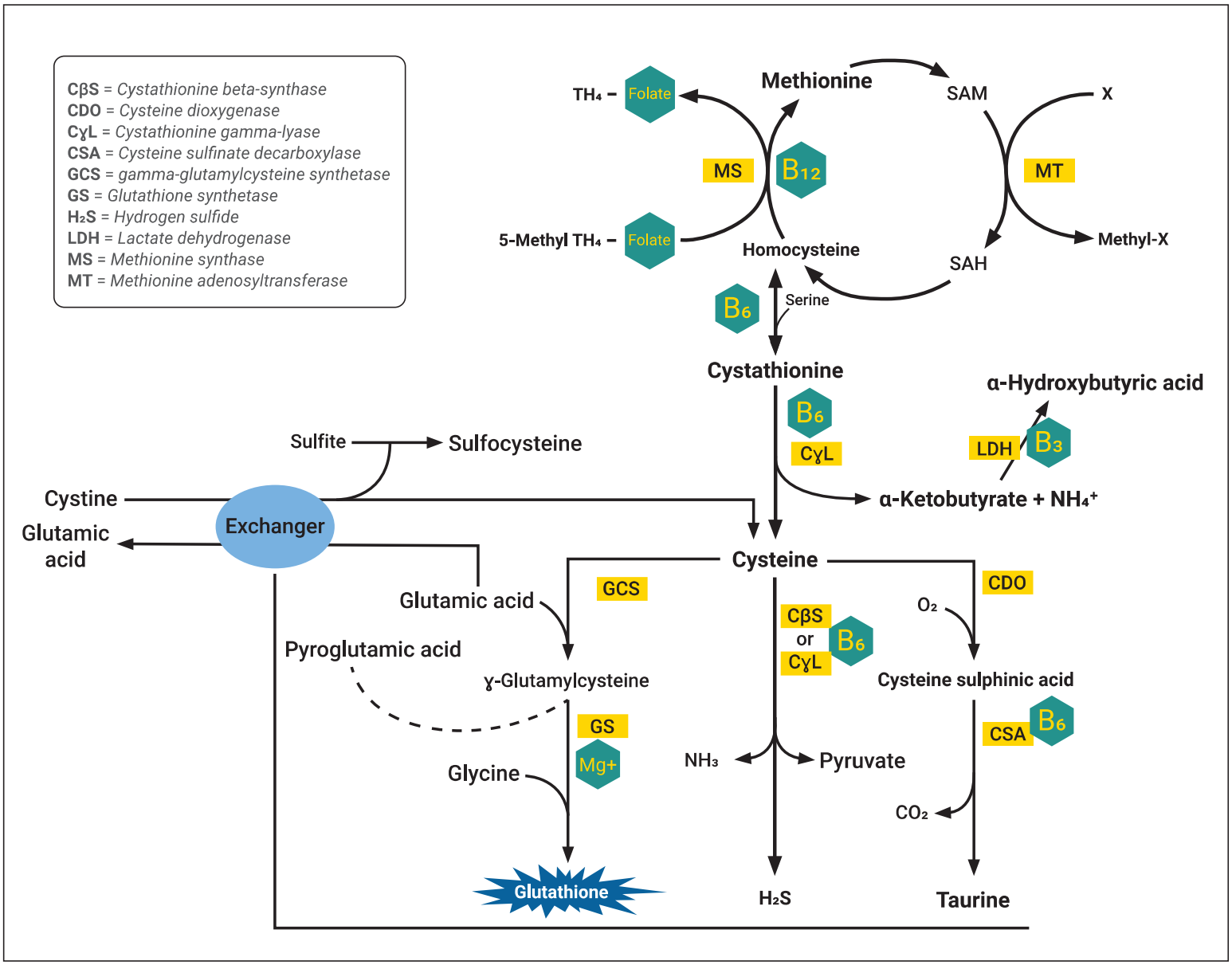
| | |
|---|---|
| **Methionine**<br>Involved in methylation; metabolism leads to glutathione production. | • Higher in those on a high protein diet; higher in fish eaters, along with tryptophan and tyrosine. Lower levels have been noted in those on a low protein diet, or significantly reduced calorie intake.<br>• Higher plasma methionine levels have been noted in liver disease.<br>• Elevated plasma methionine has been proposed to distinguish patients with homocystinuria due to in-born errors from those with B-vitamin deficiencies or renal failure, which would have normal or lower methionine levels.<br>• A *cystathionine-beta-synthase* deficiency can lead to an increase in blood methionine and cystathionine.<br>• Urine methionine significantly increased in hepatocellular carcinoma patients.<br>• Blood methionine was found lower in those with depression, multiple sclerosis. |
| **Homocystine** | • Plasma homocystine is higher in those with *cystathionine-beta-synthase* deficiency.<br>• Plasma homocystine, as well as taurine, were significantly lower in insufficient methotrexate therapy responders.<br>• Homocystine is an oxidized disulfide form of homocysteine, which gets readily converted to cystathionine. |
| **Cystathionine** | • Insufficient B6, B12, or folate have been shown to result in elevated blood levels of cystathionine.<br>• Elevations of cystathionine has been noted in neural tumors, renal defects, or defects in the conversion of homocysteine to methionine.<br>• A *cystathionine beta-synthase* (CBS) deficiency can lead to a decrease in cystathionine. |
| **Sulfocysteine** | • Sulfocysteine is the product of sulfite-dependent cleavage of cystine. In the pathway, cysteine becomes sulfite, which converts to sulfate via *sulfite oxidase* + Mo. If the pathway is blocked, sulfocysteine builds up. |
| **Taurine**<br>A semi-essential sulfur amino acid derived from methionine and cysteine. | • Higher taurine noted with increased intake of taurine-rich foods (meat, fish, dairy) and taurine supplements. Cooking results in significant loss of taurine.<br>• Taurine supplementation provided beneficial effects to elderly subjects, including reduced blood pressure.<br>• Urinary taurine and magnesium excretions were inversely related with cardiometabolic risks.<br>• Taurine depletion has been noted with long-term parental nutrition, poor quality vegan or vegetarian diets, short-bowel syndrome, intensive chemotherapy, whole-body irradiation, hepatic diseases, chronic renal failure, and type 2 diabetes. |

