Integrative Medicine Academy

How to Interpret a Great Plains Laboratory Organic Acids Test

Kurt N. Woeller, D.O.

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Lecture Overview

- Introduction to the Organic Acids Test (OAT)
- The OAT for yeast & mold toxin assessment
- The OAT for clostridia & other bacteria toxin assessment.
- The OAT for oxalate assessment
- Neurotransmitter imbalances and mitochondrial function assessment.
- Indicators of other problems: *nutrient deficiencies, fatty acid metabolites, etc*.

2

Prioritization Based On Common Findings

- The OAT can be a complicated test with many markers indicating a variety of potential problems.
- This lecture presentation focuses on what is seen *most commonly* in practice with regards to the OAT.
- The vast majority of OAT's *(approximately 80%)* that you will see from a variety of patients/clients will have similar patterns.
- Each OAT needs to be applied clinically to the patient/client and treatment not just implemented based on test markers.

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What Is The OAT?

- Organic acids are compounds with acidic properties:
  - *Carboxyl (-COOH)*
  - *Alcohol (-OH)*
  - *Thiol (-SH)*
- An accurate assessment of what is going on metabolically in the body.
- Evaluates over 70 urinary metabolites that can be useful for discovering underlying causes of chronic illness.
- Treatment based on OAT findings often leads to improved energy, sleep and mental health conditions, as well as reduced attention and concentration problems, chronic pain and digestive problems.

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GPL OAT Sections

- Yeast and Fungal Markers:
  - *Evaluates for invasive candida and mold toxicity*
- Bacterial Markers:
  - *Evaluates for dysbiosis*
- Clostridia Bacteria Markers:
  - *Evaluates for various clostridia bacteria toxins*
- Oxalate Metabolites:
  - *Evaluates for oxalic acid toxicity and endogenous oxalate production problems.*
- Glycolytic and Mitochondrial Markers:
  - *Evaluates for mitochondrial dysfunction*

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GPL OAT Sections

- Phenylalanine and Tyrosine Metabolites:
  - *Evaluates for phenylalanine and tyrosine metabolism which can lead to dopamine and norepinephrine imbalances.*
- Tryptophan Metabolites:
  - *Evaluates for issues in tryptophan metabolism which contributes to serotonin imbalance and excess quinolinic acid production.*
- Pyrimidine Metabolites:
  - *Evaluates for uracil and thymine as indicators of folate imbalance.*

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GPL OAT Sections

- Ketone and Fatty Acid Oxidation:
  - *Evaluates for fatty acid metabolism problems which can contribute to mitochondrial dysfunction.*
- Nutritional Markers:
  - *Evaluates for various nutrient imbalances*
- Indicators of Detoxification:
  - *Measures organic acids linked to glutathione status*

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GPL OAT Sections

- Amino Acid Metabolites:
  - *Measures organic acids linked to inborn errors of metabolism and other metabolic imbalances.*
- Mineral Metabolism:
  - *Measures phosphoric acid linked dietary consumption of phosphate (processed foods), as well as bone metabolism and vitamin D status.*

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Who Is The Test Beneficial For?

- Autism
- ADD/ADHD
- Autoimmune
- Chronic fatigue
- Digestive problems
- Metabolic disorders
- Mental health disorders
- Neurological disorders

Any individual with a chronic health condition where you suspect metabolic toxins may be a causative or contributing factor.

