Lab 101 : Interpreting and Using Lab Tests

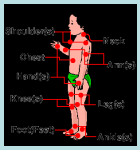
Metametrix
Laboratoire Philippe Auguste
Genova Diagnostics — Building on the Innovation of
DDI Doctors Data
The Great Plains Laboratory, Inc. — Health, Metabolism & Nutrition
LabCorp
Quest Diagnostics
EAN European Laboratory of Nutrients — Vitamin Diagnostics Inc.

Today, we are going to learn some rules about interpreting lab testing.

1. How to understand compartmentation issues
2. How to interpret reference ranges
3. How to look at the data in new ways
4. When to be sceptical about lab recommendations
5. How to see your child's results in context with others with autism

Lab tests can only tell you what is going on in one place:

Blood
Urine
Cerebral spinal fluid
Biopsied tissues
Blood cells

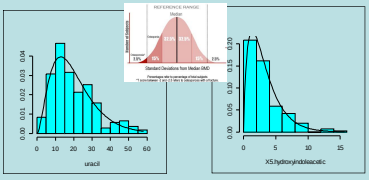


We cannot assume that blood and urine represent the whole body. These two compartments won't "agree"!

Scientific studies tell us what measuring specific things in each compartment may mean.

Urine and blood test values typically are NOT normally distributed.

Unfortunately, people who set up the standards of practice so long ago were not statisticians and did not realize that the normal distribution was an inaccurate model. This creates some snafus in making and interpreting reference ranges. More on this later.

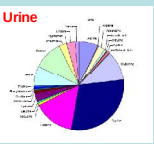


CSF

Normal levels of amino acids are not alike in different places!

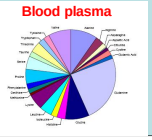
The colors represent the same amino acids in each compartment.

Urine



There are rules that govern amino acid levels in blood that are not the same as the rules that govern their levels in CSF or urine!

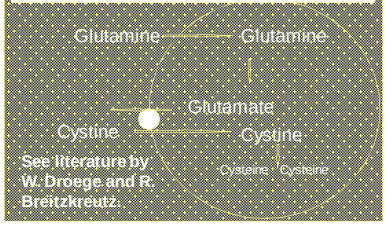
Blood plasma



These differences represent REGULATION that is specific to each compartment.

This does not often reflect nutrition.

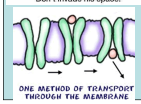
Why was glutamine so high in the CSF? Glutamine protects cells from extracellular glutamate. Glutamine is converted to glutamate inside and that allows cystine to come into cells for the formation of glutathione and may be why supplementing glutamine is so good for the gut and the brain.



See literature by W. Droege and R. Breitzkreutz.

COMPARTMENTATION

"Don't invade his space."



A compartment is a place that has boundaries. Its boundaries define a place that can be studied where a sample from that place represents the whole compartment.

Not all cells have the same needs. Liver cells have different needs from lung cells. They may "drink" from the same blood supply, but they don't "drink" the same thing.

They put some things back into blood that they don't need anymore or that is sending a message to another organ to change its priorities.

Each organ has special capabilities and knows it will be recruited to send something to other organs in response to a need that cannot be met "locally".

Specific transporters regulate the movement of substances between compartments, both in and out. These proteins make up a THIRD of our genome!

Have you ever wondered why sometimes when you give a child a supplement he really needs, that the child's symptoms will first get suddenly worse?

When cell's cannot find and bring inside enough of a substance (like a vitamin) into the cell, they dial up the transporters and receptors that service that substance. They are trying to make the most of a short supply. When you suddenly introduce a much greater quantity of that substance, it overwhelms all the adjustments the cell had made.

So, go "low and slow". Take eight days to get to a full dose.

Realize that missing another factor that now becomes important may be putting a wrench in the situation.

Never follow a hard rule like "My child doesn't tolerate B vitamins." Find out why with metabolic testing (like the organic acid test) and then see if you can fix the problem!

If you want to see where your child's metabolism needs help, DON'T get him tested when he is well unless you are getting a baseline.

Test him when he has suddenly gotten worse!

Then you can compare the good day with the bad day and see where his chemistry was struggling. Lab testing can give you some needed objectivity about these meltdowns…

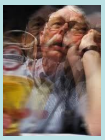
Ask your doctor to let you keep a kit at home so you will be ready to collect urine on bad days, or when he is sick!

Do you go into scrambling for a "quick fix" when your child suddenly has changed mood or behavior?

Is your quick fix repairing the body, or is it delaying correcting the problem? Lab testing can help sort that out.

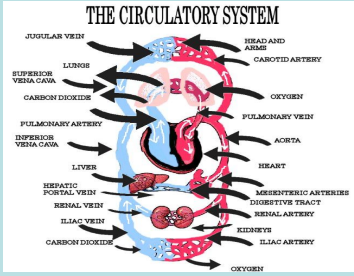
There is such a thing as a "healing crisis".

Symptoms may get worse first when you are detoxing from oxalate or even gluten, and some changes you make may stop the symptoms, but ….will it stop the process that leads to healing?



We are learning more about this with careful study of lab reports before and after therapies.

What you do to stop symptoms can either help or hurt the eventual outcome, regardless of which metabolic problem is causing the symptoms.



Sometimes, something is elevated in the blood because it is a signal. That happens a lot with amino acids. They may be increasing to give a message from one organ to another to respond to a changed condition in the body.

The way to identify a nutritional need, is to see if essential amino acids are lower than the non essential. Some non-essential amino acids become conditionally essential when certain pathways are blocked.

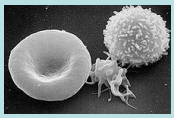
Essential	Nonessential
Isoleucine	Alanine
Leucine	Arginine*
Lysine	Aspartate
Methionine	Cysteine*
Phenylalanine	Glutamate
Threonine	Glutamine*
Tryptophan	Glycine*
Valine	Proline*
Histidine	Serine*
Tyrosine*	Asparagine*

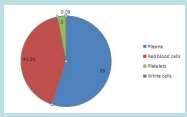
Origin of nonessential amino acids

Alanine	from pyruvate
Aspartic acid	from citric acid cycle intermediates
Asparagine	
Arginine	
Glutamic acid	
Glutamine	
Proline	
Serine	from 3-phosphoglycerate
Glycine	from serine
Cysteine	from serine (Sulfur from methionine)
Tyrosine	from phenylalanine

Labs tend to measure amino acids in blood plasma.

Red blood cells also carry amino acids and drop off them off at various organs as needed. But we don't measure them there, and they carry a different set.





Similarly, measuring nutrient levels in lymphocytes does give you ONE intracellular picture, but it cannot represent all cell types.

Will it agree with hair?

We don't know all the rules about where minerals and vitamins are stored and moved around!

Again, all this is an issue of COMPARTMENTATION. Doctor's have to go by precedents in the literature for each compartment in order to associate changes with disease processes, and there is precious little literature about hair!

Remember, the blood is a place for transportation: A highway!

Sometimes lab tests don't make distinctions that would help in interpretation.

Have you wondered why blood levels of B12 might be elevated when B12 function is extremely depressed (recognized by elevated methylmalonic acid) ?

Methyl-B12 shots can be the solution! But some doctors may think finding high B12 levels in blood could mean taking more B12 would be dangerous.

Did you know this need might have to do with a need to detoxify cyanide?

Cyanide is PARTICULARLY toxic in autism and it is made by the body normally.

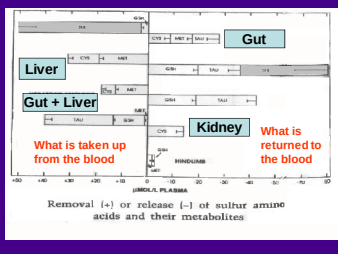
Dr. Rosemary Waring found out that in autism, an enzyme isn't working that detoxifies cyanide. Studies have shown that when this enzyme isn't working, the body will use up methylcobalamin or hydroxycobalamin to "substitute".

This produces cyanocobalamin and raises this level of "used" B12 in blood. Someone then can become functionally deficient in the key B12 cofactors.

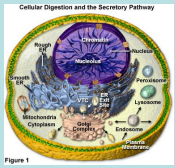
Cyanide is produced by ordinary business in the mitochondrion, and is usually detoxified there, but you can also get dietary cyanide from spinach and from nuts and seeds, especially almonds. Cyanide smells like almonds!

So when cyanocobalamin is elevated in blood (what the lab tests likely measure), that can mean that methylcobalamin and hydroxycobalamin are extremely deficient inside the cells of your body!

How sulfur amino acids move in and out of certain organ systems.



Most cells get the protein (amino acids) they need from recycling their own internal proteins, by autophagocytizing mitochondria and other organelles.



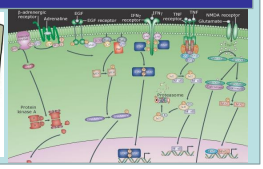
Probably, most of the time, the "maintenance" or "housekeeping" functions are adequately met by this recycling. Bringing in supplies from the outside is more important when there is some special work to do in response to a signal.