## Methionine Metabolism – *continued from previous page...*

| Glutathione Markers<br>Glutathione (GSH) is a major endogenous antioxidant that can neutralize reactive oxygen species. GSH is made up of three amino acids: glycine, cysteine, and glutamic acid. Supplementation with serine and an NAD+ precursor (like tryptophan, niacin [vitamin B3], or nicotinamide riboside), increases NAD+, and helps to support glutathione production. | |
|---|---|
| **Cystine**<br>Cystine is rate limiting for glutathione production.<br>Cystine is the oxidized form of cysteine. | • Low cystine may be reflective of reduced glutathione levels and has also been noted in those with celiac disease and lower BMD.<br>• Cysteine can be imported into cells either directly or as cystine, within the cell, cystine is immediately reduced to cysteine.<br>• Higher plasma cystine has been associated with older age, female, higher BMI, lower GFR, diabetes mellitus, metabolic syndrome, hypertension, lower total cholesterol levels, statin use, lower ejection fraction, and higher hsCRP.<br>• Higher urine cystine may be indicative of impaired amino acid reabsorption defects and has been associated with recurrent cystine kidney stones.<br>• Cystine from foods sources is considered nutritionally equivalent to cysteine (egg, beef, and whole grains, fish, lentils, and oatmeal). |
| **α-Hydroxybutyric Acid**<br>(Alpha-Hydroxybutyrate)<br>From the breakdown of alpha-ketobutyrate | • Increased glutathione results in increased formation of α-hydroxybutyric acid.<br>• Plasma levels are noted as an early marker for both insulin resistance and impaired glucose regulation.<br>• α-hydroxybutyric acid can be formed by *lactate dehydrogenase*. Urine samples with large amounts of lactic acid also had considerable quantities of α-hydroxybutyric acid.<br>• In a review of IBD, both ulcerative colitis and Crohn's disease patients had low urine α-hydroxybutyrate, as well as low succinic, citric, and hippuric acids and taurine. |
| **α-Ketobutyric Acid**<br>(Alpha-Ketobutyrate) | • α-ketobutyric acid results from the breakdown of methionine (or threonine) during glutathione production.<br>• α-ketobutyric acid is produced from cystine, along with hydrogen sulfide (H2S) as a by-product. α-Ketobutyric acid is reversibly converted to α-hydroxybutyric acid. |
| **Pyroglutamic Acid**<br>(Pyroglutamate) | • Pyroglutamic acid is the last step of the glutathione cycle and is thought to be related to cysteine insufficiency or glycine availability.<br>• Drug-induced reversible inhibition of *glutathione synthetase,* such as from acetaminophen, leads to elevated pyroglutamic acid.<br>• Researchers noted that glutathione depletion of septic patients may be noted with higher serum levels of pyroglutamate, and lower levels of *erythrocyte glutathione peroxidase.* |

## Figure 6 - Methionine Pathway

*Methionine is a sulfur-containing amino acid that functions as a methyl donor in the methylation process. It is also involved in glutathione production, an endogenous antioxidant that can neutralize reactive oxygen species. Depletion of glutathione can lead to mitochondrial dysfunction. Glutathione is made up of three amino acids, glycine, cysteine (cystine), and glutamic acid.*



## Histidine Metabolism

| | |
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| **Histidine**<br>Involved in one-carbon units for conversion of formiminoglutamic acid (FIGLU) to glutamic acid. | • High plasma histidine has been associated with increased plasma glutamic acid, alanine and glutamine, and decreased branched-chain amino acids.<br>• Elevated urine histidine means it is not available for hemoglobin production. Hemoglobin is 10% histidine.<br>• High levels have been associated with progression of type 2 diabetes after gestational diabetes.<br>• Decreased plasma histidine was associated with increased risk of ulcerative colitis relapse; a higher serum CRP in Crohn's disease; chronic kidney disease; increased inflammation; and atopic dermatitis. |
| **3-Methylhistidine** | • Synthesized in muscle myofibrillar proteins; urine marker associated with skeletal muscle.<br>• 3-methylhistidine is best measured in a diet maintaining a constant meat intake. Evaluate amino acid status and levels of activity. |
| **β-Alanine**<br>Combines with histidine to form carnosine. | • The International Society of Sports Nutrition (ISSN) found that β-alanine supplementation (4–6 g daily) significantly augmented muscle carnosine concentrations, improve exercise performance, and attenuates neuromuscular fatigue. |

## Threonine Metabolism

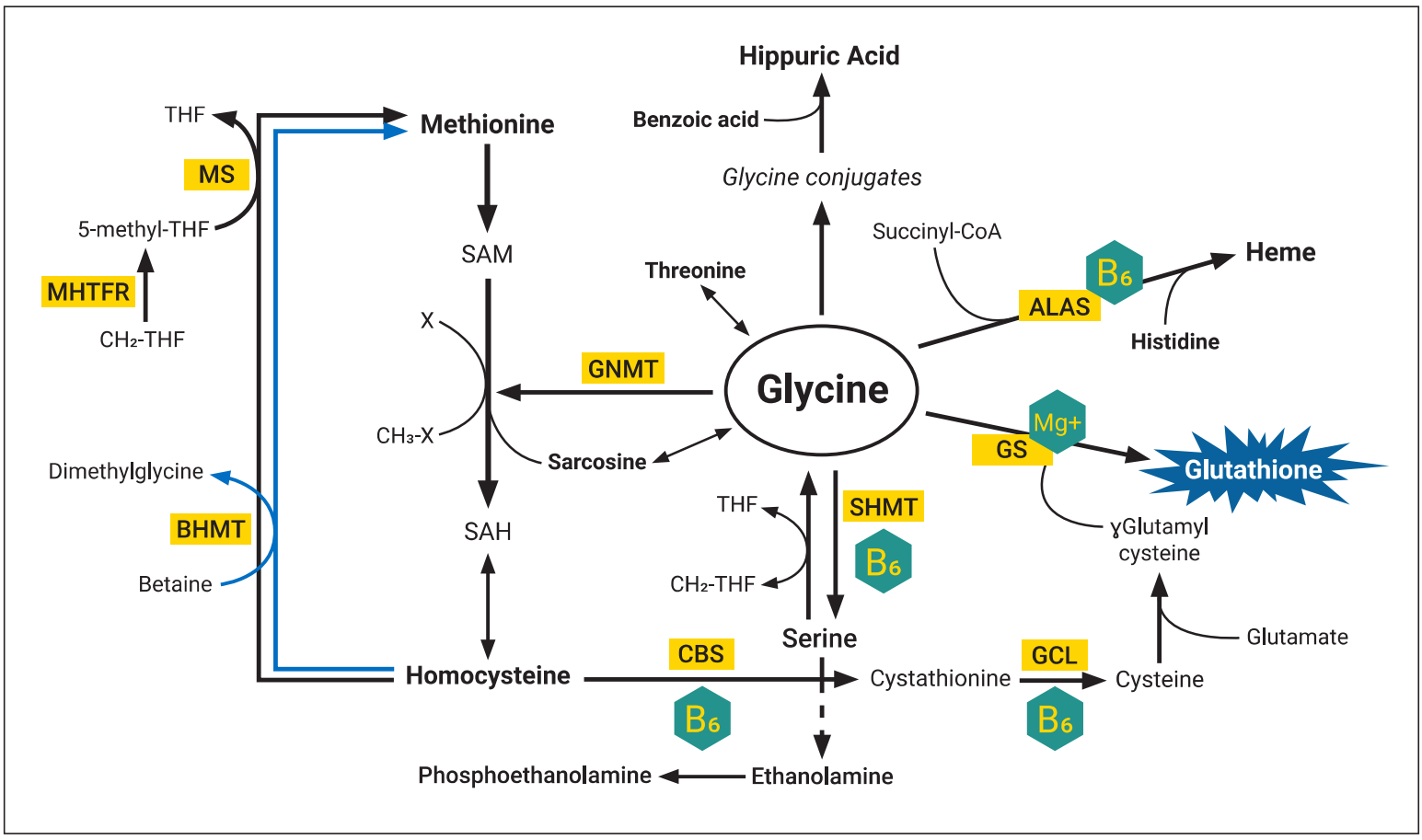
| | | |
|---|---|---|
| **Threonine**<br>Catabolized to glycine. | • Researchers found elevated plasma threonine was associated with a reduced risk of an atherogenic lipid profile (n=475).<br>• Urine threonine was higher in PCOS patients.<br>• Evaluate diet and/or vitamin B6 if threonine is elevated.<br>• Evaluate glycine, benzoic acid, and hippuric acid to establish glycine need, as high glycine need could drain threonine. | |
| **Glycine**<br>Derived from threonine and diet<br>Serine ↔ Glycine<br>Sarcosine ↔ Glycine | • Glycine helps to produce glutathione and sarcosine and is a conjugator of toxins (benzoic acid).<br>• Glycine was found highest in vegans and lowest in meat-eaters.<br>• Elevated baseline urine glycine correlated with A1C (effect size >.8) in lean subjects, and with active IBD.<br>• Plasma glycine increased after two weeks of vitamin B6 depletion; serine increased.<br>• Lower blood glycine was associated with higher risk of metabolic disorders such as obesity, type 2 diabetes, and non-alcoholic fatty liver disease/liver fibrosis. Clinical studies have suggested beneficial effects with glycine supplementation.<br>• Lower urine glycine was associated with a higher decline of free T4 and a greater risk of hypothyroidism. | |
| **Serine**<br>Serine ↔ Glycine | • Plasma serine was found higher in depression, and psychoses including schizophrenia.<br>• Methionine supplementation significantly increased plasma serine.<br>• Serine is involved in cysteine and methionine metabolism. | • Blood serine was lower in patients with hypertension.<br>• Blood serine was lower in patients with greater liver fat fractions, higher alanine transaminase (ALT) and triglyceride, in patients with fatty liver disease. |