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OAT Sample

Suggestions For Reviewing An Organic Acids Test

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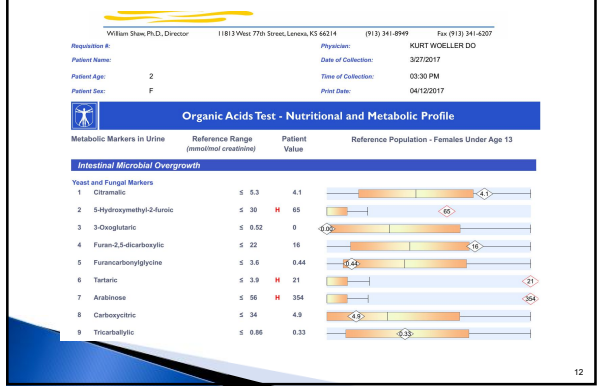
Page 1 – *Yeast and Fungal Markers*



William Shaw, Ph.D., Director 11813 West 77th Street, Lenexa, KS 66214 (913) 341-8949 Fax (913) 341-6207

Requisition #:
Patient Name:
Patient Age: 2
Patient Sex: F

Physician: KURT WOELLER DO
Date of Collection: 3/27/2017
Time of Collection: 03:30 PM
Print Date: 04/12/2017

Organic Acids Test - Nutritional and Metabolic Profile

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Value	Reference Population - Females Under Age 13
Intestinal Microbial Overgrowth				
Yeast and Fungal Markers				
1 Citramalic	≤ 5.3		4.1	4.1
2 5-Hydroxymethyl-2-furoic	≤ 30	H	65	65
3 3-Oxoglutaric	≤ 0.52		0	0.00
4 Furan-2,5-dicarboxylic	≤ 22		16	16
5 Furancarbonylglycine	≤ 3.6		0.44	0.44
6 Tartaric	≤ 3.9	H	21	21
7 Arabinose	≤ 56	H	354	354
8 Carboxycitric	≤ 34		4.9	4.9
9 Tricarballylic	≤ 0.86		0.33	0.33

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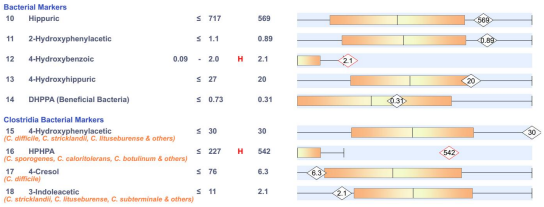
Page 1 – *Bacterial and Clostridia Markers*



Bacterial Markers							
10	Hippuric		≤ 717		569		568
11	2-Hydroxyphenylacetic		≤ 1.1		0.89		0.89
12	4-Hydroxybenzoic	0.09	- 2.0	H	2.1		2.1
13	4-Hydroxyhippuric		≤ 27		20		20
14	DHPPA (Beneficial Bacteria)		≤ 0.73		0.31		0.31
Clostridia Bacterial Markers							
15	4-Hydroxyphenylacetic *(C. difficile, C. stricklandii, C. lituseburense & others)*		≤ 30		30		30
16	HPHPA *(C. sporogenes, C. caloritolerans, C. botulinum & others)*		≤ 227	H	542		542
17	4-Cresol *(C. difficile)*		≤ 76		6.3		6.3
18	3-Indoleacetic *(C. stricklandii, C. lituseburense, C. subterminale & others)*		≤ 11		2.1		2.1

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Page 2 - *Oxalate and Mitochondrial Metabolites*

Metabolic Markers in Urine		Reference Range (mmol/mol creatinine)			Patient Value	Reference Population - Females Under Age 13
Oxalate Metabolites						
19	Glyceric	0.71	- 9.5		9.4	9.4
20	Glycolic	20	- 202		33	33
21	Oxalic	15	- 174	H	346	346
Glycolytic Cycle Metabolites						
22	Lactic	0.18	- 44		42	42
23	Pyruvic	0.88	- 9.1		5.7	5.7
Mitochondrial Markers - Krebs Cycle Metabolites						
24	Succinic		≤ 15	H	123	123
25	Fumaric	0.04	- 1.3	H	5.6	5.6
26	Malic		≤ 2.2	H	7.9	7.9
27	2-Oxoglutaric		≤ 81		8.7	8.7
28	Aconitic	11	- 35		35	35
29	Citric	59	- 440	H	1 319	1319
Mitochondrial Markers - Amino Acid Metabolites						
30	3-Methylglutaric	0.07	- 0.95	H	1.8	1.8
31	3-Hydroxyglutaric		≤ 11	H	13	13
32	3-Methylglutaconic		≤ 6.4		2.7	2.7

