Amino Acid Profiling
Clinical Guidelines for Determination of Preferred Specimen Choice
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Introduction
Profiling of amino acids in plasma and urine has been used to elucidate a rapidly growing number of aminoacidopathies since the introduction of partition chromatography methods in 1945. The question of whether plasma or urine may be the preferred specimen choice for amino acid testing is a frequent clinical concern in the evaluation of a patient's amino acid status. An informed decision must involve what principal clinical answers are sought and which amino acids are being tested. To state categorically that profiling of amino acids is best performed on plasma or urine is to oversimplify. The question of preferred specimen can be answered only when it is addressed to specific amino acids or to the specific type of information desired.

One commonly practiced method to judge the relative value of results from two specimen types is to ask which specimen has been most used for scientific studies. The majority of published studies have used plasma as the specimen for analysis (approximately a 3:1 plasma/urine ratio). This is primarily because most investigations have been concerned with essential amino acid status. Urine is typically reserved for studies of dietary protein intake, digestive adequacy, bone loss and muscle protein catabolic states. The aminoacidurias and aminoacidemias associated with metabolic disorders are approximately equally divided in the published research. Inherited metabolic disorders generally result in extreme elevations, and the abnormality is easily detected in either specimen type. The branched chain amino acids (BCAAs), for example, are elevated in both plasma and urine in maple syrup urine disease. The newer application of amino acid profiling of older children and adults to determine amino acid status in chronic degenerative diseases is more pertinent for this article.

Clinical Conditions Associated with Amino Acid Changes in:

**Plasma**
Cardiovascular Disease
Depression
Anxiety
Insomnia
Chronic Fatigue Syndrome
Multiple Sclerosis
Rheumatoid Arthritis
Epilepsy
Congestive Heart Failure
Impotence/Erectile Dysfunction
Pain syndromes
Multiple Chemical Sensitivities
Detoxification Disorders
Autistic Spectrum Disorders
Alzheimer’s Disease
Hypothyroidism
Arrhythmias
Hypertension
ADD/ADHD
Infertility

**Urine**
Inherited metabolic disorders
Starvation/Malnutrition
Protein intake/digestion
Alcoholism
Osteoporosis
Bladder tumors
Cushing’s Disease
Chronic Fatigue Syndrome
Celiac Disease
Muscle Catabolism

Amino Acid Dynamics

**Plasma**
A fasting plasma specimen reflects the state of the dynamic flux of amino acids leaving sites like skeletal muscle and flowing into sites of utilization in liver, brain, and other tissues (Figure 1). Amino acid levels in plasma reach their homeostatic balance point making a fasting specimen ideal for repeated measurement to monitor progress. The principal factors effecting changes over time are dietary intake, digestive efficiency, hepatic uptake, and the ability of skeletal muscle to maintain sufficient rates of transamination. The amount of an essential amino acid in plasma determines the rate of any dependent process in the tissues. For example, low plasma tryptophan results in reduced formation of serotonin in the brain.

![Figure 1. Plasma Amino Acid Dynamics](image)

**Urine**
Twenty-four hour urinary amino acids have been measured in the evaluation of specific clinical conditions. In many cases the research represents disruption of normal amino acid metabolism as a result of the disease process and the short-term changes in plasma amino concentration. A 24 hour urine amino acid analysis reveals amino acid metabolism throughout the period of metabolic stress of digestion and daily activity. This aspect is of particular value for evaluating those amino acids that primarily reveal tissue degradation, such as hydroxlysine and hydroxyproline, which are released from collagen of connective tissue and bone.