Rather than thinking of receptors as on/off switches, it may be closer to think of them as "tasters" that tell the cell how much of something is outside the cell. This is like what you do when you look in the refrigerator so you can plan what you want to cook.

All these receptors at the same time are giving information to the cell about the outside environment.

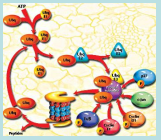
The message is all about what the cell needs to change to respond to its outside environment, like budgeting its resources.

There is a lot of information being conveyed we probably still don't know about.



Cells have two main ways to break down proteins into amino acids.

Proteasome

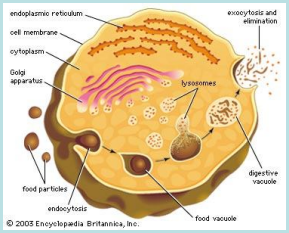


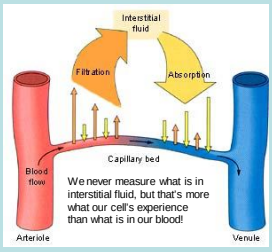
The proteasome digests proteins that have a tag on them called ubiquitin.

Lysosome

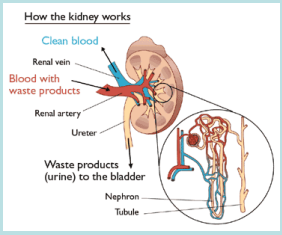
The lysosome digests globular proteins rich in cysteine.

Cells bring in outside groceries with endocytosis and get rid of wastes through exocytosis. The lysosome recycles its contents and delivers the raw ingredients back to the cell!

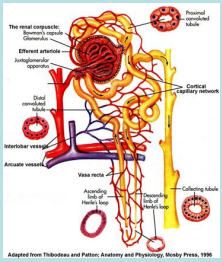




So what does urine represent?



•The urine doesn't tell us WHY we are throwing something away, or why the kidney hoards other things, but we can get a lot of hints of what is going on by noticing RATIOS of amino acids and organic acids and other substances left in urine….not absolute values.

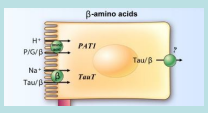


Each type of transporter has a list of substances it transports, not because they have similar biological properties, but because they are shaped the same chemically.

It is tempting to think an elevation of something marks a change in its metabolic pathway, when sometimes, it is elevated because there has been a change in levels of something else that uses the same transporter.

Also, most literature I've found on neurotransmitters in urine seems to indicate those levels tend to reflect a condition local to the kidney.

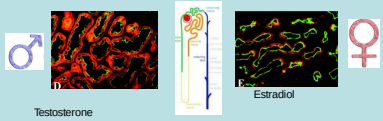
As an example. TAURINE and BETA-ALANINE travel on the same transporter. Taurine is used to balance the water inside and outside cells, so its transporter responds to signals from cells that they need a higher or lower water concentration inside the cell.



Because this also happens in the kidney, when this sort of thing is going on, beta-alanine and taurine will increase in urine. This is called "urine wasting", because it can make you deficient. Blood levels may fall. Humans get most taurine from diet so high urinary taurine is a call for more dietary taurine.

Beta-alanine being high in urine probably has nothing to do with beta-alanine's function! It just got caught in the same elevator that was called by taurine!

As another example, the OAT1 transporter (organic acid transporter) moves a whole set of organic acids in the proximal tubules of the kidney. The count of how many transporters are there to do this job is regulated by sex hormones!



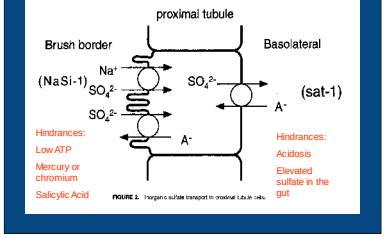
So, 4-hydroxyphenylacetic acid, 3-hydroxybutyrate, 2-oxoisocaproic, orotic, 2-hydroxyisovaleric, 4-hydroxyphenyllactic, n-acetyl aspartic acid, uracil, and 2-oxoglutarate all travel on the same "bus" across the cell membrane.

If they are high or low together, this does not necessarily indicate a change in their function, but a change in their transport.

This is the tricky thing about interpreting urine data. Just remember, it is definitely not a simple issue of low values mean you need more or high values mean you need less.

AJP - Renal Physiol July 2004 vol. 287 no. 1 F124-F138

Another example!!!

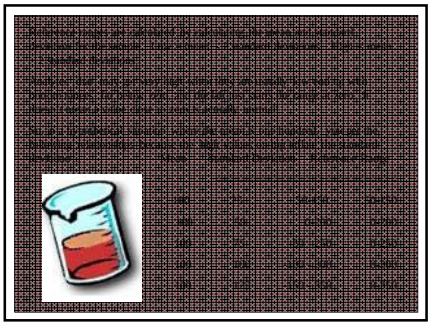


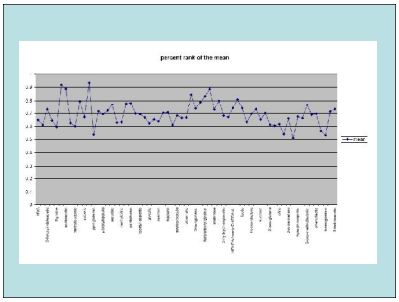
AUTISM RESEARCH INSTITUTE
Autism is Treatable

A number of years ago, I compiled a database of organic acid tests from Great Plains so I could look for patterns in the data to tell us what we needed to study to learn more about autism.

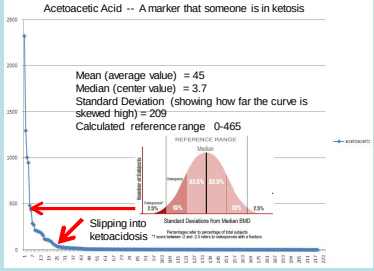
This database contains 224 organic acid tests from 106 individuals with autism.

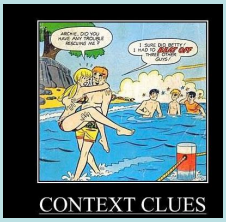
My first analysis of this data was presented to the Autism Research Institute's DAN! Thinktank, of which I've been a member almost since this thinktank was formed 16 years ago.





Acetoacetic Acid -- A marker that someone is in ketosis





On any test, the way to interpret what changes is to notice the context.

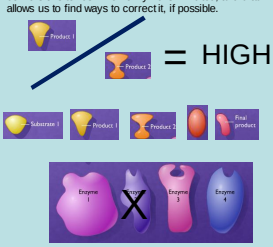
Usually all the doctor notices or the lab flags is when something is over the reference range.

Ask instead: Which analytes are high at the same time? Which analytes are low together and which are split high and low?

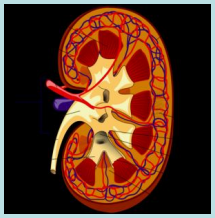
Low values are hard to notice when so many reference ranges begin at zero, but that doesn't mean that having low values is normal.

This is why I evaluate my autism data in percentile ranks. That makes it easy to spot changes or movement from test to test. Analytes can be sorted from high percentiles to low percentiles, noting ratios that are not favorable.

Ratios of substrates and products in a series of conversions tell us which enzyme is inhibited, and that allows us to find ways to correct it, if possible.

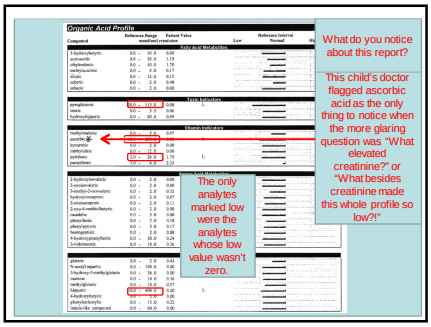


When we use urinary testing, what are the risks to interpretation?



- Do we have a way to know that changes like acidosis are not changing what is reabsorbed and what is secreted into urine?
- Is anything changing how much creatinine is making it into urine?
- Is there evidence that dilution issues are going on that are NOT corrected by creatinine but need to affect our interpretations of the results of urinary tests?
- Are the elevations of some compounds in urine caused by problems in the kidney itself?
- Could it be possible to misinterpret urinary data because we have insufficient information on the state of the kidney?

The answer to all of these questions is, "YES"!

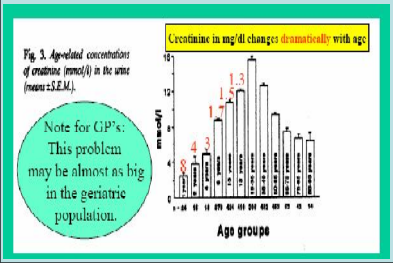


Everything on spot urine tests are reported ratioed ALREADY to creatinine.