## Threonine Metabolism – *continued from previous page...*

| | |
|---|---|
| **Glutamate/ [Serine + Glycine] GSG Index** | • The GSG index was higher in NAFLD, positively correlating with intrahepatic fat content and liver enzymes.<br>• Weight loss is linked to reduction in the GSG Index and a correlated reduction in homeostasis model assessment (HOMA) of insulin and aspartate aminotransferase (AST). |
| **Sarcosine**<br>Sarcosine ↔ Glycine | • Elevated urine sarcosine has been associated with incidence of type 2 diabetes.<br>• Blood sarcosine has been proposed as a biomarker of diet restriction and older age.<br>• Sarcosine supplementation has shown beneficial effects in schizophrenia.<br>• Krill oil supplementation increased fasting plasma sarcosine, along with betaine and choline. |
| **Ethanolamine Phosphoethanolamine** | • Plasma phosphoethanolamine was higher in infants with spinal muscular atrophy and in patients with major depressive disorder (along with plasma beta-aminoisobutyric acid, cystathionine, and homocystine).<br>• Ethanolamine becomes phosphoethanolamine; is a precursor of acetylcholine and is also a component in the phospholipid bilayer. |

## Figure 7 - Threonine-Glycine-Serine Pathway

*Glycine is involved in production of glutathione and sarcosine, conjugation of toxic compound, and in biosynthesis of bile salts, heme, creatine, and purines.*



## Lysine Metabolism

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|---|---|
| **Lysine**<br>Lysine catabolism leads to collagen and carnitine production. | • Higher plasma valine, lysine, and tyrosine were independently and positively associated with gestational diabetes mellitus and insulin activity.<br>• Increased urinary lysine was associated with a lower risk of chronic kidney disease (0.73 [0.50-0.90]).<br>• Low lysine has been associated with increased anxiety in human and animal studies. Lysine and arginine supplementation were found to reduce anxiety and basal salivary cortisol levels in adults.<br>• Lower plasma lysine and glutamine levels, and higher glutamic acid, were significantly associated with ADHD. |
| **α-Aminoadipic Acid**<br>(Alpha-aminoadipic acid)<br>(α-Aminoadipate) | • An intermediate metabolite of lysine metabolism, produced primarily under oxidative stress (metal-catalyzed oxidation).<br>• In adolescents, α-aminoadipic acid was associated with adipogenesis and insulin resistance.<br>• Higher plasma α-aminoadipic acid was associated with a 4-fold risk of future diabetes and identified risk up to 12 years before the onset of overt disease.<br>• BCAAs, cystine, α-aminoadipic acid, phenylalanine, and leucine + lysine were significantly increased in obesity, T2D, and with worsening health. |
| **Glutaric Acid** (Glutarate)<br>Endogenously produced in the catabolism of lysine and tryptophan. | • Increased Glutaric acid is associated with secondary carnitine deficiency.<br>• Glutaryl-CoA (from lysine or tryptophan) normally enters the Krebs cycle via transition to acetyl-CoA.<br>» *Glutaryl-CoA dehydrogenase (GCDH)* + glutaryl-CoA + B2 → acetyl-CoA.<br>» If *GCDH* is blocked, glutaryl-CoA + carnitine → elevated glutaric acid. |

## Glutamamic Acid & Aspartic Acid

Glutamic Acid ↔ Glutamine  Asparagine ↔ Aspartic Acid

| | |
|---|---|
| **Glutamine**<br>Glutamine ↔ Glutamic acid<br>The most abundant free amino acid and is conditionally essential during inflammation. | • Plasma glutamine, cysteine, and asparagine were significantly downregulated in psoriasis patients.<br>• Glycine and glutamine were inversely associated with risk of prediabetes or type 2 diabetes (n=8,000).<br>• PRIMED data found lower baseline levels of glutamine to be significantly associated with increased stroke risk (n=980).<br>• Major rate-limiting substrate for ammonia produced by kidney.<br>• Small intestine mucosal cells and the liver are major sites of glutamine utilization. |
| **Glutamic Acid**<br>(Glutamate)<br>Glutamic acid ↔ Glutamine | • A major excitatory neurotransmitter, glutamic acid plays an important role in the disposal of nitrogen and undergoes oxidative deamination to liberate free ammonia for the synthesis of urea. Generally lower is better.<br>• People with high plasma glutamic acid had 3 times the risk of stroke, compared to low. Those with higher plasma glutamic acid had a greater benefit from following a Mediterranean diet (n=980).<br>• Urine glutamic acid was higher in treatment-resistant than in treatment-responsive schizophrenia patients.<br>• Urine glutamic acid was lower in autistic children. |