Clinical Categories Assessed via Amino Acid Profiling

**Gastrointestinal Function**
Amino acids and their derivatives provide some useful markers that can reflect gastrointestinal function, specifically protein digestion capacity. The normal digestion of dietary...
protein results in free-form amino acids and short-chain peptides. Recent (i.e., 3 days) dietary protein intake has little influence on plasma amino acid profiling. A fasting plasma specimen highlights the dynamics of homeostatic maintenance of the free form amino acid pool, which is remarkably stable, independent of diet. In contrast, 24-hour urine analysis of amino acids more clearly elucidates recent protein intake based on the activities of the previous 24-48 hours. In feeding young men a protein mixture (patterned after egg protein) specifically devoid of methionine and cysteine for eight days, fasting plasma methionine and cystine levels showed little change during the eight-day period. Urinary levels of methionine decreased markedly within a few days after feeding of the experimental diet, suggesting urinary amino acids are more useful to monitor short-term changes in protein intake. However, plasma levels are the preferred way to assess long-term adequacy and dynamics of amino acid utilization.15

Abnormal amino acid patterns can correspond to what may be wrong in protein nourishment or digestion. The patterns seen may reflect dietary protein deficiency, and/or malnutrition. Hyperaminoacidemias and hyperaminoacidurias typically indicate genetically inherited metabolic enzyme impairments or transport problems, not digestive enzyme impairments or insufficient stomach acid secretion. Low levels measured among the essential and some of the semi-essential amino acids reflect dietary and uptake problems. For example, the essential amino acid histidine is required to make histamine, an important digestive function, which occurs early in the stomach. Low plasma or urinary histidine may then suggest impaired ability for optimal protein digestion. Low levels of the aromatic amino acids tryptophan, phenylalanine, and tyrosine — may indicate inadequate stomach acid (HCl) secretion as this is critical to activate pepsin-mediated protein digestion. Clinicians must remember to consider renal function in evaluation of urinary amino acids, however, as patients with renal failure may show decreased creatinine measurements, resulting in skewed levels upon measurement and subsequent interpretation.

In select circumstances, elevations in urine amino acids can serve as disease markers. For example, hydroxyproline appears to be a hallmark for celiac disease and other malabsorption states, with the greatest hydroxyproline excretion occurring in those patients with the most pronounced steatorrhoea.6 This is believed to reflect an increased turnover of collagen and may be related to the osteomalacia sometimes accompanying malabsorption.

Cellular Energy Production
Fatigue may be one of the most commonly reported medical complaints heard by clinicians today. Amino acid deficits may be related to the cause of fatigue. Amino acids undergo transamination reactions which supply intermediates to the citric acid cycle in order to facilitate mitochondrial oxidative phosphorylation; or more meaningful to the patient, cellular energy production.7 Citric acid cycle intermediates are produced from aspartate, tyrosine, phenylalanine, isoleucine, valine, methionine, glutamine, histidine, arginine, proline, glutamate, and beta-alanine. Despite a significant lack of clinical research on urinary amino acids for assessment of fatigue syndromes, one study of interest has emerged in which strong associations of beta-alanine in urine with chronic fatigue symptom expression has suggested a possible molecular basis in the development of an objective test for chronic fatigue syndrome.8

Amino Acid Profiling

There has been increasing interest in the mechanisms behind central (brain-related) fatigue, particularly in relation to changes in brain monoamine metabolism and the influence of specific amino acids on fatigue.9 Central fatigue has been implicated in both chronic fatigue syndrome10 and postoperative fatigue.11 Evidence continues to emerge demonstrating increased ratios of plasma tryptophan to branched-chain amino acids may be responsible for the central fatigue seen in long, sustained exercise and post-surgery.12-14 The literature abounds with clinical studies on fatigue, with an overwhelming preponderance of these studies utilizing measurements of plasma amino acids.

Detoxification
Determination of detoxification capacity is an important clinical issue for many patients with chronic illness, especially if suspected to be environmentally induced. While the role of amino acids in phase II hepatic conjugation reactions is well established, assessment of amino acid availability for optimal conjugation warrants further clarification. Of particular interest are the amino acids, glycine, cysteine, glutamic acid, taurine, methionine, glutamine, and aspartate. As urinary levels are best reserved for evaluation of short-term dietary changes or protein digestion capability, profiling of plasma pool availability is relevant to detoxification capacity. Highly targeted urinary amino acid derivatives however, such as hydroxyproline, may serve as useful biomarkers of exposure to pollution.15,16

