Clinical Applications of Urinary Organic Acids. Part 1: Detoxification Markers

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Abstract

Modern instrumentation allows the measurement of organic acids in urine in their physiological concentration ranges. Eight of the compounds that are reported can serve as markers for specific toxicant exposure or detoxification challenges. Xylene exposure causes elevation of 2-methylhippurate, and orotic acid elevation reveals ammonia challenge that exceeds the capacity of the urea cycle. General hepatic detoxification stimulation by natural compounds, drugs, or xenobiotic compounds causes elevated levels of glucaric acid. Abnormalities of α-hydroxybutyrate, pyroglutamate, and sulfate can indicate up-regulated glutathione biosynthesis, impaired reformation of glutathione in the γ-glutamyl cycle, and depleted total body glutathione status, respectively. Patterns of these compounds measured in a simple overnight urine specimen help to identify focal areas of clinical concern and monitor patient responses to detoxification interventions. *(Altern Med Rev* 2008;13(3):205-215)

Introduction

A group of urinary organic acids serve as biomarkers of specific environmental exposures and detoxification system functions. Patterns of marker abnormalities provide insight into diseases possibly caused or complicated by toxin accumulation and detoxification responses. Glutathione plays an important role as both an antioxidant and an essential component of the detoxification mechanism. Multiple markers allow for a functional assessment of glutathione status and the dynamics of its synthesis and utilization by the body. Since environmental pollution is becoming recognized as a significant health factor, evaluation of the body's exposure and ability to handle this stress is vital for proper patient care.

2-Methylhippurate: A Marker of Xylene Exposure

Methylhippurates are produced when human detoxification systems operate on methylbenzene isomers. Many consumer products contain the common solvent grade of xylene, which is primarily a mixture of the ortho isomer with smaller amounts of the meta and para isomers. Exposure to ortho-xylene (1,2-dimethylbenzene) results in excretion of 2-methylhippurate.[1,2] The first detoxification step is oxidation via hepatic P450 oxidase enzymes to 2-methylbenzoate. This organic acid is then conjugated with glycine to form the peptide product, 2-methylhippurate (Figure 1). The conjugation reaction requires formation of an acyl ester with coenzyme A. The potential for enhancing clearance by assuring adequacy of the substrate (glycine) and the essential precursor of coenzyme A (pantothenic acid) is discussed in more detail below.

Xylene exposure may be traced to paint thinners, building products, fuel, exhaust fumes, and industrial degreasers and solvents. Spray-painting workers show elevated methylhippurate, indicating recent exposure to xylene.[3] Both spot urine specimens collected at the end

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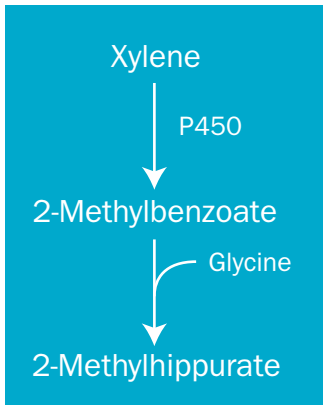
Figure 1. Conversion of Xylene to 2-Methylhippurate



of the workday and 24-hour urine specimens demonstrate high correlation with time-averaged paint-worker exposures.[4] Patient counseling in avoidance is indicated. In addition, the combination of smoking and drinking by exposed workers suppresses the conversion of xylene to methylhippurates.[5] The importance of screening for methylhippurates in human urine to evaluate occupational exposure is indicated by the development of special analytical methods for their measurement, such as micellar electrokinetic capillary chromatography and liquid chromatography/mass spectrometry-mass spectrometry (LC/MS-MS).[6,7]

Benzoate and Hippurate: Markers of Glycine and Pantothenic Acid Insufficiency

Benzoic acid, or benzoate, was one of the compounds first found to be elevated in urine from patients with intestinal bacterial overgrowth of various origins. Many patients with intestinal bacterial overgrowth resulting from cystic fibrosis, unclassified enteritis, celiac disease, or short bowel syndrome have elevated benzoate along with varying degrees of elevated phenylacetate, p-hydroxybenzoate, and p-hydroxyphenylacetate.[8] These products were thought to be derived from unabsorbed phenylalanine or tyrosine from dietary protein. Later reports demonstrated that bacterial catabolism of dietary polyphenols may be the predominant origin of benzoate, which is normally conjugated with glycine in the liver to form hippurate, in a manner similar to the detoxification of xylene.[9] Dietary polyphenols such as those contained in green tea generally persist into the lower small intestine because they are resistant to degradation by digestive fluids and persist in the regions of the gut where bacterial populations are abundant.[10]