## Glutamamic Acid & Aspartic Acid – *continued from previous page...*

| | |
|---|---|
| **Glutamine/Glutamic Acid Ratio** | • Glutamic acid has been associated with higher BMI, blood pressure, and insulin resistance, while glutamine levels were inversely associated.<br>• A high plasma glutamine-to-glutamic acid ratio was associated with lower risk of diabetes in the Framingham Heart Study (n=1015).<br>• Higher glutamine-to-glutamic acid ratio was associated with a better cardiometabolic-risk profile over 10 years in the PRIMED study (n=1879). |
| **Asparagine**<br>**Aspartic Acid**<br>Asparagine is converted to aspartic acid, then to glutamic acid. | • Asparagine is a nontoxic carrier of residual ammonia. A byproduct of asparagine metabolism is oxaloacetate.<br>• Higher levels of asparagine were associated with lower rates of diabetes, insulin, and HOMA.<br>• Plasma asparagine and the tyrosine/phenylalanine ratio were found to be protective against depression.<br>• Higher asparagine, aspartic acid, and citrulline were associated with higher rates of physical frailty and sarcopenia.<br>• Plasma glutamine, cysteine, and asparagine were significantly downregulated in psoriasis patients. |

## Collagen Catabolism

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| **Proline**<br>Collagen contains proline, hydroxyproline, and glycine. | • Sarcopenia (low muscle mass) was associated with higher plasma proline.<br>• Proline was significantly lower in esophageal cancer patients compared to the healthy controls.<br>• Hydroxyproline and proline together constitute around 25% of residues and allow for stability and twisting of collagen.<br>• Dietary intake increases levels of proline and hydroxyproline.<br>• Proline and hydroxyproline both negatively correlated with a higher likelihood of anxiety, depression, and psychoses. |
| **Hydroxyproline**<br>Hydroxyproline is the key factor in stabilizing collagens. | • Hydroxyproline is abundant in meat and low in plant-based foods. Meat intake increases levels of proline and hydroxyproline.<br>• Increased hydroxyproline has been found in collagen catabolism (bone resorption, increased reactive oxygen species [ROS]), tissue degradation, muscle damage, or other conditions such as Paget's disease or Alzheimer's disease.<br>• Proline and hydroxyproline both negatively correlated with a higher likelihood of anxiety, depression, and psychoses.<br>• Plasma hydroxylproline may be reduced with fatigue (caused by deprivation of rest and sleep; a physical stress condition) or oxidative stress. |
| **Glycylproline** | • Patients with pressure sores had significantly increased glycylproline, finding positive predictive value for pressure sores of 70%.<br>• In an older (1964) review of bone markers of patients with bone disease, researchers found glycylproline only in patients with severe active rickets.<br>• Urine glycylproline and hydroxylysine  patients with pressure sores, compared to controls. |

# 3 – Nutrition

## B-Vitamin Complex (B1, B2, B3, B5, LA)

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|---|---|
| **Total Branched-Chain Keto Acids**<br><br>**α-Ketoglutaric Acid**<br><br>**Pyruvic Acid** | • Branched-chain α-keto dehydrogenase (BCKD) is dependent on B vitamins, a lack of which may decrease pathway function, possibly leading to an elevation of the branched-chain keto-acids.<br>• Women were divided into three groups based on their level of branched-chain keto-acid excretion. The women with the highest levels of excretion had the greatest reduction after taking a B-vitamin complex. Dietary B-vitamin intakes were all similar, indicating that many individuals may require more than recommended amounts.<br>• Higher α-ketoglutaric acid levels are seen in B1 deficiency: α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and branched-chain ketoacid dehydrogenases may also be impacted. |

## Vitamin B-12

| | |
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| **Methylmalonic Acid**<br>(MMA)<br>(Methylmalonate) | • Methylmalonic acid has been used as a marker for need of vitamin B12 and in the diagnosis of methylmalonic acidurias.<br>• A linear relationship between methylmalonic acid concentrations in serum and urine.<br>• Insufficient B12 or folate has also been shown to result in elevated blood levels of cystathionine. |

## Folate

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|---|---|
| **Formiminoglutamic Acid**<br>(FIGLU) | • The intermediate metabolite in the pathway that converts histidine to glutamic acid. Histidine provides one-carbon units for conversion of FIGLU to glutamic acid.<br>• FIGLU can become elevated in those with a folate deficiency, even without a histidine load, and in inborn errors. |

## Vitamin B-6

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|---|---|
| **Pyridoxic Acid**<br>(4-Pyridoxate)<br><br>**Xanthurenic Acid**<br>(Xanthurenate) | • Pyridoxic acid is a catabolic product of vitamin B6 that is excreted in the urine. Pyridoxic acid represents > 90% of vitamin B6 species excreted in the urine, and 40-60% of dietary vitamin B6 intake. Urine 4-pyridoxic acid correlated with plasma PLP and RBC PLP.<br>• 4-Pyridoxic acid level varies according to vitamin B6 intake and responds within 1–2 weeks to vitamin B6 depletion and repletion. Very low levels (<dl on the report) may indicate B6 need, and very high levels may identify excess intake.<br>• Increased xanthurenic acid after a tryptophan load may occur in vitamin B6-deficient individuals.<br>• In a mathematical model without a tryptophan load, xanthurenic acid and kynurenine increased at a more pronounced deficiency. Kynurenic acid may be more sensitive but may also result in a slight decrease. |

## Biotin

| | |
|---|---|
| **3-Hydroxyisovaleric Acid** | • Increased urinary excretion of 3-hydroxyisovaleric acid is used as an indicator of biotin deficiency.<br>• Urinary excretion of 3-hydroxyisovaleric acid was greater in the smokers.<br>• People with multiple acyl-CoA dehydrogenase deficiency (MADD) shows elevations of ethylmalonic acid, glutaric acid, 3-hydroxyisovaleric acid, and other key markers.<br>• Animal research found feeding a ketogenic-type diet, low in carbohydrates and high in fat, exaggerated biotin deficiencies. A ketogenic diet may increase biotin need. |

## Plant Components

Low levels identify low intake of these plant components. Evaluate in context of overall polyphenol intake, and polyphenol microbial metabolism in Section 6.

