A look at Organic Acid Analysis as a Functional Tool

Organic acids, also called carboxylic acids comprise key intermediary compounds of virtually all biochemical pathways as well as exogenous compounds. The citric acid cycle (Kreb’s cycle) in the mitochondrion, for example, comprised of nine organic acids and eight enzymes, is the central metabolic pathway for all fuel molecules; dietary carbohydrates, proteins, and fats. Deficiencies in any of the Kreb’s cycle enzymes causes an inefficient cycling of the acid intermediates - any number of which consequently increase in the urine of the affected individual. Metabolic defects such as these are commonly known as organic acidurias; a well-established group of disorders classified under the term of Inborn Errors of Metabolism (IEM’s). In these patients, urinary organic acid profiling reveals elevated excretion of acid intermediates due to a block in a metabolic pathway; a block which may arise from a defect in the required enzyme or transport protein of the pathway in question. Inborn errors of metabolism are generally rare, occurring in 1 in 5000 live births. Presentation may occur at any age with a broad range of clinical manifestations of which may be due to either toxic accumulations of substrates upstream of the metabolic block, deficiencies of products downstream of the block, or from intermediates of alternative biochemical pathways.¹

Capillary gas-liquid chromatography/mass spectrometry (GC/MS) of trimethylsilylated derivatives has been the primary method of organic acid analysis, and has contributed greatly to the understanding of many organic acidurias. GC/MS profiling has proven to be an essential method of analysis in clinical chemistry, without equivocation, during the last three decades, primarily for its high-efficiency resolution, and reproducibility.

GC/MS profiling for disorders other than IEM’s has become a useful tool in medicine, especially for the functional investigation into metabolic changes associated with a number of defects. GCMS profile analysis of ketone bodies and other organic acids in diabetes mellitus, for example, has helped recognize this defect as a disorder of glucose, fatty acid ands amino acid metabolism.² This method of analysis has also helped greatly in the identification of a number of vitamin/nutrient deficiencies. The utility of organic acid profiling for the assessment of vitamin insufficiencies is not a new concept, and has been used extensively for example, in the assessment of biotin deficiency, represented by elevations in urinary 3-hydroxyisovaleric acid, and vitamin B12 deficiency as represented through elevations of urinary methylmalonic acid. Clearly, a comprehensive analysis of organic acids from urine holds the potential for a wealth of information on the physiological and pathophysiological status of different metabolic pathways and their interrelationships in the body, of which may provide a wealth of clinically relevant information for patient care.

For a few considerations to put this complex yet very useful functional test in perspective, consider a few scenarios.

As any clinician knows, the clinical significance of any result, as it relates to the patient, is at the doctors discretion taking into consideration a full subjective history and presenting symptoms, in addition to other signs.

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With regards to a low value for citrate, for example, we need to consider how the other analytes in this cycle present. If the other analytes in the TCA cycle are low as well. This may suggest an overall depletion in cellular energy production through aerobic respiration. On the other hand, an isolated depletion of one analyte, citrate, may simply reflect a deficiency in the associated enzyme complex and/or cofactor upstream of this analyte, resulting in a relative deficiency downstream in the metabolic pathway.

Another example, a low homovanillate, or other catecholamines, may warrant further evaluation based on other presenting signs and symptoms for competency in catecholamine output, and hence, adrenal status.

Again, the QRG (Quick Reference Guide) presents considerations primarily for elevations of these analytes in the urine, as it is believed that under ideal situations the levels of these analytes in the urine should be negligible. Also, the QRG is not a chart of diagnoses but a list of suggestions that the doctor may want to investigate with further research pertaining to the patient’s particular case.

Another example, quinolinate, a metabolite of the essential amino acid tryptophan in the kynurenine pathway. This pathway is chiefly activated by IFN - gamma and IFN - alpha. Quinolinate is markedly elevated in the CNS following trauma or inflammation, and is implicated in neuronal injury through activation of the N-methyl-D-aspartate (NMDA) receptor.

Another example, pyroglutamate, involved in the biosynthesis and degradation of glutathione. Individuals with a deficiency in this pathway usually present with elevated pyroglutamic acid (5-oxoprolinuria) with resulting low intracellular levels of glutathione. Because pyroglutamate also has other roles in physiology - component of thyrotropin releasing hormone, and a component of heavy chains of immunoglobulins - a clinical consideration into the health status in these arenas may be an indication in a certain instance.

Another example, as listed in the QRG, elevated orotate may suggest insufficient detoxification of ammonia load through the urea cycle suggesting therefore a hyperammonemia. As you know, orotic acid is an important indicator of disorders of ammonia metabolism. Literature on Inborn Errors of Metabolism defines orotic aciduria as a disorder of the urea cycle that lead to accumulation of carbamoyl phosphate in mitochondria (i.e.- ornithine transcarbamoylase deficiency). The medication, allopurinol also produces an orotic aciduria due to inhibition of the enzyme orotidine-5-phosphate decarboxylase (Bhagavan, Medical Biochemistry, 1992. Blau et al. Physician’s Guide to the Laboratory Diagnosis of Metabolic Diseases, 2003). The bottom line here - hyperammonemias may be caused by a number of factors in which ammonia buildup exceeds its clearance - IEM's, organic acidemias, medications, liver pathology, and neurodegenerative disorders, in particular ALS with abnormal glutamate metabolism (not listed on Flow Chart) where ammonia is not detoxified sufficiently.

Below is a sampling (not inclusive) of references in no particular order that may be of interest.

- Plaitakis Andreas, Shashidharan P. Amyotrophic Lateral Sclerosis, Glutamate, and Oxidative Stress. 2000.
- Sweetman L. Organic Acid Analysis. ibid.


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