Detoxification of ammonia is an important responsibility of the liver. The urea cycle involves a series of biochemical steps in which ammonia, a waste product of protein metabolism, is removed from the blood, converted to urea, and excreted in urine. In urea cycle dysfunction, ammonia (a highly toxic substance) accumulates, and is not removed from the body efficiently. Ammonia accumulation in the general circulation may go on to reach the brain, where it may cause neurologic damage and in severe cases can lead to irreversible brain damage and/or death. Mild hyperammonemia conditions are often seen as low plasma glutamic acid levels and high glutamine levels.17 Symptoms include headache, irritability, fatigue, mental confusion, poor concentration, and food intolerance reactions, particularly to high protein foods. At the other end of the spectrum of urea cycle dysfunction are inherited urea cycle disorders. A urea cycle disorder is a distinct genetic disease caused by a deficiency of one of the enzymes in the urea cycle, which is responsible for removing ammonia from the bloodstream.

Removal of ammonia via the urea cycle can be an important clinical issue. A case of infantile autism has been associated with inefficient ammonia detoxification as evidenced by elevated plasma ammonia and elevated plasma and urine levels of gamma-aminobutyric acid (GABA). It was postulated that elevated ammonia levels may result in higher GABA concentrations and that a link between plasma ammonia and plasma GABA exist where the concentration of GABA in the plasma is directly related to plasma ammonia concentration.18 Meanwhile, in elderly subjects, patients with Alzheimer's disease (vs. healthy controls) exhibited altered plasma ornithine and arginine concentrations,19 perhaps highlighting the long term effect of altered urea cycle function on neurodegeneration.
Amino Acid Profiling

> Neurotransmitter Metabolism

The aromatic amino acids – phenylalanine, tyrosine, and tryptophan – are converted to catecholamines and serotonin by enzymes in adrenal, intestinal, and neuronal tissue. GABA and glutamic acid exert CNS-active neurotransmission effects without any modification of their chemical structures. Plasma levels of these amino acids are known to influence CNS concentrations of the respective neurotransmitters. Schizophrenia treatments (and etiologic mechanisms) have been linked to the glutamatergic and dopaminergic excitatory amino acid systems. Alterations in plasma levels of aspartate, glutamate, glycine, and taurine have been suggested as neurochemical markers of epilepsy.

Plasma tyrosine has been proposed as a useful assessment of thyroid function. Low plasma levels of tyrosine have been associated with hypothyroidism. Tyrosine has been used as a treatment for depression and blood pressure modulation. Possible additional symptoms of low plasma tyrosine would be chronic fatigue, learning, memory or behavioral disorders, and autonomic dysfunction. High levels of stress lead to depletion of phenylalanine. The inherited metabolic disorder of phenylketonuria results in greatly elevated phenylalanine in plasma and urine. Excessive protein intake or a metabolic block in the conversion of phenylalanine to tyrosine can also elevate phenylalanine in plasma or urine.

Numerous studies have demonstrated that plasma tryptophan is an indirect marker of changes in brain serotonin synthesis. Tryptophan has been shown to help induce sleep in insomniacs due to increased serotonin production in the brain stem. Plasma tryptophan levels are increased with sleep deprivation because of decreased utilization. Low plasma levels of tryptophan have been reported in depressed patients and are correlated with the degree of depression. Used alone or with amitriptyline, the amino acid is effective against depression in general practice.

Serine is also a critical component in the biosynthesis of acetylcholine, an important CNS neurotransmitter used in memory function and mediator of parasympathetic activity. Patients suffering from episodic acute psychosis display a disturbance of serine-glycine metabolism, and a higher serine/glycine ratio is observed in depressed individuals.