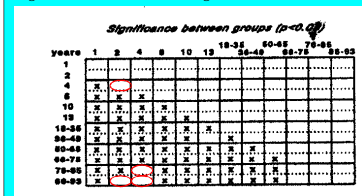
Creatinine was adopted as a "standard" for calculating the dilution of the urine for several reasons:

1. It was thought that creatinine was filtered in the kidney and that it was not secreted or reabsorbed in the tubule cells.. That turned out incorrect.
2. It was thought to be solely determined by the amount of muscle mass in an individual, which was thought to be stable, and not subject to changes except when someone increased in age as a child or lost muscle during aging.
3. The existence of other things that would change creatinine were not discovered until fairly recently , but creatinine had been used as the standard for decades.
4. Once a practice is in place, changing that practice is very difficult.
5. This issue is very important to autism because the conditions that change creatinine seem to be very specific to autism.

Creatinine changes with the size of your muscles and that changes with age.



Between the age groups indicated below, where the intersection of ages is marked with an X, there is a significant difference in the expected creatinine for those ages. That means each of these ages needs its own reference range.



Source: Simeckova A, Zamrazil V, Cerovska J. Calciuria, magnesiuria and creatininuria--relation to age. Physiol Res. 1998;47(1):35-40.

Years ago, when making adjustments for age in these reports, I realized that something far more powerful than age was changing the average level of all the analytes, and changing them quickly and dramatically from test to test.

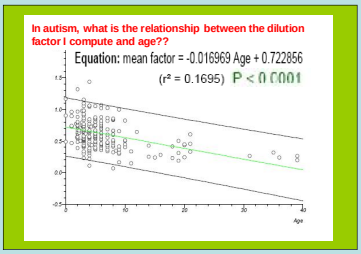
I began putting the analytes in ratio to each other to get rid of the creatinine correction. The "adjustment factor" I derived from the test itself recognizes an issue that is specific to kidney function and regulation..

THIS COMPLETELY CHANGES THE INTERPRETATION !!!!

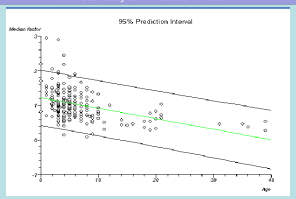
It also can change your impressions about whether an analyte got higher or lower after treatment because some treatments drastically lower the urine concentration for everything on the test because they changed creatinine itself.

Now we know that creatinine is actively secreted from the kidney responding to changed conditions, and that lowers the concentration of everything on the test, making tests really impossible to interpret without adjusting for this problem.

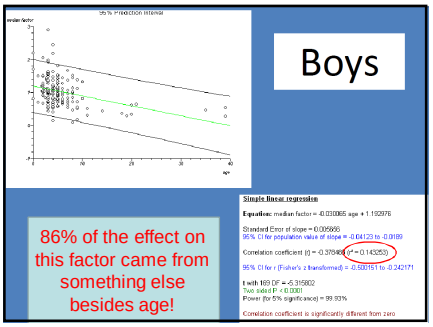
In autism, what is the relationship between the dilution factor I compute and age??

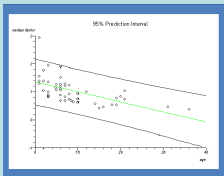


Two years ago I went back and recomputed the dilution factor ratioing each analyte to its own **median** from my database of 224 tests. After doing that, the factor produced more normal curves and data that tied much more understandably to clinical conditions.



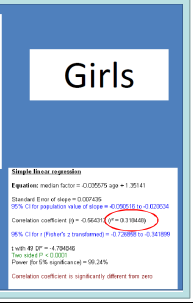
This factor eliminated the creatinine ratio and the effect of age so it was easier to compare people of different ages as they got older on repeat tests.



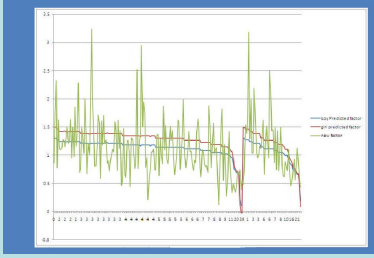


Girls

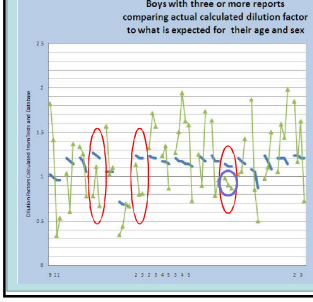
Only 69% of the effect on this factor is from something else besides age with girls!



These are how the "dilution factors" vary in each age group and by sex



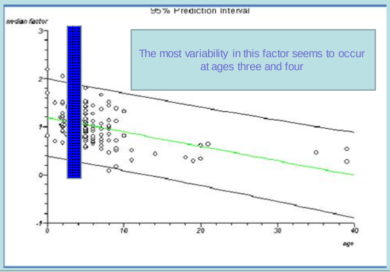
Boys with three or more reports comparing actual calculated dilution factor to what is expected for their age and sex



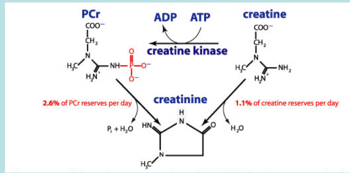
So, if my database is representative of autism, then it is more normal in autism for the dilution to shift dramatically than not to shift!

That also suggests that this shifting might be part of what is wrong in autism!

Insufficient control data!!!!



WHAT BESIDES AGE CAN CHANGE CREATININE?



These studies found out what changed creatinine…

Despite rumors to the contrary, creatinine IS among the substances actively secreted from the tubule cells into the urine.

A test meal of 80 grams of protein led to more than a doubling of uinary creatinine and an increase of tubular secretion of creatinine of up to 3.4 times its previous rate.

J Herrera and B Rodriguez-Iturbe Nephrology Dialysis Transplantation, Vol 13, 1998 Issue 13 623-629.

Gut flora metabolize creatinine that comes from blood to intestinal cells, but the amount of blood creatinine that is converted there in normal healthy controls is usually nothing, but the rate in those with untreated kidney disease is as high as 42%…

"The data…confirm the hypothesis that creatinine is converted into other metabolites, probably by action of the gut flora. "

Hankins DA, Babb AL, Uvelli DA, Scribner BH.Int J Artif Organs. 1981 Jan;4(1):35-9

An IV of vitamin C seems to have changed urine creatinine quickly.

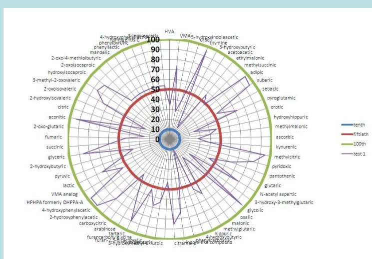
A child with autism went to a doctor who collected urine for a GPL OAT.

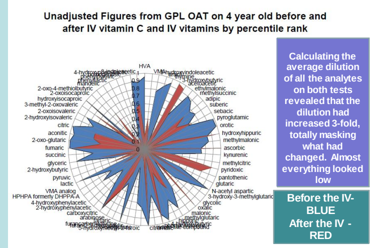
That day, the child was given an IV containing vitamin C and B vitamins.

Within hours the child was in metabolic crisis and in the emergency room.

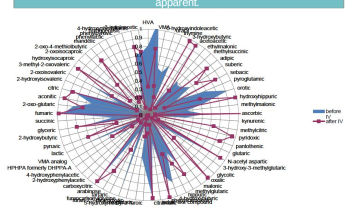
His parents collected urine for another OAT at 10:30 that night, and the results of those two tests on the same day follow.

First, let's talk about how to read this kind of graph!

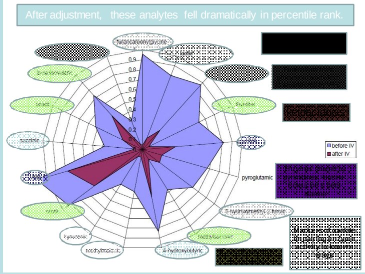




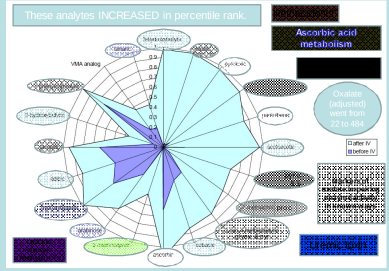
After adjusting for the changed dilution, huge shifts became apparent.

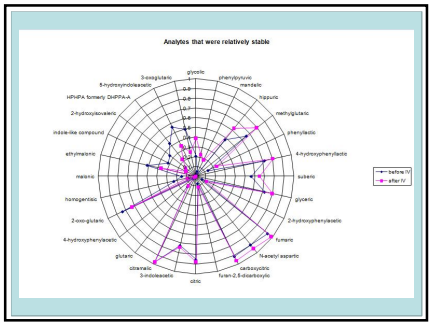


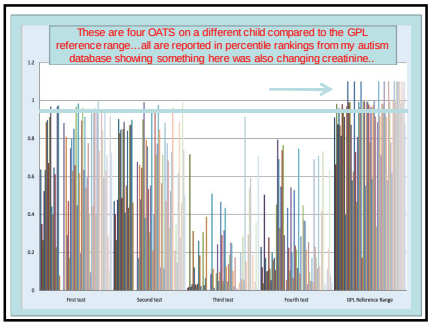
After adjustment, these analytes fell dramatically in percentile rank.