Benzoic acid, a common food component used as a preservative in packaged foods such as pickles and lunch meats, also occurs naturally in cranberries and other fruits,[11] a factor to take into account when interpreting elevated hippurate levels in urine. Whether the source is dietary intake or jejunal bacterial metabolism, benzoate should be rapidly converted to hippurate by conjugation with glycine. Glycine and pantothenic acid can be limiting factors in this process. Availability of glycine is easily limited when demand is high. Elevated benzoate is a confirmatory marker for inadequacy of glycine or pantothenic acid for conjugation reactions.[12,13] Abnormalities of urinary benzoate and hippurate may reveal clinically significant detoxification or dysbiosis. High benzoate indicates poor detoxification via phase II glycine conjugation. Interpretations of other scenarios are summarized in Table 1.

Orotate: A Marker of Ammonia Accumulation

When there is insufficient capacity for detoxifying ammonia via the urea cycle, carbamoyl phosphate leaves the mitochondria and stimulates the synthesis of orotic acid (orotate)[14] (Figure 2). Increased orotate production is a sensitive indicator of arginine deficiency.[15] Symptoms that develop following arginine deprivation can largely be accounted for by a decreased efficiency of ammonia detoxification and reduced formation of nitric oxide. Common symptoms of ammonemia are listed in Table 2. Mild or late-onset forms of inborn errors of urea-cycle enzyme defects, such as ornithine transcarbamylase (OTC) deficiency, may also lead to increased orotate and ammonia levels.[16,17] Increased orotate biosynthesis is observed with increasing ammonia concentrations in rat, mouse, and human liver. Orotate production is reduced by *in vitro* arginine supplementation due to stimulation of urea cycle activity.[18,19]

Table 1. Patterns of Benzoate and Hippurate

Benzoate	Hippurate	Other Bacterial Markers	Interpretation
Low	Low	No elevations	Low intake of benzoate and precursors, plus normal or low dietary polyphenol conversion by intestinal microbes
		Multiple elevations	Low intake of benzoate and precursors with intestinal microbial overgrowth of species that do not metabolize dietary polyphenols (very rare)
High	Low	No elevations	Glycine conjugation deficit (possibly genetic polymorphic phenotype if hippurate is very low); dietary benzoate or precursor intake
		Multiple elevations	Glycine conjugation deficit; presume benzoate is at least partially from intestinal microbial action on dietary polyphenols
Low	High	No elevations	Normal hippurate production via active glycine conjugation; no indication of microbial overgrowth
		Multiple elevations	Normal hippurate production via active glycine conjugation; presume hippurate is at least partially derived from intestinal microbial action on dietary polyphenols
High	High	No elevations	Very high dietary benzoate or precursor intake with partial conversion to hippurate
		Multiple elevations	Very high benzoate load, some, or all, of which is contributed by intestinal microbial action on dietary polyphenols

Hyperammonemic attacks and urinary orotate excretion were both decreased significantly following arginine supplementation in patients with late-onset OTC deficiency.[15] Magnesium deficiency can also have adverse effects on urea cycle enzymes[20] and can be a cause of reversible renal failure.[21] In addition, magnesium deficiency favors the accumulation of orotate because magnesium is required for further metabolism of orotate to form pyrimidines. Although a normal urinary orotate level does not indicate magnesium sufficiency, high levels indicate a significant possibility of intracellular magnesium insufficiency, especially in patients with kidney failure.[22,23]

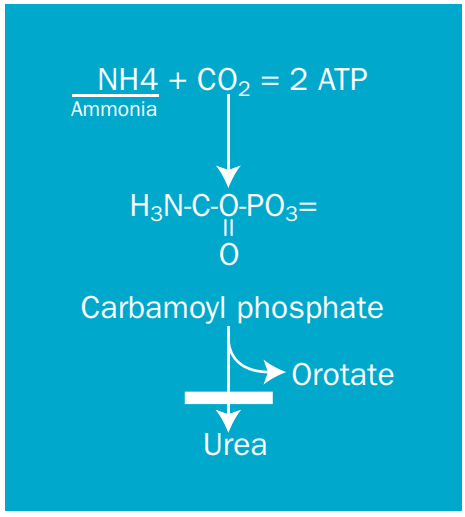
Intestinal bacterial overgrowth should always be suspected in cases of chronic ammonemia. Although cases of overgrowth severe enough to produce cirrhosis or coma are rare,[24-26] mild, transient ammonemia can be revealed by elevated urinary orotate. Oral glutamine supplementation at levels above 10 g/day in adults can result in elevation of urinary orotate due to