Muscle Catabolism

Specific amino acids measured in urine provide insight into protein catabolism. Urinary 1-methylhistidine (1-MeHis) is a marker of beef, chicken and poultry consumption. High urinary excretion of 3-methylhistidine (3-MeHis), a component of muscle, indicates active catabolism of muscle and is an abnormal marker for excessive muscle breakdown. It has been used as such a marker in studies of clinical conditions associated with nitrogen loss, including trauma, surgery, infection and in uncontrolled diabetes. A study in Sweden looked at 3-MeHis levels to evaluate effect of alphaketoglutarate-enriched enteral feeding on protein metabolism after major surgery. Other numerous studies utilized urinary 3-MeHis in cases where limiting catabolism is the outcome being studied. Urine 3-MeHis was used to evaluate the anabolic effectiveness of supplementation with exercise. Muscle breakdown in resistance exercisers trying various post-exercise beverages was assessed via urinary 3-MeHis.

Collagen

Proline is required for protein synthesis and is metabolized into hydroxyproline, an important component in connective tissue. Therefore, high urinary levels may reflect inadequate connective tissue synthesis. Low levels of proline can indicate a poor quality protein diet and consequently prevent optimal connective tissue maintenance. Hydroxyproline is a component of collagen. High levels in 24-hour urine or plasma correlate with the increased osteocalcin secretion that is characteristic of high bone turnover. Also involved with collagen synthesis in connective tissue is the amino acid hydroxylysine (HLys), a derivative of lysine. HLys and Hydroxyproline are indicators of liver disease, however elevated HLys seems to be a stronger index of hepatic collagen metabolism in chronic liver disease.

Nutritional Markers

Abnormal levels of amino acids in plasma and urine can also indicate insufficiencies of nutrients. Specific vitamins and minerals are required for amino acid metabolism. Abnormal results from amino acid profiling may be due to deficiencies of the nutrients required as cofactors for transformation into other compounds. Low levels of essential amino acids may indicate inadequate pancreatic enzyme activity. Because zinc is required as a cofactor in several digestive enzymes, a deficiency of this element can affect overall plasma amino acid levels. Individual amino acid abnormalities are indicators of specific nutrient insufficiencies.

Because the catabolism of amino acids is a heavily utilized pathway in the liver, breakdown of branched chain amino acids (BCAAs) affords an opportunity for detecting interruptions in the pathway caused by inadequacy of vitamin B6, thiamin and/or other B vitamins. Leucine, isoleucine and valine are initially metabolized utilizing a pyridoxal-5-phosphate dependent enzyme. Continued deamination into keto-acids requires vitamins B1, B2, B3, B5 and B6. Plasma homocysteine elevations indicate a demand for vitamins B6, B12 and folate, necessary cofactors for the metabolism of this amino acid. A limitation of homocysteine as a marker for any one component in this vitamin triad is the fact that homocysteine will rise in the absence of B6, B12 and/or folate.

One study performed on cobalamin deficient rats, serine (Ser) and threonine (Thr) levels in plasma and urine were significantly elevated. After two weeks of B12 supplementation, in addition to decreased urinary methylnalonic acid, was normalization of plasma Ser and Thr. It appears that cobalamin deficiency results in impaired metabolism of Thr and Ser due to minimization of the enzymes responsible for the conversion of Ser and Thr to pyruvate.

Vitamin C is the main cofactor involved in collagen synthesis – namely the conversion of proline to HPro. Acute or chronic deficiency of vitamin C produces a significant increase in the proline/HPro ratio in urine. Supplementation with vitamin C has been used to successfully treat certain types of collagen disorders and to stimulate collagen synthesis.

Vascular Function

Vascular tension involves the cell regulator nitric oxide (NO) and its precursor arginine. A sequence of events in the endothelial cells results in NO release. NO penetrates into the underlying layer of muscle cells where it elicits release of the final modulator of muscle relaxation, cyclic guanosine monophosphate. Many of the reported effects of arginine in human health are due to NO-related cell responses. Impairment of endothelium-dependent coronary microvascular
function due to aging in particular, can be restored by L-arginine supplementation. NO plays a role in vascular homeostasis influencing vascular tone and structure. NO-mediated pathways are also investigated in understanding erectile dysfunction. In evaluating vascular function plasma arginine and/or urinary nitrates are measured. Plasma asymmetric-dimethylarginine, a NO inhibitor is another index used in similar studies. However, measurement of urine amino acids in assessment of vascular health is minimal. Homocystinuria, a genetic disorder caused by a cystathionine beta-synthase deficiency, is associated with vascular events as a result of markedly elevated circulating homocysteine. Human studies have shown that high levels of homocysteine are associated with impaired endothelial-dependent vasodilation in healthy subjects indicating that the bioavailability of NO is decreased in those with hyperhomocysteinemia. Plasma homocysteine levels are preferred in studies investigating related disorders.