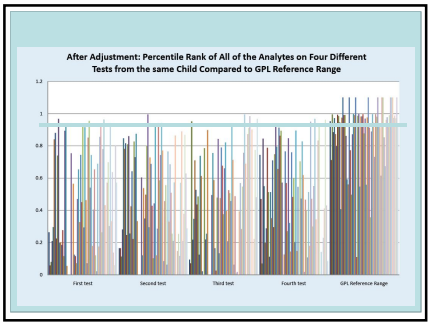


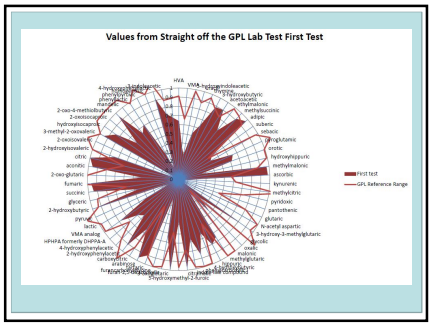
These analytes INCREASED in percentile rank.

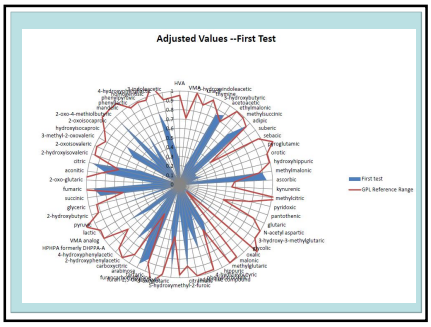


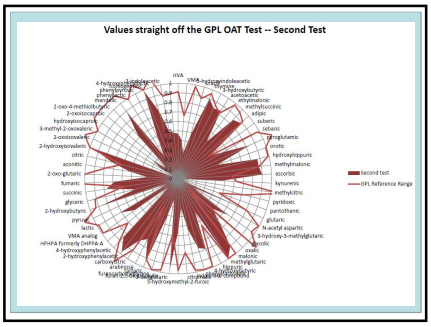

Analytes that were relatively stable
before IV
after IV

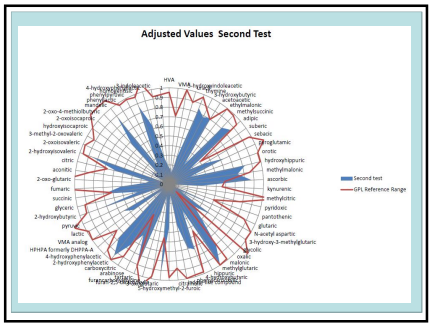

These are four OATS on a different child compared to the GPL reference range…all are reported in percentile rankings from my autism database showing something here was also changing creatinine..
First test
Second test
Third test
Fourth test
GPL Reference Range

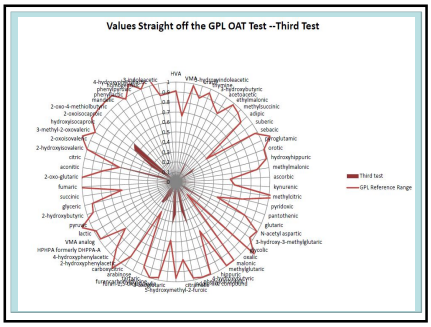

After Adjustment: Percentile Rank of All of the Analytes on Four Different Tests from the same Child Compared to GPL Reference Range
First test
Second test
Third test
Fourth test
GPL Reference Range

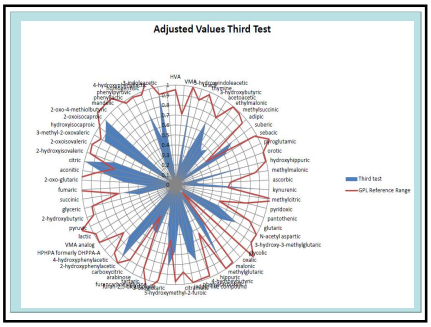

Values from Straight off the GPL Lab Test First Test
First test
GPL Reference Range

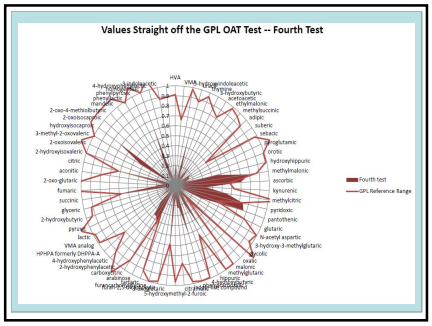

Adjusted Values --First Test
First test
GPL Reference Range

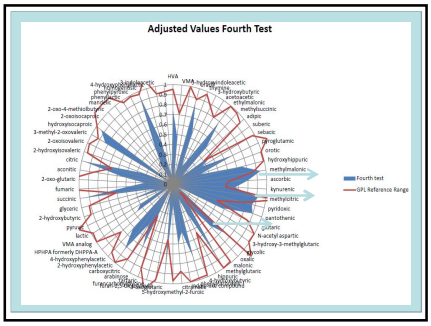

Values straight off the GPL OAT Test -- Second Test
Second test
GPL Reference Range

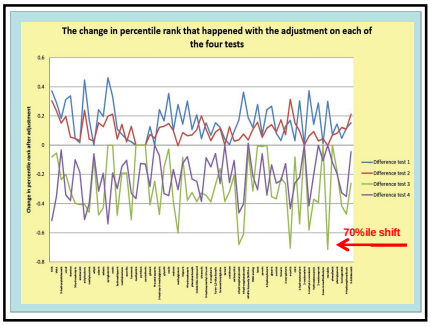

Adjusted Values Second Test
Second test
GPL Reference Range


Values Straight off the GPL OAT Test --Third Test
Third test
GPL Reference Range


Adjusted Values Third Test
Third test
GPL Reference Range







Our project found a different source for this shift when a little girl with autism had a doctor who ordered ten organic acid tests from Great Plains over about two years.

The whole time, this girl had been on a low oxalate diet.

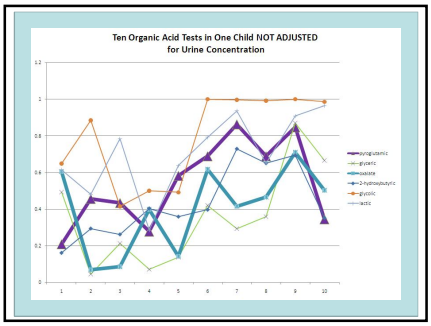
In the first year, the oxalate came down as expected, but something began to look very different in the second year.

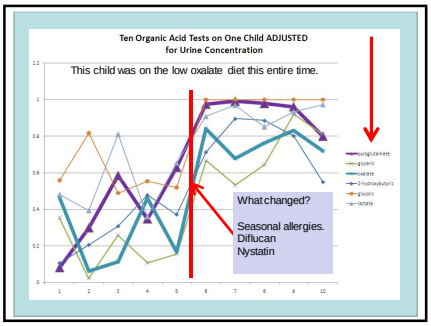
She had seasonal allergies and was treated with medications and an OAT showed up with high arabinose.

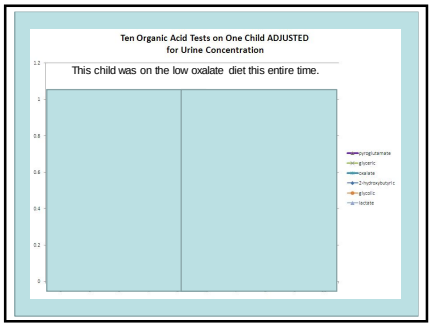
Her doctor found markers on a stool test suggesting increased yeast, so this child was treated with alternating diflucan and nystatin.

Right before her last OAT, she was taken off the antifungal medicines and began using natural antifungals.

The results of her testing were sent to me for analysis. Values are adjusted for dilution and expressed as percentile ranks.







Looking for an explanation for what changed in this little girl's labwork, a study I found and other studies that fit in with it showed there is a relationship between:

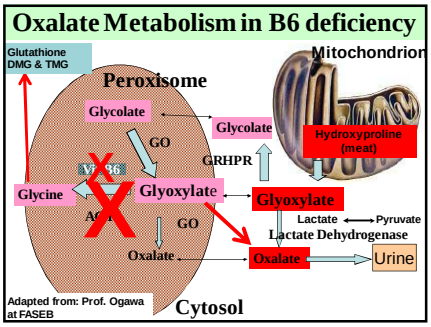
- **Oxidative stress**
- **Changes in urine creatinine**
- **B6 depletion which could lead to making endogenous oxalate**

This data suggested why in autism, there can be:

- **A need for much higher levels of pyridoxine**
- **A need for much higher levels of glutathione and other antioxidants**
- **A need for much higher levels of biotin than are typically given.**

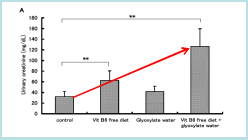
We found information about useful markers to tell us when this is so!