Table 2. Symptoms of Hyperammonemia

Anorexia
Irritability
Heavy or rapid breathing
Lethargy
Vomiting
Disorientation
Somnolence
Asterixis (rare)*
Combativeness
Obtundation
Coma
Cerebral edema

* a tremor of the wrist on extension; a.k.a. a "flapping tremor" because it resembles a bird flapping its wings

Figure 2. The Shunting of Ammonia from Urea to Orotate



increased ammonia generated from hepatic oxidative deamination reactions.[27] Although high-protein diets might be suspected of contributing to ammonemia, studies in rats have shown high-protein intake induces sufficient glutamate synthesis in perivenous hepatocytes to assist with increased ammonia production.[28] To the extent a patient with high urinary orotate is able to mount such responses, they may tolerate high-protein diets. However, genetic polymorphic effects predict that such ability may not be found in all patients.

Glucarate: A Marker of Hepatic Phase I and II Activity

Hepatic phase II conjugation reactions convert fat-soluble substances to water-soluble forms for elimination. The major phase II pathways generate mercaptan (glutathione) sulfate, glycine, and glucuronide conjugates. Markers for these pathways may be found in a profile of organic acids in urine. Assessment of the metabolic status of these major detoxification processes assists in understanding the body's capacity to detoxify foreign substances and thereby prevent long-term damage from continued exposure. Measurement of glucarate in urine serves as a specific biomarker for glucuronidation.[29] Elevations in urinary glucaric acid (glucarate) specifically suggest exposure to pesticides, herbicides, fungicides, petrochemicals, alcohol, or drugs.

Glucarate is a by-product of the predominant liver hepatic phase I detoxification reactions involving cytochrome P450 oxidation of glucose to glucuronic acid, the substrate for phase II conjugation reactions. Thus, glucarate production can reflect both the oxidation and conjugation phases of detoxification. Hepatic output of glucarate is accurately reflected by urinary glucarate, and glucarate excretion is an indicator of overall hepatic detoxification demand.[30]

The clinical significance of endogenous glucarate production should not be confused with the cancer-protective role of oral supplementation with glucarate salts, which decreases the enterohepatic circulation of carcinogens.[31] ß-Glucuronidase produced by intestinal bacteria can increase the enterohepatic circulation of carcinogens.[32] Oral d-glucarate is converted into the potent ß-glucuronidase inhibitor d-glucaro-1,4-lactone under the influence of stomach acid. Urinary glucarate is influenced by oral glucarate supplementation (usually as calcium d-glucarate) because most absorbed glucarate is cleared in urine.[33]

The liver produces glucuronate for use in phase II conjugation reactions;[34,35] for example, conjugation of glucuronate with bilirubin for urinary excretion. Many drugs, food components, and products of gut microbial metabolism are prepared for excretion by glucuronidation. The by-product glucarate can become elevated as an indication of enzyme induction due to potentially toxic exposures that induce greater rates of glucose oxidation to glucuronic acid.[36-38] Metabolic challenges that result in stimulation of hepatic P450 activity tend to produce increased excretion of glucarate. Urinary d-glucarate, for example, is elevated during rifampicin/streptomycin treatment[39] and in pesticide-exposed groups;[40] both exposures induce mixed function oxidase activity. Glucarate measurements have been advanced as useful biomarkers of xenobiotic exposure, particularly as a screening tool for xenobiotic exposure in reproductive epidemiology.[41]

Long-term exposure to environmental pollutants and continued stress on systems of detoxification can lead to oxidative stress, high levels of P450 activity, and reduced capacity for phase II conjugation reactions. Patients suffering from toxic burdens may experience a wide range of symptoms, including fatigue, headaches, muscle pain, mood disorders, and poor exercise tolerance. Researchers have reported many chronic fatigue syndrome patients have disordered liver detoxification ability and show signs of increased toxic exposure.[42]