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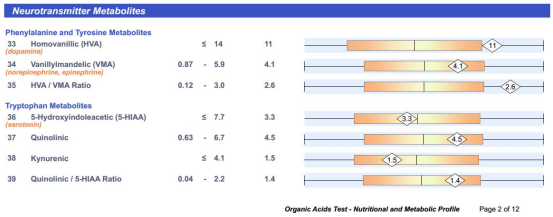
Page 2 – *Neurotransmitter Metabolites*



Neurotransmitter Metabolites						
Phenylalanine and Tyrosine Metabolites						
33	Homovanillic (HVA) *(dopamine)*		≤ 14		11	11
34	Vanillylmandelic (VMA) *(norepinephrine, epinephrine)*	0.87	- 5.9		4.1	4.1
35	HVA / VMA Ratio	0.12	- 3.0		2.6	2.6
Tryptophan Metabolites						
36	5-Hydroxyindoleacetic (5-HIAA) *(serotonin)*		≤ 7.7		3.3	3.3
37	Quinolinic	0.63	- 6.7		4.5	4.5
38	Kynurenic		≤ 4.1		1.5	1.5
39	Quinolinic / 5-HIAA Ratio	0.04	- 2.2		1.4	1.4

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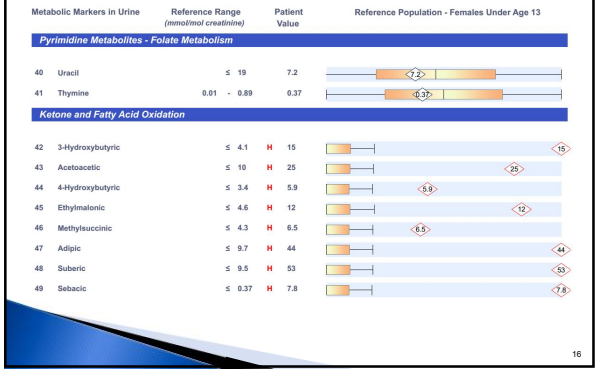
Page 3 – *Pyrimidines and Fatty Acids*



Metabolic Markers in Urine		Reference Range (mmol/mol creatinine)		Patient Value	Reference Population - Females Under Age 13
Pyrimidine Metabolites - Folate Metabolism					
40	Uracil	≤ 19		7.2	7.2
41	Thymine	0.01 - 0.89		0.37	0.37
Ketone and Fatty Acid Oxidation					
42	3-Hydroxybutyric	≤ 4.1	H	15	15
43	Acetoacetic	≤ 10	H	25	25
44	4-Hydroxybutyric	≤ 3.4	H	5.9	5.9
45	Ethylmalonic	≤ 4.6	H	12	12
46	Methylsuccinic	≤ 4.3	H	6.5	6.5
47	Adipic	≤ 9.7	H	44	44
48	Suberic	≤ 9.5	H	53	53
49	Sebacic	≤ 0.37	H	7.8	7.8

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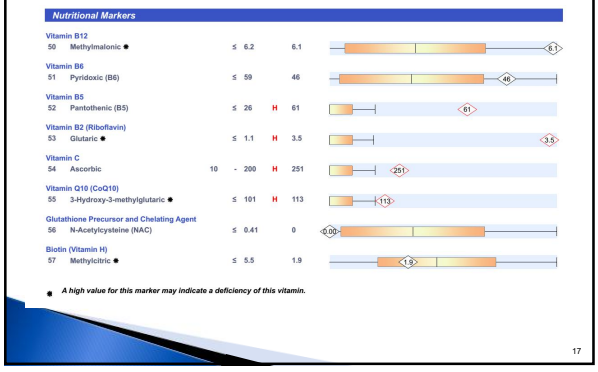
Page 3 – Nutritional Markers