Effect of vitamin B6 deficiency on glyoxylate metabolism in rats with or without glyoxylate overload
Biomedical Research 27 (3) 93-98, 2006

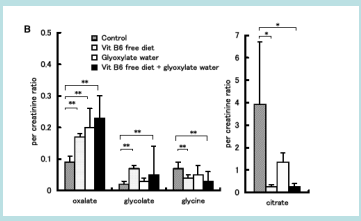


In the study I found on B6 deficiency, rats began to make excess oxalate because of B6 deficiency and being exposed to oxalate's precursor, glyoxylate, at the same time. This produced a four-fold increase in the amount of creatinine that was in each deciliter of urine.

That means, since creatinine is used as the standard to compute urine concentration, this biological change would ARTIFICIALLY LOWER all the analytes in a urine test by four-fold. High items would look like they moved into normal territory, when in reality, the levels might have gotten higher! In my autism database, a more typical change in urine concentration was three-fold.

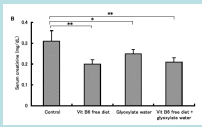


HIGH LEVELS OF OXALATE APPARENTLY CHANGE THE HANDLING OF CREATININE IN THE KIDNEY CAUSING HUGE INCREASES IN THE AMOUNT OF CREATININE THAT IS PRESENT IN A SPOT URINE SAMPLE.



These researchers, if they had removed the creatinine correction, could have reported that the oxalate increased 4.6 fold in B6 deficiency and 12.7 fold when B6 deficiency met an increased supply of glyoxylate. They also could have reported that glycolic acid increased 6.8 fold, and 9.8 fold respectively.

The paper also showed how this created TROUBLE for using Traditional Markers of Kidney Disease and Liver Damage when there is B6 deficiency



Elevations of **serum creatinine** are considered to be a marker of kidney disease.

In these B6 deficient rats, serum creatinine instead of rising actually fell by a third because creatinine was being removed from blood in the kidney raising urine levels four-fold.

Elevations of **AST and ALT in serum** are considered to be the markers of **liver toxicity** that would tell you if diflucan , oxidative stress, or something else was stressing the liver.

In B6 deficiency, ALT and AST FELL!

AST particularly fell like a stone!

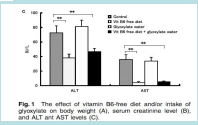
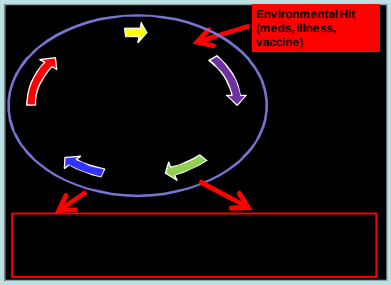
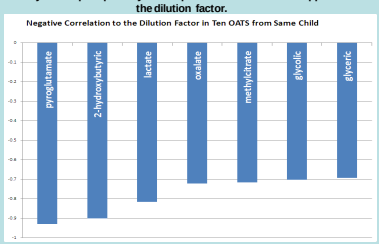


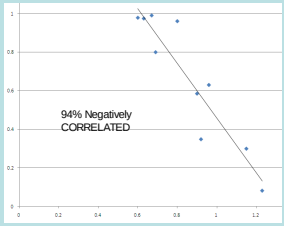
Fig. 1 The effect of vitamin B6-free diet and/or intake of glyoxylate on body weight (A), serum creatinine level (B), and ALT and AST levels (C).



Hidden by the artifactually lowered levels for all analytes on the OAT, this is the set of analytes that ROSE significantly while the urine concentration of other analytes fell precipitously. Their percentile ranks moved opposite to the the dilution factor.



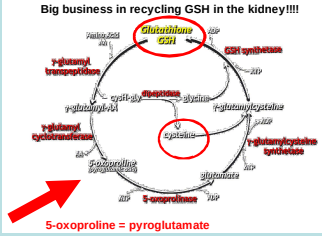
DILUTION FACTOR



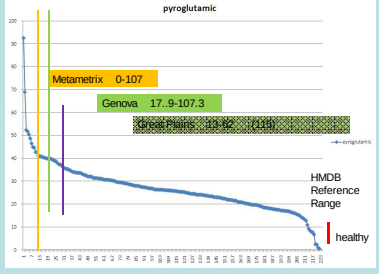
The most striking change was the way the dilution factor for each test went lower as pyroglutamate soared. Pyroglutamate being high is a specfic marker for glutathione depletion.

Gamma-glutamyl cycle:

Big business in recycling GSH in the kidney!!!!



5-oxoproline = pyroglutamate



J Autism Dev Disord. 2011 Apr 26. **129 ARTICLES on autism and ox.stress**

Metabolic Imbalance Associated with Methylation Dysregulation and Oxidative Damage in Children with Autism.

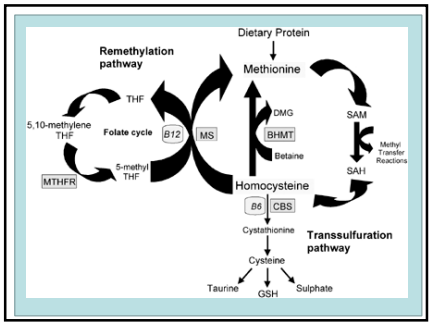
Melnyk S, Fuchs GJ, Schulz E, Lopez M, Kahler SG, Fussell JJ, Bellando J, Pavliv O, Rose S, Seidel L, Gaylor DW, Jill James S.

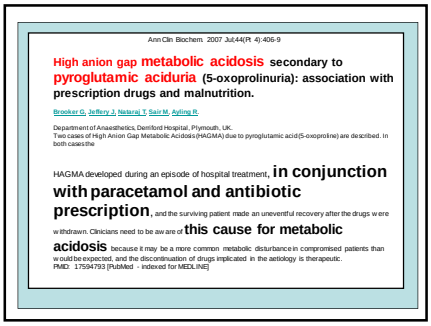
Source

Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Abstract

Oxidative stress and abnormal DNA methylation have been implicated in the pathophysiology of autism. We investigated the dynamics of an integrated metabolic pathway essential for cellular antioxidant and methylation capacity in 68 children with autism, 54 age-matched control children and 40 unaffected siblings. The metabolic profile of unaffected siblings differed significantly from case siblings but not from controls. Oxidative protein/DNA damage and DNA hypomethylation (epigenetic alteration) were found in autistic children but not paired siblings or controls. These data indicate that the deficit in antioxidant and methylation capacity is specific for autism and may promote cellular damage and altered epigenetic gene expression. Further, these results suggest a plausible mechanism by which pro-oxidant environmental stressors may modulate genetic predisposition to autism. PMID: 21519954





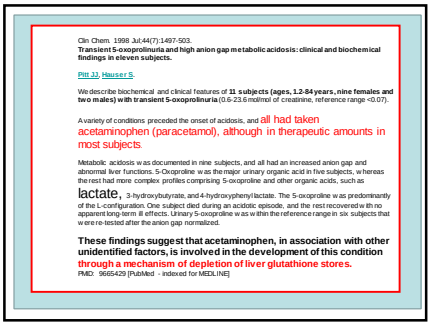
Ann Clin Biochem 2007 Jul;44(Pt 4):406-9

High anion gap metabolic acidosis secondary to pyroglutamic aciduria (5-oxoprolinuria): association with prescription drugs and malnutrition.

Brooker G, Jeffery J, Nataraj T, Sair M, Ayling R

Department of Anaesthetics, Derriford Hospital, Plymouth, UK.
Two cases of High Anion Gap Metabolic Acidosis (HAGMA) due to pyroglutamic acid (5-oxoproline) are described. In both cases the

HAGMA developed during an episode of hospital treatment, **in conjunction with paracetamol and antibiotic prescription**, and the surviving patient made an uneventful recovery after the drugs were withdrawn. Clinicians need to be aware of **this cause for metabolic acidosis** because it may be a more common metabolic disturbance in compromised patients than would be expected, and the discontinuation of drugs implicated in the aetiology is therapeutic.
PMID: 17594793 [PubMed - indexed for MEDLINE]



Clin Chem. 1998 Jul;44(7):1497-503.
Transient 5-oxoprolinuria and high anion gap metabolic acidosis: clinical and biochemical findings in eleven subjects.

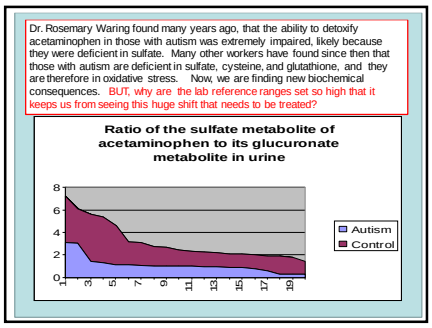
Pitt JJ, Hauser S.

We describe biochemical and clinical features of **11 subjects (ages, 1.2-84 years, nine females and two males) with transient 5-oxoprolinuria** (0.6-23.6 mol/mol of creatinine, reference range <0.07).

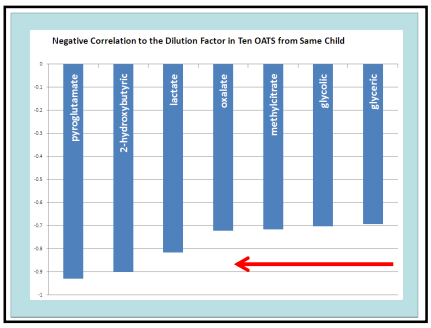
A variety of conditions preceded the onset of acidosis, and all had taken acetaminophen (paracetamol), although in therapeutic amounts in most subjects.