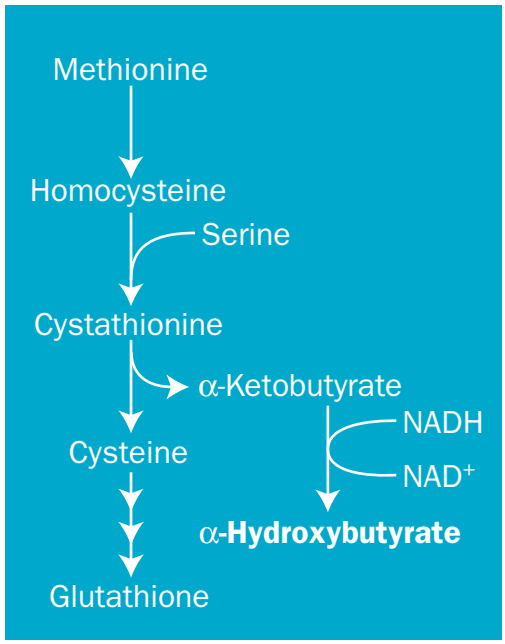
The ability of the laboratory to measure glucarate is determined by the type of sample preparation chosen. Glucarate is extremely water soluble and does not move into the organic solvent layer when methods requiring solvent extraction are used. Newer methods utilizing LC-MS/MS technology can provide accurate measures of glucarate because no solvent extractions are performed.

Three Markers of Glutathione Status

α-Hydroxybutyrate (2-Hydroxybutyrate)

α-Hydroxybutyrate (AHB) is produced as a by-product during the conversion of cystathione to cysteine in the methionine-to-glutathione pathway (Figure 3). The activity of this pathway is highly variable, changing in response to demands for protection against oxidative stress. As oxidative stress increases, the flow of homocysteine shifts away from transmethylation to methionine toward transulfuration to cystathionine, in order to increase the flux of cysteine into glutathione synthesis.[43] Thus, AHB production is directly related to the rate of hepatic glutathione synthesis.

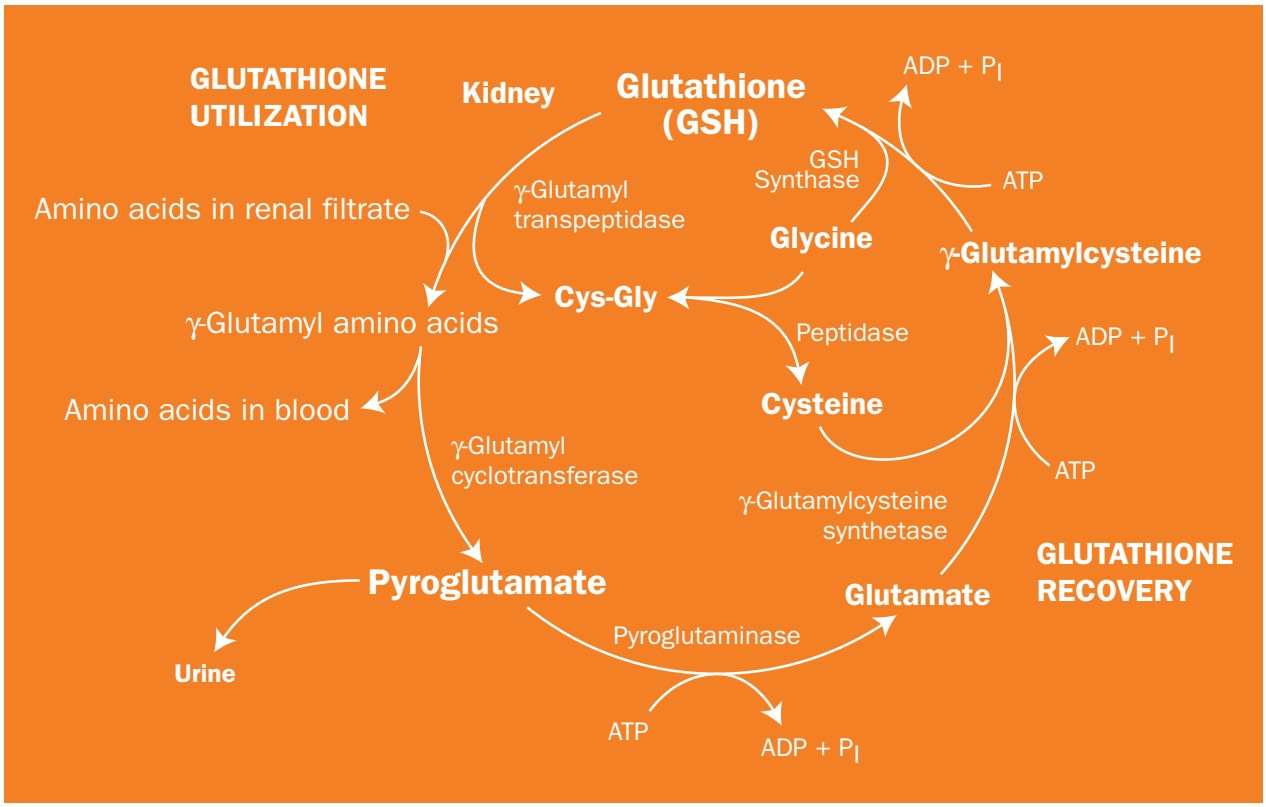
Figure 3. α-Hydroxybutyrate, a By-Product of the Pathway from Methionine to Glutathione