| | |
|---|---|
| **Quercetin** | • Research has noted antidiabetic, anti-inflammatory, antioxidant, antimicrobial, anti-Alzheimer's, antiarthritic, cardiovascular, and wound-healing effects.<br>• Quercetin and its derivatives are flavonoids that undergo a substantial intestinal phase-II metabolism. Quercetin intake is excreted and reaches a steady state after 3–4 days and has been suggested as a marker of absorbed quercetin. Urinary quercetin can be used as a marker of regular intake.<br>• Evaluate intake of foods high in quercetin, such as onions, cruciferous vegetables, and dark berries; evaluate supplementation status. |
| **Tartaric Acid** | • Tartaric acid is a compound found in plant foods. It has been identified as a biomarker of grape intake, though it has also been identified in other foods. Tartaric acid levels peak at 4–8 hours after intake. Levels in foods vary significantly between types of foods and within individual foods.<br>• Tartaric acid cannot be processed by humans and is either excreted or utilized by gut bacteria as a carbon source. Some bacteria have genes for tartaric metabolizing enzymes, so levels can be impacted by gut microbiome. The process starts once tartaric acid is released (i.e., grapes are crushed or are invaded by pathogens), making it susceptible to catabolic enzymes from microorganisms, which may reduce it to oxaloacetate, glyceric acid, and pyruvic acid. |

## Meat Intake

| | |
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| **Carnosine & Anserine** | • Carnosine is generally very low and increases proportionally after meat intake. Urine levels peak after ~5 hours and are completely excreted within 20–25 hours.<br>• Urine carnosine increased with albuminuria in patients with diabetes. Patients with eGFR < 30 ml/mi had higher urine-carnosine excretion rates compared to eGFR (> 90 ml).<br>• Psoriasis patients had elevated asparagine, carnosine, and phosphoserine. They were all positively associated with psoriasis. |
| **1-Methylhistidine** | • Total meat intake was associated with plasma anserine, carnosine, 1-methylhistidine.<br>• 1-Methylhistidine is derived mainly from anserine, from meat, fish, and primarily poultry. |

## Fructose Intake

| | |
|---|---|
| **Fructose** | • Emerging research seems to show a relationship between the rise in metabolic diseases and the increased consumption of fructose—particularly consumption of non-natural sources of fructose found in sugar-sweetened beverages and other processed foods.<br>• Elevated fructose levels should be further investigated. Dietary fructose intake should be determined, modified if excessive, and monitored for metabolic changes. |

# 4 – Stress & Mood

## Neurotransmitter

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|---|---|
| **γ-Aminobutyric Acid**<br><br>**Gamma Amino-Butyric Acid** (GABA) | • γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter that acts in the nervous system and the immune system. γ-aminobutyric acid affects control of cortisol.<br>• Dysregulation of γ-aminobutyric acid has been associated with cognition, oxidative stress, glucose tolerance, and mood disorders.<br>• GABA levels were diminished in currently depressed patients in a meta-analysis (n=373). Plasma GABA levels have varied in psychiatric disorders.<br>• Urine GABA was significantly increased in metabolic syndrome patients compared to controls.<br>• GABA blood levels were significantly lower in osteoporotic patients.<br>• Foods containing γ-aminobutyric acid include tea leaves, mulberry leaves, tomato, lactic acid bacteria, yeasts and molds, fermented milk and soy products, sourdough, sprouts of brown rice, barley and beans, and kimchi, or dietary supplements. |

## Catecholamine Turnover

| | |
|---|---|
| **Homovanillic Acid** (HVA)<br>(Homovanillate) | • HVA levels have shown to be related to neurotransmitter metabolism in subjects suffering from occupational stress; levels were lower in workers with higher coworker support.<br>• Higher scores on the Beck Depression Inventory-II, plus a decrease in the levels of plasma dopamine and urine dopamine, 3,4-dihydroxyphenylacetic acid, HVA, norepinephrine, serotonin, and hydroxyindoleacetic acid, were seen in pesticide exposed workers. |
| **Vanillylmandelic Acid** (VAM)<br>(Vanillylmandelate) | • VMA levels are related to neurotransmitter metabolism in subjects suffering from occupational stress. Levels were lower in workers with higher coworker support.<br>• Stress reduction found that lower-stress groups had lower levels of mood disturbance and anxiety, and lower levels of both VMA and 5-HIAA. The low-stress group practiced meditation. |

## Serotonin Turnover

| | |
|---|---|
| **5-Hydroxyindoleacetic Acid** (5-HIAA) | • Research has noted that patients with depression often show a significant reduction in 5-HIAA.<br>• 5-HIAA increased after tryptophan-enriched cereal intake, and improvement in anxiety and depression symptoms was observed.<br>• 5-HIAA was higher in hyperserotonemic autistic subjects compared to controls.<br>• 5-HIAA may increase significantly after eating banana, pineapple, tomato, kiwi fruit, and walnuts. |

## Steroid Hormone

| | |
|---|---|
| **Cortisol and Cortisone** | • Elevated cortisol can alter or disrupt the functions such as the digestive, reproductive systems, and immune systems, and growth processes<br>• Higher cortisol levels have been noted in acute stress situations and some medications, excess licorice or caffeine intake, aspartame use, and smoking.<br>• A morning spot urine sample gave comparable results to a 24-hour collection samples.<br>• Research of those with CVD risk factors, such as dyslipidemia, hypertension, or hyperglycemia, had higher urinary cortisol. |
| **Aldosterone** | • Aldosterone's primary function is to act on the kidney to impact sodium absorption and potassium excretion. It can impact blood pressure. It is higher in the morning.<br>• If elevated, check blood pressure, presence of sleep apnea, and note impact of age – levels are lower in those >50 years old compared to those <30.<br>• Clinical evidence has established strong associations between aldosterone excess, resistant hypertension, and obstructive sleep apnea. Primary hyperaldosteronism (PA) is widely recognized as the most common form of secondary hypertension. A single urine sample is not used for diagnosis.<br>» Depression and anxiety disorders are common symptoms associated with primary aldosteronism. It is more common in women.<br>» A clinically relevant spectrum of subclinical primary aldosteronism has been noted in normotension. |

# 5 – Toxic Impacts

## Oxidative Damage

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| **8-Hydroxy-2' Deoxyguanosine** (8-OHdG or 8-OH-2-DG) | • 8-hydroxy-2'-deoxyguanosine is a biomarker of oxidative stress. It is a DNA repair product. 8-OHdG is considered a biomarker of cellular oxidative stress throughout the body and has been associated with chronic inflammation, nutrient deficiency, cancer, atherosclerosis, diabetes, arsenic exposure, insomnia, schizophrenia, and bipolar disorder, but not in depression, medical ionizing radiation exposure, etc.<br>• A baseline 8-OHdG level has been proposed as part of an annual check-up. |