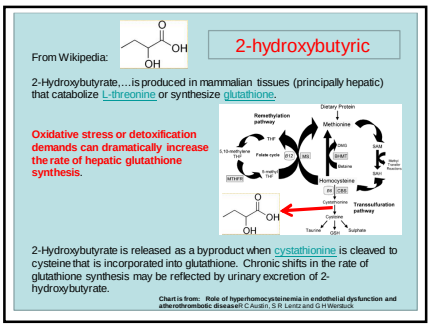
Metabolic acidosis was documented in nine subjects, and all had an increased anion gap and abnormal liver functions. 5-Oxoproline was the major urinary organic acid in five subjects, whereas the rest had more complex profiles comprising 5-oxoproline and other organic acids, such as lactate, 3-hydroxybutyrate, and 4-hydroxyphenyllactate. The 5-oxoproline was predominantly of the L-configuration. One subject died during an acidotic episode, and the rest recovered with no apparent long-term ill effects. Urinary 5-oxoproline was within the reference range in six subjects that were re-tested after the anion gap normalized.

These findings suggest that acetaminophen, in association with other unidentified factors, is involved in the development of this condition through a mechanism of depletion of liver glutathione stores.
PMID: 9665429 [PubMed - indexed for MEDLINE]



Dr. Rosemary Waring found many years ago, that the ability to detoxify acetaminophen in those with autism was extremely impaired, likely because they were deficient in sulfate. Many other workers have found since then that those with autism are deficient in sulfate, cysteine, and glutathione, and they are therefore in oxidative stress. Now, we are finding new biochemical consequences. BUT, why are the lab reference ranges set so high that it keeps us from seeing this huge shift that needs to be treated?





From Wikipedia:

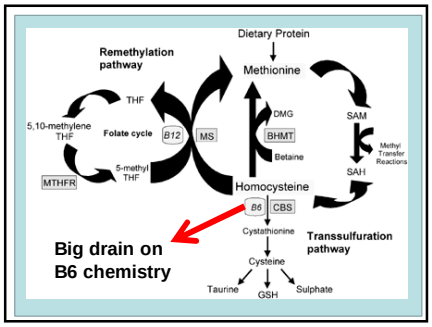
2-hydroxybutyric

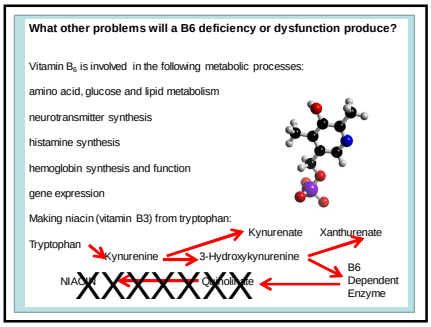
2-Hydroxybutyrate,...is produced in mammalian tissues (principally hepatic) that catabolize L-threonine or synthesize glutathione.

Oxidative stress or detoxification demands can dramatically increase the rate of hepatic glutathione synthesis.

2-Hydroxybutyrate is released as a byproduct when cystathionine is cleaved to cysteine that is incorporated into glutathione. Chronic shifts in the rate of glutathione synthesis may be reflected by urinary excretion of 2-hydroxybutyrate.

Chart is from: **Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease**R C Austin, S R Lentz and G H Werstuck





What other problems will a B6 deficiency or dysfunction produce?

Vitamin B_6 is involved in the following metabolic processes:

amino acid, glucose and lipid metabolism

neurotransmitter synthesis

histamine synthesis

hemoglobin synthesis and function

gene expression

Making niacin (vitamin B3) from tryptophan:

Tryptophan → Kynurenine → 3-Hydroxykynurenine → Quinolinate → NIACIN

Kynurenate

Xanthurenate

B6 Dependent Enzyme

Am J Psychiatry 1978; 135:472-475
Copyright © 1978 by American Psychiatric Association

REGULAR ARTICLES

The effect of high doses of vitamin B6 on autistic children: a double- blind crossover study

B Rimland, E Callaway and P Dreyfus

The authors used data from an earlier nonblind study to identify 16 autistic-type child outpatients who had apparently improved when given vitamin B6 (pyridoxine). In a double-blind study each child's B6 supplement was replaced during two separate experimental trial periods with either a B6 supplement or a matched placebo. Behavior was rated as deteriorating significantly during the B6 withdrawal.

The Role of Vitamins and Minerals in Psychiatry

Integrative Medicine Insights, by Cornish, S., and Mehl_Madrona L., published in 2008, summarized Jun 4, 2009

Vitamin B6 and magnesium therapy may help some children with *autism*.

In one older study, 42% of children with *autism* had low levels of *vitamin B6*. These results prompted *doctors* to try *vitamin B6 therapy* for patients with *autism*. *Parents* of some children with *autism* reported improvements in behavior with *vitamin B6 treatment* alone. Some children also showed improvement after combined *treatment* with *vitamin B6 and magnesium*. A recent large study (Rimland and Edelson) of 5,780 *autistic* children and *adults* reported clinical improvement in 47% of the patients who received combined *vitamin B6 and magnesium therapy*.

Studies of High Dosage Vitamin B6 and often with Magnesium in Autistic Children and Adults
1965 - 2005
(Twenty-one of twenty-two studies yielded positive results, including 13 double-blind placebo-controlled trials; even minor adverse effects rarely were seen)

Doses of B6 used in these studies ranged from a daily dose of:

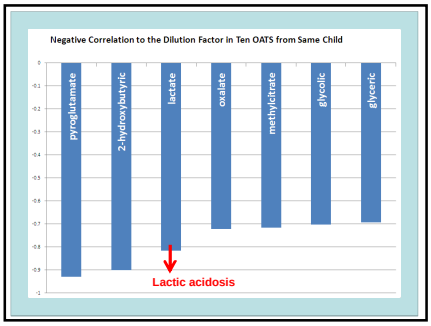
30	10 mg/kg/day
75	14 mg/kg/day
100	20 mg/kg/day
150	30 mg/kg day
300	
400	
420	
450	
500	
900	
1000	
1125	
3000	

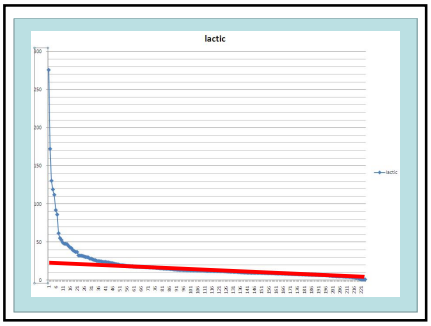
The kidney community only recommends 5 mg/kg/day to correct AGT deficiency, keeping glycolic acid and oxalate lower.

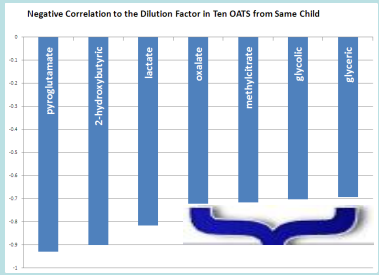
Autism may need more than this because this B6 is being shared with the sulfur chemistry and tryptophan/niacin/serotonin chemistry.

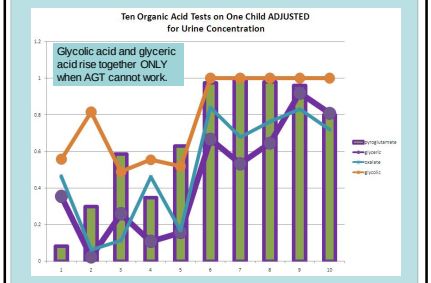
My suspicions are that glycolate levels in urine can and should be used as the standard for titrating up to an effective dose.

http://www.autism.com/pro_b6_studies.asp









Plasma and urine glycolate assays for differentiating the hyperoxaluria syndromes.

Marangella M, Petrarulo M, Vitale C, Cosseddu D, Linari F.

Source

Renal Stone Laboratory, Ospedale MaurizIano Umberto I, Turin, Italy.

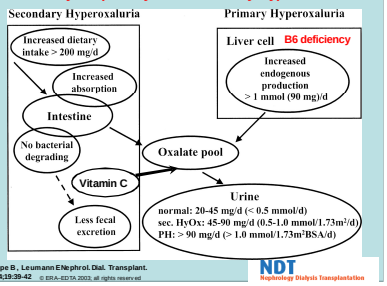
Abstract

....Glycolate levels were normal in 5 patients with enteric hyperoxaluria.

We conclude that glycolate assay is essential for identifying patients with primary hyperoxaluria [and pyridoxine dependency] and may represent a valuable tool for differentiating hyperoxaluria.

PMID: 1507356

Pathways of primary and secondary hyperoxaluria.