The enzyme α-hydroxybutyrate dehydrogenase (AHBD) catalyzes oxidation of AHB to acetate, especially when the NADPH/$NADP^+$ ratio is low. However, studies on rat liver show that mitochondria maintain a high NADPH/$NADP^+$ ratio needed to recycle glutathione to the reduced state.[44] Elevation of this ratio has been proposed as the explanation of very high AHB excretion in extreme situations such as heavy alcohol consumption.[45] Any conditions that result in high activity of carbohydrate oxidation can raise the NADH/NAD^+ ratio, because the cytosolic oxidative pathways increase NADH. Alcohol consumption and insulin-stimulated glucose uptake raise the NADH/NAD^+ ratio, generating reductive stress.

The activity of AHBD on the second day after a myocardial infarction is a marker for estimates of infarct size and a measure of reperfusion effectiveness.[46] The intense energy demand of cardiac muscle is likely the reason for such high concentrations of AHBD in

Figure 4. The γ-Glutamyl Cycle of Amino Acid Recovery Production



that tissue, because AHB strongly inhibits mitochondrial energy metabolism as measured by CO_2 production.[47]

Smoking, poor diet, and lack of exercise significantly inhibit the activity of AHBD, suggesting that urinary elevation of AHB may be related to these factors via increased glutathione demand.[48] High AHB is also found during phases of increased lymphocyte destruction in infectious diseases such as measles.[49] Elevated AHB is found in birth asphyxia and in inherited metabolic diseases such as "cerebral" lactic acidosis, glutaric aciduria type II, dihydrolipoyl dehydrogenase (E3) deficiency, and propionic acidemia.[47] All of the conditions associated with increased AHB excretion may be related to increased rates of hepatic glutathione synthesis from methionine.

Elevated AHB thus shows increased flow through the hepatic glutathione synthesis pathway as required during times of increased oxidative stress or for detoxification functions. Other signs, such as urinary pyroglutamate and sulfate discussed below, must be assessed to determine a patient's ability to sustain this flow. It is also possible to find some patients in late stages of chronic glutathione and methionine depletion who have such low capacity to generate the transulfuration flow that their AHB is normal. These patients generally have low plasma methionine and taurine.

Pyroglutamate (5-Oxoproline)

Pyroglutamate is created in the γ-glutamyl cycle (GGC), a pathway highly active in renal tubules and anywhere there is a high demand for glutathione (Figure 4). When cytosolic glutamate is abundant, the GGC functions without forming γ-glutamyl amino acid bonds to achieve net glutathione synthesis. The amino acid reabsorbing function of the GGC may be unique to renal tissue. When γ-glutamyl peptide bonds are broken the reaction produces the cyclic form of glutamate

(pyroglutamate) that must be opened by 5-oxoprolinase to regenerate glutamate. In healthy individuals, a very modest amount of pyroglutamate is spilled in the urine because most of it is reconverted to glutathione.[50] The GGC is also active in the liver, and the enzyme that initiates the cycle, γ-glutamyl transpeptidase (GGT), is very abundant in hepatocytes. This fact has led to the use of serum GGT activity as a primary differential diagnostic tool for liver disease because damage to hepatocytes causes release of the enzyme into the blood.[51,52] Although liver pathology is indicated when serum GGT is elevated, other signs appear in cases of GGT deficiency.