## Toxins

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| **2-Methylhippuric Acid** (2-Methylhippurate) | • 2-Methylhippuric acid is a metabolite of xylene, which is an aromatic hydrocarbon widely used as a solvent. High levels have been noted in mitochondrial fatty acid beta-oxidation disorders. It is primarily noted as a marker of xylene exposure.<br>• Elevated levels found in workers exposed to xylene, and from tobacco smoke, gasoline, paint, varnish, shellac, rust preventative, air fresheners. |
| **Mandelic Acid**<br><br>**Benzoylformate** (Phenylglyoxylic Acid) | • Styrene is a key component in consumer products. Occupational exposure has been associated with increased rates of pulmonary, neurological, genetic, ocular, and reproductive complications, plus leukemia. Mandelic acid and benzoylformate are major metabolites of styrene and ethylbenzene exposure.<br>» Styrene can be found in polystyrene packaging and can migrate into packaged food.<br>• Benzoylformate has been associated with metabolism of adrenaline and noradrenaline, and phenylketonuria.<br>• In a review of 2005–2006 and 2011–2012 NHANES data (N=4690), smokers had 2-fold and 1.6-fold higher levels of both markers.<br>• Eating more vegetables and fruit was associated with decreased levels. |
| **Glucaric Acid** (Glucarate) (D-Glucaric Acid) | • Urinary glucaric acid has been used as an indicator of induced hepatic drug-metabolization and elevated with exposure to xenobiotics. Levels may indirectly represent P-450 activity or an end-product of the glucuronidation pathway. Calcium-D-glucarate is the calcium salt of D-Glucarate.<br>• Dietary glucaric acid and supplementation with calcium-D-glucarate may suppress cell proliferation and inflammation, induce apoptosis, and to have anticancer properties.<br>» Glucaric acid from dietary plants may act as a nontoxic β-glucuronidase inhibitor. Glucaric acid is normally in equilibrium with D-glucaro-1,4-lactone, and an increase in dietary glucaric acid increased D-glucaro-1,4-lactone, which suppresses blood and tissue beta-glucuronidase activity. Vegetarians may have higher levels.<br>• It has been found increased with increased PCBs, toxins, and medications. |

## Urea Cycle

The urea cycle converts waste nitrogen from protein into urea for excretion to prevent the build up of ammonia. It consists of four key markers and six consecutive enzymatic reactions.

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| **Arginine** | • Arginine is a precursor of urea, nitric oxide, polyamines (putrescine, spermidine, spermine and agmatine), proline, glutamate, and creatine.<br>• Levels may drop with increased need, or in renal or small intestine dysfunction.<br>• Synthesis of arginine depends on citrulline levels and is less regulated by dietary arginine. Supplementation with citrulline increases plasma arginine and the production of nitric oxide (NO). Arginine supplementation may lower blood pressure.<br>• Metabolism of elevated arginine levels is dependent on glycine availability.<br>• Plasma arginine and ornithine increased, while citrulline remained stable, following watermelon juice intake, a rich source of citrulline. |
| **Citrulline** | • Citrulline comes from dietary sources and plasma amino acid precursors such as arginine, ornithine, glutamine, glutamate, or proline.<br>• Enterocytes are the main site of citrulline production, making small intestine function a key determinant of plasma citrulline levels.<br>• Decreased blood citrulline was associated with impaired enterocyte function and small bowel absorptive capacity, increased risk of diabetes, a marker of physical frailty and sarcopenia, and multiple sclerosis. |
| **Ornithine** | • It is a key substrate for the synthesis of proline, polyamines, and citrulline.<br>• Higher ornithine blood levels were associated with lower breast cancer risk; also found higher in those with Alzheimer's and Parkinson's disease.<br>• Ornithine supplements have been utilized for NH3 detoxification in liver disease. |
| **Homocitrulline** | • Protein carbamoylation is a non-enzymatic modification that carbamylates isocyanate with lysine to form homocitrulline. Carbamoylation has been proposed as a promoter of molecular aging and has been associated with conditions such as atherosclerosis or immune system dysfunction.<br>» Homocitrulline has been proposed to reflect the intensity of protein carbamoylation rate.<br>• Homocitrulline was higher in patients with chronic renal disease, concentrations were positively correlated with urea concentration.<br>• Serum homocitrulline was significantly higher in coronary artery disease patients. |
| **Arginosuccinic Acid** | • It is not normally detectable in healthy adults. Adult argininosuccinate lyase insufficiencies are typically treated with a lower protein/higher carbohydrate diet, arginine supplementation, and avoidance of fasting. |

## Kidney Impacts

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| **Orotic Acid** (Orotate) | • Urinary excretion of orotic acid is an intermediate in pyrimidine biosynthesis (nitrogenous bases in DNA and RNA) and is increased in many urea cycle disorders such as hyperammonemia, hereditary orotic aciduria (uridine-5'- monophosphate synthase deficiency) or disorders of arginine.<br>» Elevated urinary orotic acid may identify arginine depletion or assess the efficacy of repletion.<br>• Orotic acid is found in bovine milk and dairy products.<br>• Elevated excretion of orotic acid has also been observed in patients with Reye's syndrome, lysinuric protein intolerance, or patients receiving treatment with allopurinol. |
| **Microalbumin** (Albumin) | • Albumin is not normally found in the urine. Temporary dysfunction of the filtration barrier can occur under certain conditions including fever, dehydration, a urinary tract infection, and after vigorous exercise, allowing small amounts of albumin through the barrier. Recommendations for follow-up include three measurements one month apart. Although microalbuminuria does have relatively benign causes, its presence in urine should be further evaluated for serious and chronic conditions.<br>• Many factors affect levels including gender, race, blood pressure, time of day, exercise, dehydration, smoking, hypertension, diabetes, muscle mass, and amount of food, water, and salt intake, producing up to a 40% daily variation.<br>• Endothelial dysfunction is likely to be involved in the initiation and development of microalbuminuria, initially reversible but becoming fixed with increasing vascular structural changes. |
| **Phosphate** | • Maintaining phosphate homeostasis in the body includes regulation of intestinal absorption from the diet, the rate of bone turnover, and urinary excretion.<br>• A major source of dietary phosphorus in the American diet comes from phosphate additives in processed foods and drinks.<br>• Urine levels are considered a marker of intestinal phosphate absorption. |
| **Creatinine** | • As a waste product, creatinine is filtered out of the blood by the kidneys and removed from the body in urine. The amount of creatinine formed daily is based on muscle mass, which varies with age, gender, and ethnicity. It is usually produced at a fairly constant rate in each person.<br>• Elevated creatinine can indicate kidney issues and should be evaluated with additional kidney-function testing; however, it is not an early marker for diagnosis of early disease and estimated GFR has become a more useful measurement. |
| **Oxalic Acid** | • Dietary oxalic acid intake and endogenous production of oxalates are important in the pathophysiology of calcium oxalate stone disease.<br>• Endogenous oxalic acid production occurs primarily in the liver and is influenced by dietary intake of precursors, notably ascorbic acid (vitamin C). |

# 6 – Microbial Metabolites

## Amino Acid Microbial Metabolites

Gut-bacteria fermentation of colonic protein is referred to as proteolytic fermentation. Proteolytic metabolites have been implicated in metabolic disease, including obesity, diabetes, colitis, and non-alcoholic fatty liver disease. The aromatic amino acids, phenylalanine, tyrosine, and tryptophan are microbial metabolites known to have significant impacts on health and function. Proteolytic metabolites impact both the gut and systemic circulation, initiating negative reactions, inflammatory responses, and tissue permeability, among others.