Other Conditions
Urinary amino acids have been measured in the evaluation of specific clinical conditions. In many cases the research represents disruption of normal amino acid metabolism as a result of the disease process and the short-term changes in plasma amino concentration.

Patients with Cushing's disease exhibit changes in urine and serum concentrations, and renal clearance of amino acids with relationship to glucose tolerance. Normalization of cortisol levels restores amino acid status. Investigation of aminoaciduria of subjects with different types and severity or traumatic injuries shows that many amino acids are involved and that the aminoaciduria is correlated with a reduced total serum calcium. Changes in plasma and urinary amino acids were seen during diabetic keto-acidosis (DKA). A strong correlation was found between the urinary excretion of several amino acids and that of the beta-2-microglobulin characterizing tubular dysfunction, thus reflecting altered metabolic state and renal function due to DKA. Urinary phosphoethanolamine (PEA) is typically elevated in the first few weeks of life and declines throughout childhood and adolescence. Higher than normal levels of urinary PEA were seen in infants and children with impaired central nervous systems, systemic skeletal affections and hepatopathies. Urinary beta-aminoisobutyric acid has been used in several studies as a marker of urinary tract tumors and at helping to predict recurrences, while other studies have correlated this amino acid derivative in urine with leukemias and lymphomas.

Clinical Application
For evaluation of overall amino acid body status, plasma testing emerges as the method of choice. Urine amino acid assays appear to be most commonly used to diagnose genetic metabolic disorders. Muscle protein and collagen catabolism and integrity are evaluated by certain amino acids elevated in urine. Urine amino acids are typically not measured to indicate nutrient demands. For example, folate deficiency leads to increased catabolism of histidine and consequent increased urinary histidine excretion and/or its metabolites. Although an elevated histidine may indicate need for folate, the urinary organic acid formiminoglutamate is a more specific marker for folate status within the tissues.

Amino Acid Profiling

Organic Acids in Urine
There are various methods of acquiring data about vitamin status. Concentrations of vitamins can be measured in serum or blood cells. The excretory products formed from vitamins may be measured in urine. Thirdly, functional adequacy of a particular vitamin can be revealed by the urinary levels of specific metabolic intermediates controlled by the action of the vitamin. For routine clinical purposes, the most useful assay gives a clear answer to the question of whether body pools are adequate to meet current tissue demands.

To demonstrate, increased plasma or urine isoleucine or appearance of significant levels of the branched chain keto acids (not BCAAs) in urine, are markers of thiamin deficiency. Ultimately, the combination of markers most useful in assessing an individual need for a specific nutrient such as thiamin is plasma or urine isoleucine, urine pyruvate, alpha-ketoisovalerate, alpha-ketoisocaproate, and alpha-keto-beta-methylvalerate. In addition, urinary levels of organic acids formed from amino acid catabolism can be extremely useful as markers of functional adequacy of amino acids. This should be considered when answering the question of specimen selection for direct testing of amino acids. The combination of amino acids in plasma with organic acids in urine provides a more complete picture of amino acid abnormalities and becomes an exciting prospect to further assess an individual's specific nutritional needs.

Conclusion
The overall conclusion to be drawn from this discussion is that a great majority of reports documenting clinically useful information from evaluation of essential amino acids have evaluated plasma levels. We can also say that for most, but not all clinical situations, the greatest array of useful information is derived from the measurement of plasma amino acids. Plasma is especially favored when the prime consideration is the supply of the essential amino acids for optimum balance to maintain or restore health. Amino acid testing is extremely valuable in establishing nutritional therapies and understanding cellular and metabolic needs of a patient. The choice of specimen for testing should be based upon what clinical information is being investigated.

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References
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