Gene defects are known to cause GGT deficiency. Cell regulation by leukotriene production is affected in individuals with GGT deficiency because lack of transpeptidase activity causes failure of cysteinyl-leukotriene C4 (LTC(4)) to be cleaved to LTD(4), producing a total lack of LTD(4).[53] Growth failure, shortened life span, and infertility are found in mice with genetic GGT deficiency.[54] A separate gene defect causes defective activity of the enzyme 5-oxoprolinase that converts the ring form to the open-chain glutamate. Hereditary 5-oxoprolinase deficiency can produce profound pyroglutamic aciduria. Developmental delay has been reported in human cases of 5-oxoprolinase deficiency.[55,56]

Glutathione synthase deficiency is the most common genetic defect seen in the GGC pathway.[57] The autosomal recessive form of this defect in the GGC pathway causes recurrent kidney stones; whereas, the homozygous expression results in vomiting, diarrhea, and abdominal pain.[58,59] Extreme pyroglutamic aciduria (also known as 5-oxoprolinuria) is found in patients with a polymorphism in glutathione synthase characterized by metabolic acidemia, hemolytic anemia, and central nervous system damage.[60] This enzymatic defect causes decreased levels of cellular glutathione that greatly stimulates the synthesis of γ-glutamylcysteine. Since this intermediate cannot be utilized due to the defective enzyme, it is cleaved to cysteine that can be utilized for protein and peptide synthesis along with the pyroglutamate that simply spills into the urine. Enzyme polymorphisms are often mild enough to escape detection until late in life, if at all. Complaints of malaise and anorexia by a 48-year-old man were idiopathic until organic acid profiles revealed the cause of recurrent high anion gap as consistent pyroglutamic aciduria, indicating a genetic alteration in the γ-glutamyl cycle.[61] Other cases of mild impairment may become noticed only when drugs like acetaminophen place stress on hepatic glutathione pools and pyroglutamic aciduria causes elevated anion gap.[62]

Chronic failure to recover glutathione via the γ-glutamyl pathway causes increased flux of hepatic cysteine to glutathione that leads to reduction in sulfate excretion (see below). Studies showing an age-related decline in expression of glutathione synthase in rat liver suggests this problem may be more prevalent in older patients.[63] Preformed glutathione or supplemental N-acetylcysteine (NAC) may be used along with antioxidant supplementation to build glutathione levels.[64-66] In addition, ATP (as Mg^{++}-ATP) is required in three steps of the GGC. Compromised mitochondrial energy production can contribute to elevated urinary pyroglutamate. Alternatively, drugs that require glutathione conjugation can cause failure to sustain glutathione adequacy. This effect has been reported in acetaminophen toxicity.[67] Elevated pyroglutamate due to such drug use indicates a mild polymorphism that is revealed only under increased detoxification stress. In mammary tissue the γ-glutamyl cycle may serve as a signaling pathway where pyroglutamate stimulates amino acid metabolism. The over-use of NAC and other glutathione inducers may cause adverse metabolic stress, so monitoring of progress with laboratory evaluation is warranted.[68]

Burn patients excrete higher than normal amounts of pyroglutamate, as blood levels of glutathione are lowered and rate of glycine synthesis is decreased.[69] The possibility of limitation of glutathione reformation by glutathione synthase being affected by the supply of glycine has been demonstrated in several ways. Dietary glycine restricts glutathione formation similarly to the effect produced by limiting sulfur amino acid availability.[70] Urinary pyroglutamic aciduria has been proposed as a marker for glycine deficiency.[71] During dietary protein restriction, glutathione synthesis is limited. If concurrent glycine conjugation demand is increased in healthy human subjects, then pyroglutamate excretion increases because of lowered availability of glycine. Under these conditions urinary pyroglutamate is positively related to urinary sulfate in a linear manner over the entire sulfate concentration range.[72] These results indicate the diversion of methionine-derived cysteine

from glutathione synthesis to sulfur synthesis as glycine limitation worsens.