Nutritional Markers					
Vitamin B12					
50	Methylmalonic ✹	≤ 6.2		6.1	6.1
Vitamin B6					
51	Pyridoxic (B6)	≤ 59		46	46
Vitamin B5					
52	Pantothenic (B5)	≤ 26	H	61	61
Vitamin B2 (Riboflavin)					
53	Glutaric ✹	≤ 1.1	H	3.5	3.5
Vitamin C					
54	Ascorbic	10 - 200	H	251	251
Vitamin Q10 (CoQ10)					
55	3-Hydroxy-3-methylglutaric ✹	≤ 101	H	113	113
Glutathione Precursor and Chelating Agent					
56	N-Acetylcysteine (NAC)	≤ 0.41		0	0.00
Biotin (Vitamin H)					
57	Methylcitric ✹	≤ 5.5		1.9	1.9

✹ *A high value for this marker may indicate a deficiency of this vitamin.*

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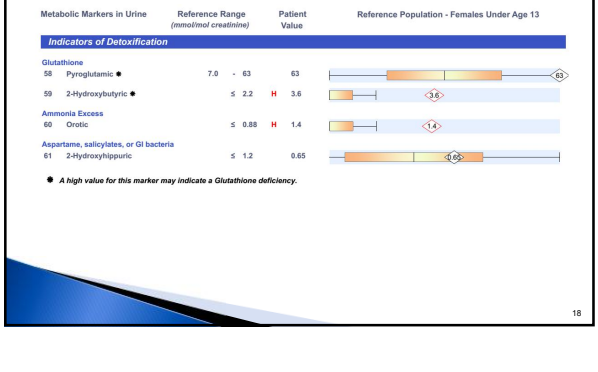
Page 4 – *Indicators of Detoxification*



Metabolic Markers in Urine		Reference Range (mmol/mol creatinine)		Patient Value	Reference Population - Females Under Age 13
Indicators of Detoxification					
Glutathione					
58	Pyroglutamic ✹	7.0 - 63		63	63
59	2-Hydroxybutyric ✹	≤ 2.2	H	3.6	3.6
Ammonia Excess					
60	Orotic	≤ 0.88	H	1.4	1.4
Aspartame, salicylates, or GI bacteria					
61	2-Hydroxyhippuric	≤ 1.2		0.65	0.65

✹ *A high value for this marker may indicate a Glutathione deficiency.*

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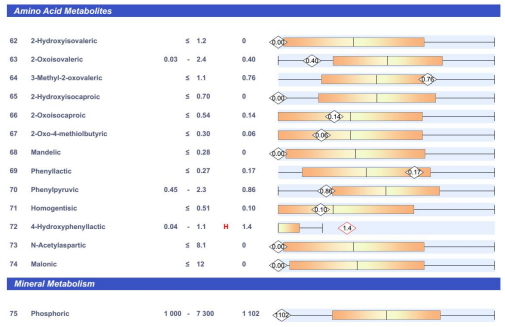
Page 4 – *Amino Acid Metabolites and Phosphoric Acid*



Amino Acid Metabolites

62	2-Hydroxyisovaleric		≤ 1.2		0	0.00
63	2-Oxoisovaleric	0.03	- 2.4		0.40	0.40
64	3-Methyl-2-oxovaleric		≤ 1.1		0.76	0.76
65	2-Hydroxyisocaproic		≤ 0.70		0	0.00
66	2-Oxoisocaproic		≤ 0.54		0.14	0.14
67	2-Oxo-4-methiolbutyric		≤ 0.30		0.06	0.06
68	Mandelic		≤ 0.28		0	0.00
69	Phenyllactic		≤ 0.27		0.17	0.17
70	Phenylpyruvic	0.45	- 2.3		0.86	0.86
71	Homogentisic		≤ 0.51		0.10	0.10
72	4-Hydroxyphenyllactic	0.04	- 1.1	H	1.4	1.4
73	N-Acetylaspartic		≤ 8.1		0	0.00
74	Malonic		≤ 12		0	0.00

Mineral Metabolism

75	Phosphoric	1 000	- 7 300		1 102	1102

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Indicator of Fluid Intake

76	*Creatinine	242	mg/dL

*The creatinine test is performed to adjust metabolic marker results for differences in fluid intake. Urinary creatinine has limited diagnostic value due to variability as a result of recent fluid intake. Samples are rejected if creatinine is below 20 mg/dL unless the client requests results knowing of our rejection criteria.