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| **4-Hydroxyphenylacetic Acid** (p-Hydroxyphenylacetic Acid) | • 4-Hydroxyphenylacetic acid is primarily a tyrosine breakdown product. *Acinetobacter, Clostridium, Klebsiella, Pseudomonas,* and *Proteus* act on food high in tyrosine or tyramine (fermented foods, such as sausages, marmite, soybean products, fish sauce, beers, and wine).<br>» *C. difficile* is known to decarboxylate 4-hydroxyphenylacetic acid to produce *p*-cresol.<br>• 4-Hydroxyphenylacetic acid is found higher in olives, American cranberries, grape wines; lower in corn, beers, oats, cocoa beans, milk (cow), evening primrose. 4-Hydroxyphenylacetic acid was higher in urine of those adhering to a Mediterranean diet.<br>• If elevated, evaluate protein intake, digestion, and gut bacteria. Quercetin has been proposed to reduce radicals of 4-hydroxyphenylacetic acid.<br>• Though the perfect test for small bowel bacterial overgrowth (SIBO) has yet to be devised, limited research has correlated 4-hydroxyphenylacetic acid with SIBO. |
| **Indoleacetic Acid** | • A product of tryptophan fermentation. If elevated, decrease protein intake and address digestion and GI issues. *Bacteroides, Clostridia*, and *E. coli* ferment tryptophan to produced indoleacetic acid.<br>• It has been found elevated in liver disease, ASD, and cancer, and has been noted as a marker of microbial activity.<br>• Indoleacetic acid can be degraded by *Bacillus subtilis,* or *Pseudomonas aeruginosa.*<br>• Indoleacetic acid has been found in many foods, such as lettuce, cherry tomato, Chinese bayberry, and okra. |

## Polyphenol Microbial Metabolites

Polyphenols are constituents found in fruits and vegetables. Most polyphenols are not well absorbed in the small intestines and undergo microbial fermentation in the large intestines, producing a significant number of metabolites. Polyphenols are excreted in the urine, after undergoing phase II conjugation. The interaction of polyphenols and gut microbiota is a two-way interaction, with polyphenols supporting the growth of beneficial bacteria or hampering the increase of pathogenic bacteria, while microbiota may act on polyphenols to increase their bioavailability and produce beneficial compounds such as polyphenol metabolites.

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| **3,4-Dihydroxy-hydrocinnamic Acid** (3,4-Dihydroxyphenyl-propionate) | • 3,4-dihydroxyphenylpropionic acid found in red beetroot, common beet, olives, and correlated with coffee intake.<br>• One of the most abundant phenolates, formed by microbial transformation of dietary polyphenols and endogenous metabolites such as dopamine, phenylalanine, tyrosine, and tryptophan. 3,4-dihydroxyphenylpropionic acid is highly correlated with homovanillic acid (HVA).<br>• 3,4-dihydroxyphenylpropionic acid has antioxidant properties and significantly inhibited the secretion of pro-inflammatory cytokines. |

## Polyphenol Microbial Metabolites – *continued from previous page...*

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| **3,5-Dihydroxybenzoic Acid** (3,5-DHBA) | • 3,5-Dihydroxybenzoic acid was highly correlated with intake of whole-grain bread and breakfast cereals, and a primary metabolite of alkylresorcinols, a biomarker for whole-grain intake. Alkylresorcinols are a naturally occurring type of phenolic lipid found in high concentrations in the outer layer and bran of cereal grain, primarily wheat and rye. |
| **4-Hydroxybenzoic Acid** (p-Hydroxybenzoate or 4-HB) | • One of the most abundant phenolates formed by the microbiota. It is a product of microbial transformation of dietary polyphenols and endogenous metabolites such as dopamine, phenylalanine, tyrosine, and tryptophan.<br>• It is found in red huckleberry, coriander, blueberry, Swiss chard, carrots, olive, sour cherries.<br>• 4-hydroxybenzoic acid increased on a low FODMAP diet, and is positively associated with *Firmicutes, Verrucomicrobia,* and *A. muciniphilia*, and negatively with *Actinobacteria.*<br>• 4-hydroxybenzoic acid is the common metabolite of all parabens, structurally related benzoic acid (without the OH group) and has potential endocrine activity. |
| **Benzoic Acid** (Benzoate) | • Benzoic acid is primarily made endogenously by gut bacteria acting upon dietary polyphenols. Benzoic acid acts as an acidifier and can inhibit pathogenic microorganisms. Benzoic acid is found in broccoli, pepper (C. annuum), fruits, corn. It is also an additive.<br>• Hippuric acid is the main metabolite of benzoate.<br>» Benzoic acid + *butyrate-CoA ligase* → butyryl-CoA + glycine + *glycine N-benzoyltransferase* (GLYAT) → hippuric acid<br>• Phenylacetic acid and benzoic acid have been proposed as a way to modulate release of glycine, glutamine/glutamic acid, and taurine, as a neuroregulatory process. Phenylacetic acid and benzoic acid have both been used clinically to scavenge glycine and glutamine for the purpose of excess nitrogen excretion in urea cycle defects. |
| **Hippuric Acid** (Hippurate) | • Benzoic acid is metabolized to hippuric acid and excreted. Hippuric acid is a normal urinary metabolite associated with microbial degradation of certain dietary components. Levels of hippuric acid rise with the consumption of fruit juice, tea, and wine, which are converted to benzoic acid.<br>• Though a defect in the enzymatic conjugation of benzoic to hippuric acid has been noted in Crohn's disease patients, research implicates altered gut microbial metabolism as the cause of decreased hippuric acid. Other research has found a positive association between *Clostridia* spp. and hippuric acid levels.<br>• Hippuric acid has been positively associated with gut diversity.<br>• If elevated, evaluate benzoic acid and glycine levels. Support with glycine if needed. |

## Isoflavone Microbial Metabolites

Naturally occurring chemical constituents that may interact with estrogen receptors to produce estrogenic or anti-estrogenic effects and are composed of a wide group of nonsteroidal compounds similar in structure and function to human estrogens. The three primary phytoestrogen groups are isoflavones, prenylflavonoids, and lignans. Phytoestrogen concentrations in spot-urine samples correlated strongly with those in serum.