Hoppe B., Leumann ENephrol. Dial. Transplant. 2004;19:39-42 © ERA-EDTA 2003; all rights reserved

NDT Nephrology Dialysis Transplantation

One of the central problems in autism is oxidative stress. Once oxalatemoves from blood into cells, it competes with glutathione for entry into the mitochondrion, and impairs so many enzymes there that the situation would be recognized clinically as mitochondrial dysfunction.

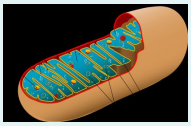
OXALATE→ CELLULAR DISTRESS AND OXIDATIVE STRESS

This will:

- •deplete glutathione
- •turn on inflammatory cycles.
- •Disrupt remethylation

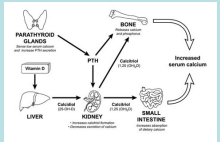
May raise pyruvate

Alter mitochondrial DNA



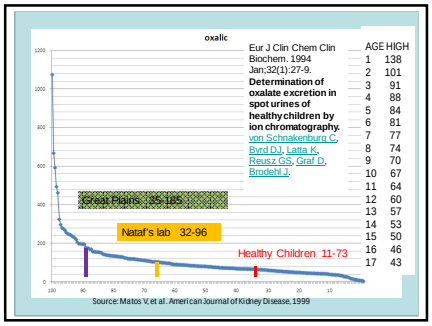
"it can be concluded that mitochondrial damage is an essential event in hyperoxaluria" **Veena et al., 2008.**

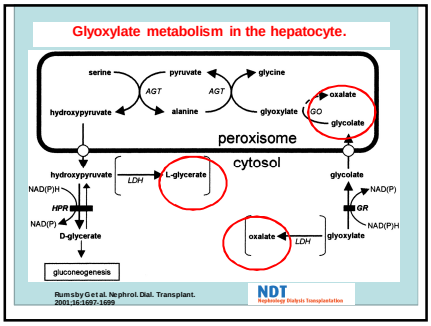
At the same time this oxidative stress is increasing in the mitochondrion, this may impair the conversions of vitamin D that are necessary in the kidney., making blood levels of vitamin D shrink even when your child is in the sun!

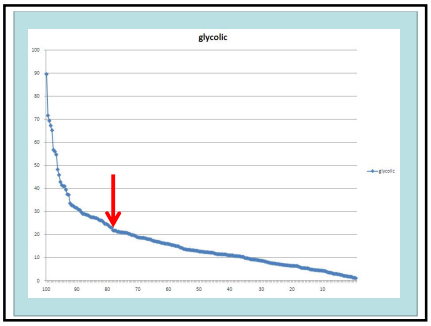


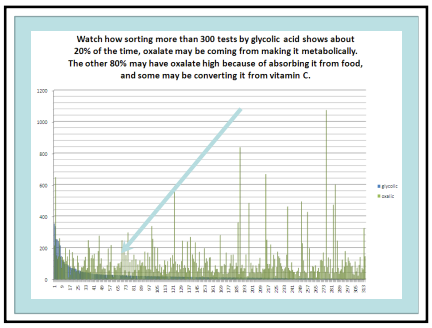
From Arch. Biochem. Biophys 1988 May 1;262(2):471-80:

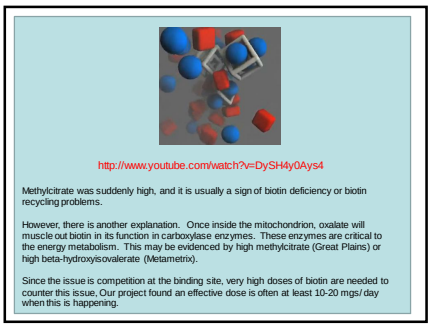
"The increase in cellular oxidative stress was paralleled by decreases in both 1-alpha- and 24-hydroxylase activities toward 25-OH D3.

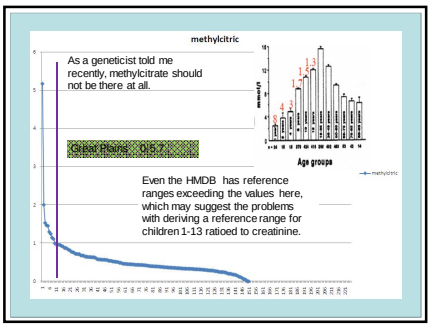

oxalic
Eur J Clin Chem Clin Biochem. 1994 Jan;32(1):27-9.
Determination of oxalate excretion in spot urines of healthy children by ion chromatography.
von Schnakenburg C, Byrd DJ, Latta K, Reusz GS, Graf D, Brodehl J.
AGE HIGH
1 138
2 101
3 91
4 88
5 84
6 81
7 77
8 74
9 70
10 67
11 64
12 60
13 57
14 53
15 50
16 46
17 43
Great Plains 35-185
Nataf's lab 32-96
Healthy Children 11-73
Source: Matos V, et al. American Journal of Kidney Disease. 1999

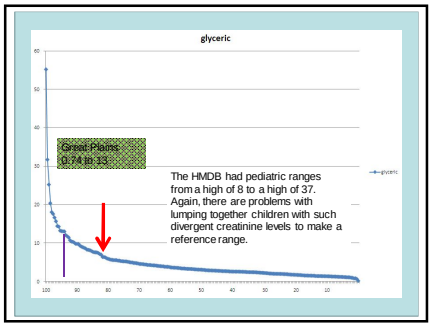

Glyoxylate metabolism in the hepatocyte.
serine
pyruvate
glycine
AGT
AGT
hydroxypyruvate
alanine
glyoxylate
oxalate
GO
glycolate
peroxisome
cytosol
hydroxypyruvate
LDH
L-glycerate
glycolate
NAD(P)H
HPR
NAD(P)
D-glycerate
NAD(P)
GR
NAD(P)H
oxalate
LDH
glyoxylate
gluconeogenesis
Rumsby G et al. Nephrol. Dial. Transplant. 2001;16:1697-1699
NDT

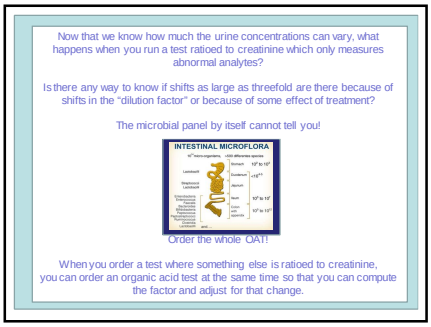

glycolic

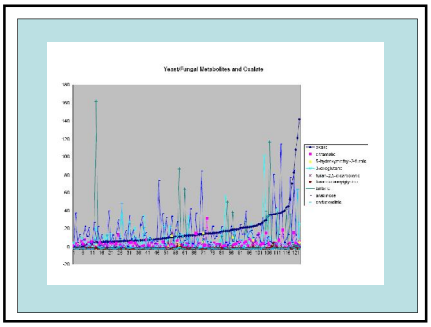

Watch how sorting more than 300 tests by glycolic acid shows about 20% of the time, oxalate may be coming from making it metabolically. The other 80% may have oxalate high because of absorbing it from food, and some may be converting it from vitamin C.

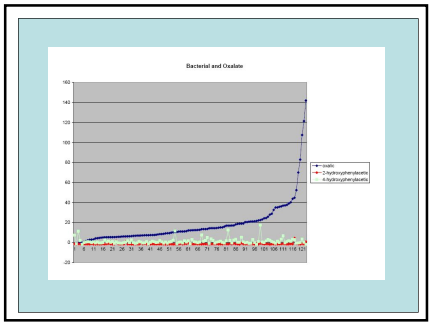

http://www.youtube.com/watch?v=DySH4y0Ays4
Methylcitrate was suddenly high, and it is usually a sign of biotin deficiency or biotin recycling problems.
However, there is another explanation. Once inside the mitochondrion, oxalate will muscle out biotin in its function in carboxylase enzymes. These enzymes are critical to the energy metabolism. This may be evidenced by high methylcitrate (Great Plains) or high beta-hydroxyisovalerate (Metametrix).
Since the issue is competition at the binding site, very high doses of biotin are needed to counter this issue, Our project found an effective dose is often at least 10-20 mgs/ day when this is happening.

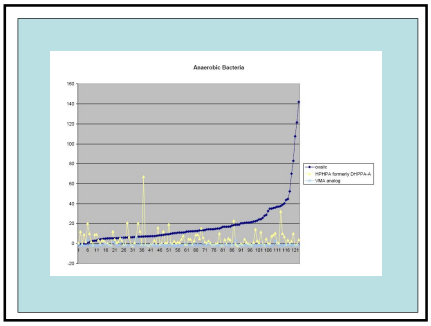

methylcitric
As a geneticist told me recently, methylcitrate should not be there at all.
Great Plains 0-5.7
Even the HMDB has reference ranges exceeding the values here, which may suggest the problems with deriving a reference range for children 1-13 ratioed to creatinine.
Age groups

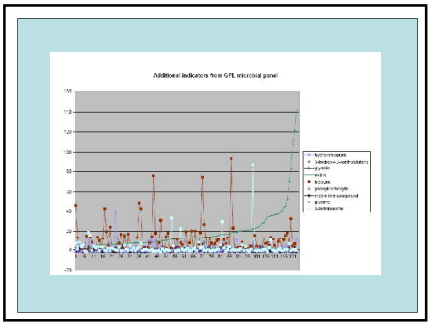

glyceric
Great Plains 0-74 to 35
The HMDB had pediatric ranges from a high of 8 to a high of 37. Again, there are problems with lumping together children with such divergent creatinine levels to make a reference range.