Explanation of Report Format

The reference ranges for organic acids were established using samples collected from typical individuals of all ages with no known physiological or psychological disorders. The ranges were determined by calculating the mean and standard deviation (SD) and are defined as ± 2SD of the mean. Reference ranges are age and gender specific, consisting of Male Adult (≥13 years), Female Adult (≥13 years), Male Child (<13 years), and Female Child (<13 years).

There are two types of graphical representations of patient values found in the new report format of both the standard Organic Acids Test and the Microbial Organic Acids Test.

The first graph will occur when the value of the patient is within the reference (normal) range, defined as the mean plus or minus two standard deviations.

The second graph will occur when the value of the patient exceeds the upper limit of normal. In such cases, the graphical reference range is "shrunk" so that the degree of abnormality can be appreciated at a glance. In this case, the lower limits of normal are not shown, only the upper limit of normal is shown.

In both cases, the value of the patient is given to the left of the graph and is repeated on the graph inside a diamond. If the value is within the normal range, the diamond will be outlined in black. If the value is high or low, the diamond will be outlined in red.

Example of Value Within Reference Range

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Result	Reference Range - Males Age 13 and Under
Intestinal Microbial Overgrowth				
HPHPA (Clostridia marker)	<	219.9	212	212

Mean
+1SD
-2SD
Patient value
Lower limit of normal
Upper limit of normal

Example of Elevated Value

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Result	Reference Range - Males Age 13 and Under
Intestinal Microbial Overgrowth				
HPHPA (Clostridia marker)	<	219.9	H 3894	3894

Mean
+1SD
-2SD
Patient value
Upper limit of normal

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Invasive Candida Assessment

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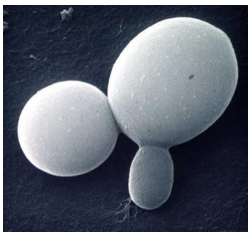
Unicellular Candida



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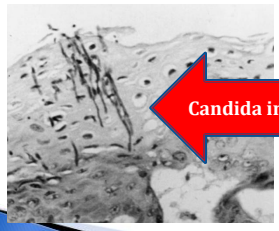
François L. Mayer, et. Al. *Candida albicans pathogenicity mechanisms.* Virulence. Feb 15, 2013; 4(2): 119–128.



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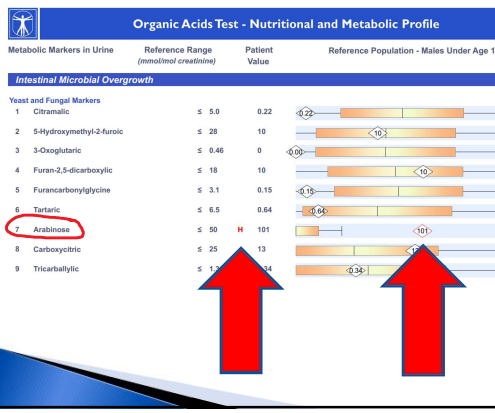