Small amounts of pyroglutamate are always present in overnight urine because it is produced as an intermediate in a cycle used in the active transport of amino acids in renal tubules. Since the appearance of a micromole of pyroglutamate in urine is accompanied by the recovery of a micromole of amino acids into the renal blood supply, glutathione is essentially "wasted" in order to prevent massive essential amino acid loss. Up to one-third of the glutathione circulating in blood may be used in this amino acid recovery process.[57]

Since pyroglutamate can be formed by heating of foods that contain high amounts of glutamic acid, some urinary pyroglutamate may have dietary origins. Foods high in glutamic acid include artificial diets that use glutamate as a flavor enhancer and high-protein foods, such as meats, eggs, and dairy products. This effect is of particular concern in neonatal diagnosis that compares normal infants consuming breast milk with infants on heated formulas.[73] Urinary pyroglutamate reference ranges established on large out-patient adult populations will reflect normal intake of dietary pyroglutamate. NAC is an effective oral agent for rebuilding total body glutathione; oral taurine supplementation also enhances glutathione levels by sparing cysteine demand while providing an effective antioxidant to assist glutathione.

Sulfate

Sulfate is the ionic form of an inorganic rather than organic acid. It may be included on panels of urinary organic acids because of the important information it provides about detoxification status. For example, protein synthesis disruption associated with zinc deficiency causes decreased incorporation of cystine into proteins, with concurrent large increases in urinary sulfate and taurine from cysteine degradation.[74] The rise in sulfate parallels the depth of zinc deficiency, indicating that, if other sulfate sources are normalized, elevated urinary sulfate can be a metabolic marker of the severity of zinc deficiency.

The sulfation pathway is used in phase II liver detoxification for biotransformation of many drugs, steroid hormones, phenolics, and other classes of compounds. The addition of a sulfate group increases water solubility of hydrophobic compounds in preparation for urinary excretion. The ratio of urinary sulfate to creatinine is used to assess total body reserve of sulfur-containing compounds (especially glutathione) used in phase II pathways.[75] When the ratio of sulfate to creatinine is low, these stores need replenishment. Glutathione administration with oral NAC, taurine, and salts of sulfate are used in combinations to replenish sulfur pathways and restore the hepatic supply of inorganic sulfate.[76]

Severe depletion of organic sulfur sources will cause simultaneous high pyroglutamate and low sulfate excretion. High pyroglutamate with normal sulfate indicates inadequate organic sulfur sources for production of cysteine required for glutathione synthesis. Only organic sulfur in the form of compounds such as NAC or methionine, along with adequate glycine, will restore normal glutathione levels. Normal urinary pyroglutamate with low sulfate levels can occur in individuals with impaired sulfate activation. In these cases, rapid replenishment of hepatic sulfate may be accomplished with either sulfur donors like NAC or inorganic sulfate such as sodium sulfate.[75]

On the other hand, a well-nourished patient under temporary metabolic stress (for example, detoxification from use of acetaminophen) may have elevated α-hydroxybutyrate, signaling the increased rate of hepatic glutathione synthesis. However, such a patient may have no need for amino acid or glutathione therapy due to normal pyroglutamate and sulfate levels. An individual with limited ability to produce glutathione may show the glycine depletion pattern of high pyroglutamate and low sulfate. This patient is a candidate for glutathione administration along with glycine and NAC or methionine. In addition, taurine status should be monitored since depletion of other sulfur-containing compounds is likely. Elevation of urinary sulfate has also been shown to reflect intake of sulfating agents widely used as food additives.[77]

Conclusion

The eight compounds discussed here make up one category of functional markers from a full profile of urinary organic acids. Insufficient ammonia disposal and poor glutathione status are among the most frequent detoxification issues that contribute to symptoms

of chronic disease. Four of the markers in the detoxification category reveal these issues, while the other four indicate xenobiotic exposure, general hepatic detoxification up-regulation, or glycine conjugation deficits. Abnormalities in these compounds may be tied to other organic acidurias, such as elevated markers indicating mitochondrial inefficiency that restricts ATP to drive the urea cycle. Such patterns will be explained in future articles. The full power of organic acid profiling is the ability to identify clinically relevant problems by the reinforcing patterns. The full profile can reveal much about the metabolic, nutritional, and toxicological aspects of human health. Identifying abnormal patterns can assist clinicians in successfully targeting therapies to a patient's individual need, thereby improving outcomes.

Disclosure

Metametrix Clinical Laboratory provides urinary organic acids testing. The authors, Richard Lord, PhD, and Alexander Bralley, PhD, CCN, are chief science officer for Metametrix Institute and founder and CEO of Metametrix Clinical Laboratory, respectively.

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