Now that we know how much the urine concentrations can vary, what happens when you run a test ratioed to creatinine which only measures abnormal analytes?
Is there any way to know if shifts as large as threefold are there because of shifts in the "dilution factor" or because of some effect of treatment?
The microbial panel by itself cannot tell you!
INTESTINAL MICROFLORA
Order the whole OAT!
When you order a test where something else is ratioed to creatinine, you can order an organic acid test at the same time so that you can compute the factor and adjust for that change.


Yeast/Fungal Metabolites and Oxalate







What best serves the child in unmasking that child's issues compared to other children with autism?

Viewing the data on these children in the context of all the other data on the OAT, and in context with other tests done at the same time, can unmask issues you've never been able to see before.

So far, this adjustment has unmasked children with fatty acid and other metabolic disorders that were obscured before because everything had shifted low and even revealed that some things thought to be abnormal were normal!

Expressing these values as percentile rankings allowed us to see things that distinguish children from others with autism.

Shifts to low values were as important as shifts to high values. This had been obscured to us by the reference ranges that kept us from noticing relative shifts of the analytes to each other compared to other children.

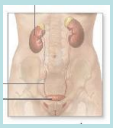
Correcting the data from the lab by putting the values in ratio to each other helps in preventing unnecessary treatment. It also helps us realize that what may have changed the most after treatment was a shift in concentration of the whole test.

The evidence is that an unadjusted shift lower may have reflected a problem rather than an improvement in the health of the child in the area we were focused upon!

SUMMARY ON URINARY TESTS

It is time that we understand the condition and regulation of the kidneys in autism and understand the effects of their health and regulation on our interpretations of urinary testing.

These data point out problems with making too many assumptions about urinary testing using organic acid tests.



What about other spot urine tests?

Do they have enough normal values to calculate a factor?

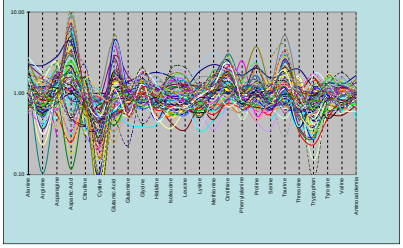
No…not usually!

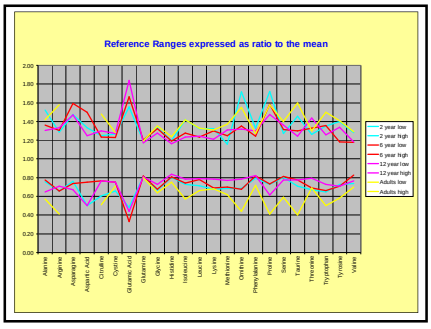
Again, it may be reasonable to order an organic acid test in order to calculate this factor when other urine tests are ordered at the same time whenever 24 hour testing is impossible.

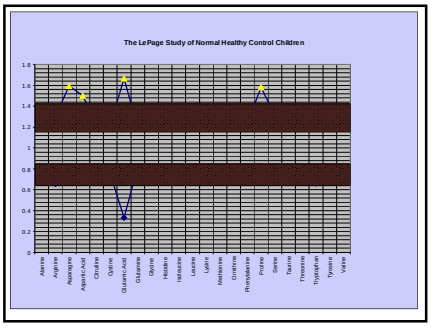
Issues to Be Resolved and Strategies

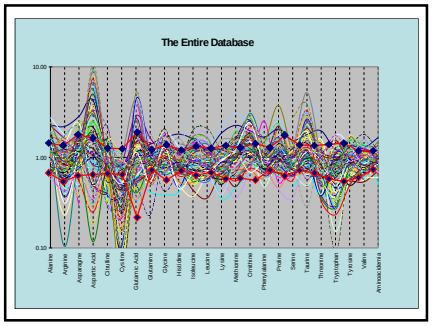
1. We really need labs to have large numbers of completely healthy unrelated control children of the same age as patients as the basis for their reference ranges.
2. Comparing against a percentile rank gives more useful information than seeing whether a value falls within a reference range.
3. Until labs have a reason to spend money testing healthy children, the reference ranges will represent sick children.
4. Perhaps now, the best place of comparison for doctors is their own patients…those whose history they know and can correlate to labwork.
5. Encourage your doctor to get someone to collect the data and adjust for changes in urinary concentration, building his own database against which he can compare your child.
6. He will be far more able that way to find children with special problems in their metabolism that were invisible before because it will be so much easier to see how patients differ from each other.

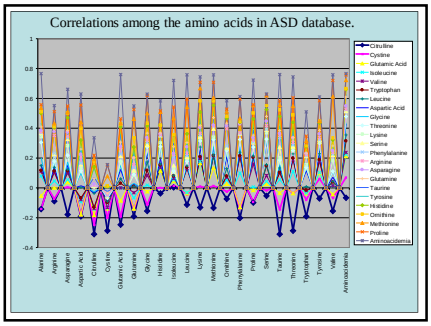
Lessons Learned from Collecting Amino Acid Data in Autism

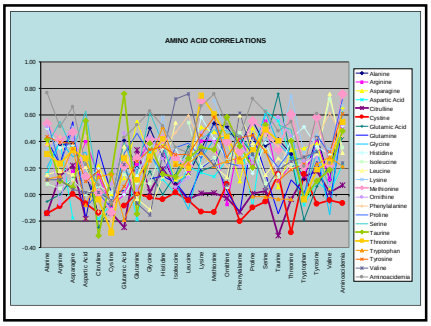


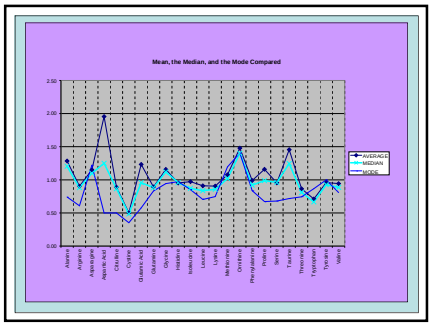


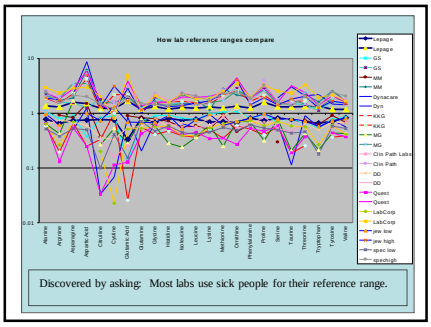


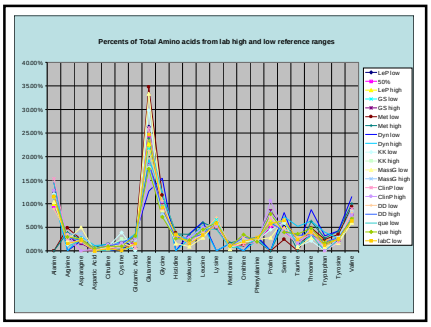

The Entire Database

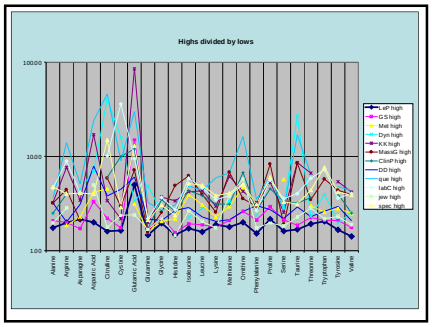

Correlations among the amino acids in ASD database.

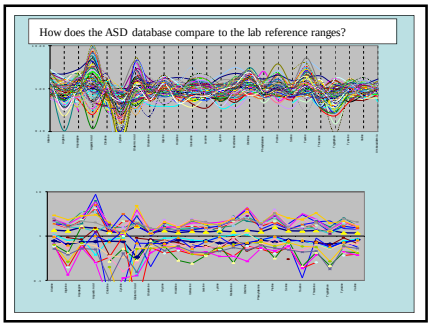

AMINO ACID CORRELATIONS


Mean, the Median, and the Mode Compared


How lab reference ranges compare
Discovered by asking: Most labs use sick people for their reference range.


Percents of Total Amino acids from lab high and low reference ranges


Highs divided by lows


How does the ASD database compare to the lab reference ranges?


AUTISM OXALATE PROJECT
216 West Rio Grande Street
Garland, TX 75041
Phone: 972-840-8709
Cell: 214-450-9645
Fax: 972-220-0059
Susan Owens, MAIS
www.lowoxalate.info
Project Head — Autism Oxalate Project
Independent Research in
Autism, Sulfur Issues, and Oxalate-related disease
Autism Research Institute
Thinktank member
Defeat Autism Now!
I also recommend for further exploration of organic acid tests, a powerpoint made by Metametrix which can be downloaded here:
http://www.slideshare.net/metametrix/organix-profile