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| **Equol** | • Equol is a bacterial-derived metabolite with estrogenic and antioxidant activity. Reductase enzymes secreted by the gut microbiota convert daidzein into equol. Daidzein is an isoflavone from soy, tofu, soy milk, tempeh, miso.<br>• The ability to produce equol varies among individuals, because only people who possess the intestinal bacteria capable of producing equol are regarded as equol producers. Vegetarians reported significantly higher rates of equol production.<br>• Spot-urine equol levels have been found to correlate strongly with serum concentrations.<br>• Women with PMS had a significantly higher risk of being an equol non-producer. Intake of daidzein from soy have been associated with reductions of estrogen-dependent and aging-associated disorders. Isoflavonoid-rich herbal supplement (included daidzein) improved intima media thickness of carotid arteries (CIMT) and inhibited growth of existing atherosclerotic plaques of post-menopausal women. |

## Fungal Assessment

There are some yeast strains, such as Candida, that possesses the ability to biotransform arabinose, glucose, or glycerol to arabinitol.

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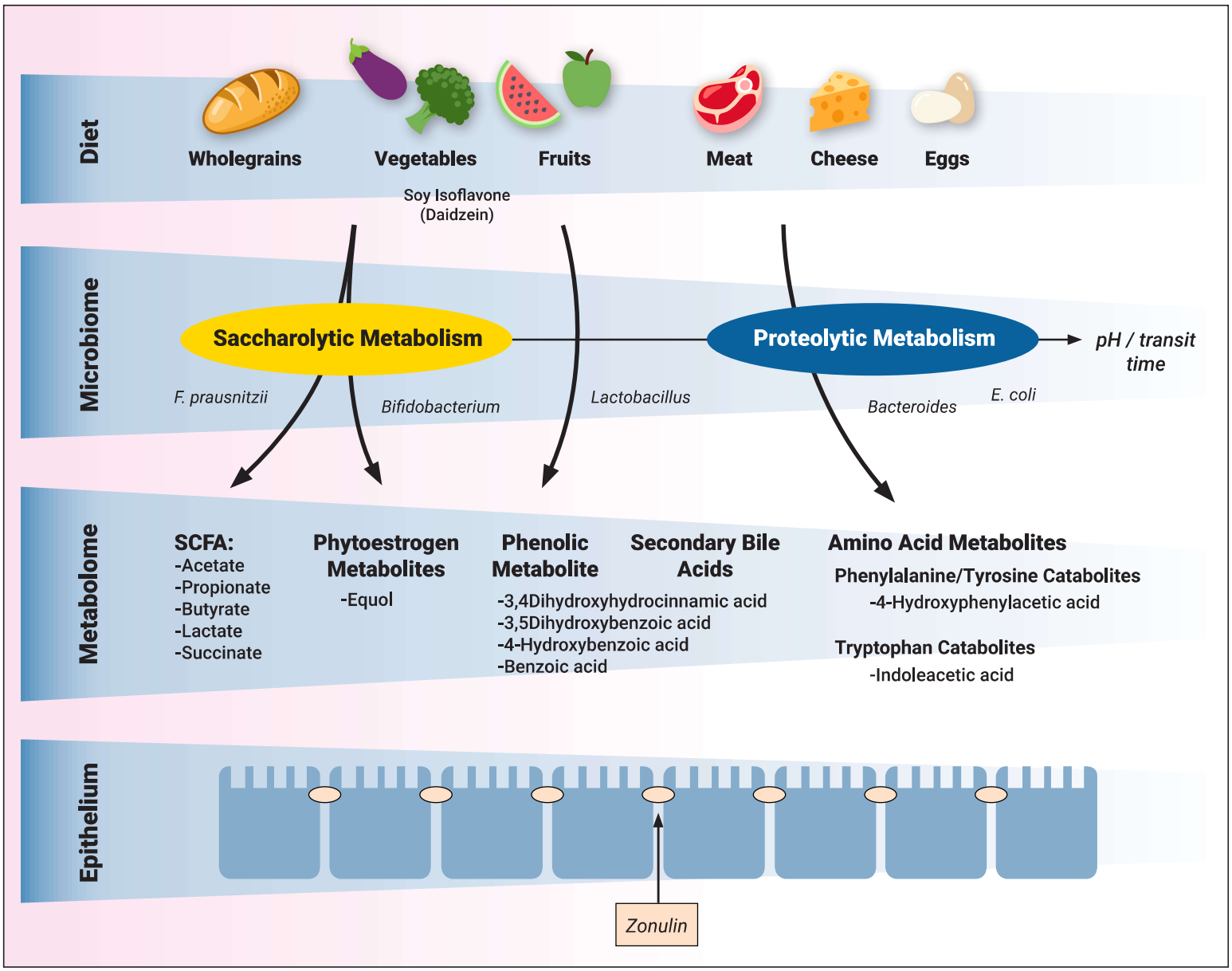
| **Arabinitol** | • Evaluate for consumption of foods and pharmaceuticals that contain arabinitol.<br>• Because a common substrate for the production of arabinitol in the body is glucose, reduced intake of dietary sugars is a key therapeutic area for elevated arabinitol.<br>• Urinary arabinitol has been noted as a marker for invasive candidiasis or infection by *Candida* fungal species, though other genera are capable of production.<br>• Microbiome analysis is a reasonable next step if high levels of arabinitol are found in the urine. Treatment of an imbalanced microbiome can help reduce overgrowth of pathogenic species that have been found to produce arabinitol. |

## Figure 8 - Microbial Metabolites Pathway

*Gut-bacterial fermentation of colonic protein is referred to as proteolytic fermentation. Proteolytic metabolites have been implicated in metabolic disease, including obesity, diabetes, colitis, and non-alcoholic fatty liver disease. Polyphenols are constituents found in fruits and vegetables. Most polyphenols are not well absorbed in the small intestines and undergo microbial fermentation in the large intestines. The interaction of polyphenols and gut microbiota is a two-way interaction, with polyphenols supporting the growth of beneficial bacteria or hampering the increase of pathogenic bacteria, while microbiota may act on polyphenols to increase their bioavailability and produce beneficial compounds. Metabolism of polyphenols by microbiota varies based on level and combination of polyphenols consumed, and level and variety of intestinal microbiota, as well as by age, gender, Body Mass Index (BMI), renal function, and genetics.*



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