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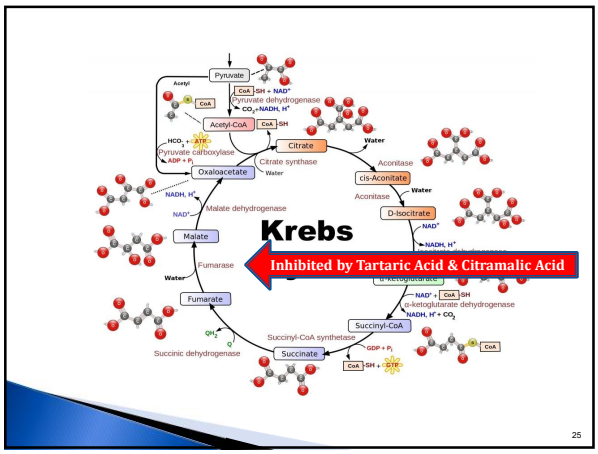
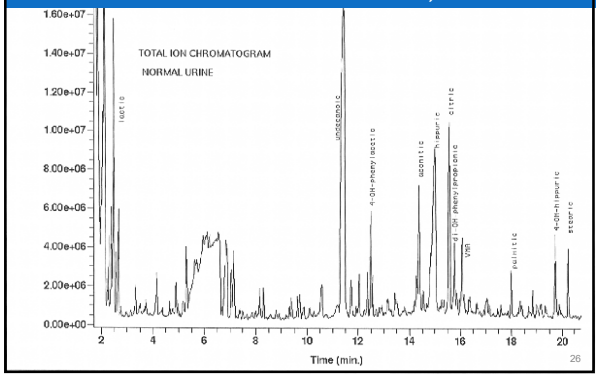


Shaw, W Increased Urinary Excretion of Analogs of Krebs Cycle Metabolites and Arabinose in Two Brothers with Autistic Features. Clin Chem 41:1094-1104, 1995



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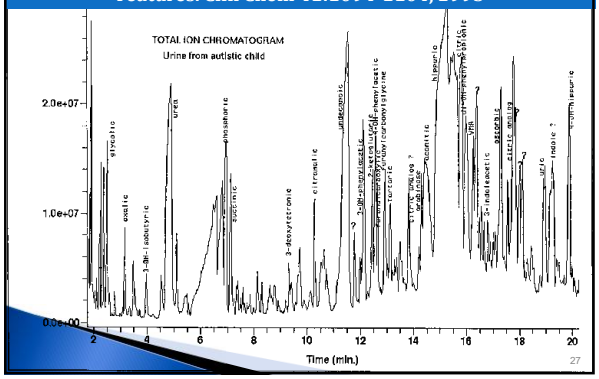
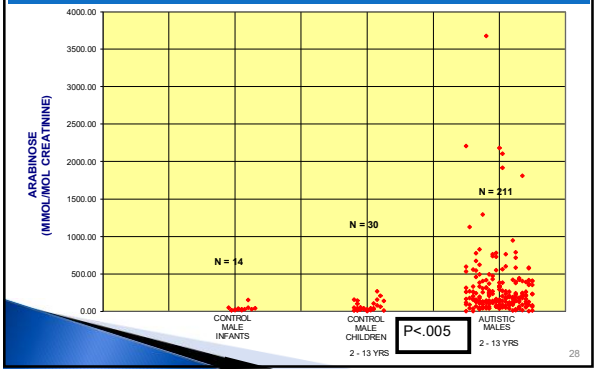
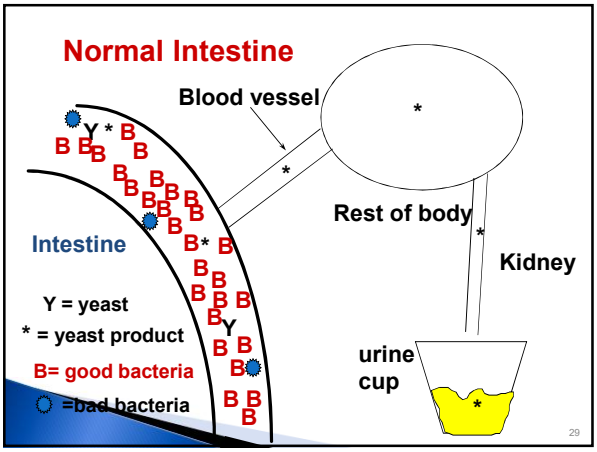
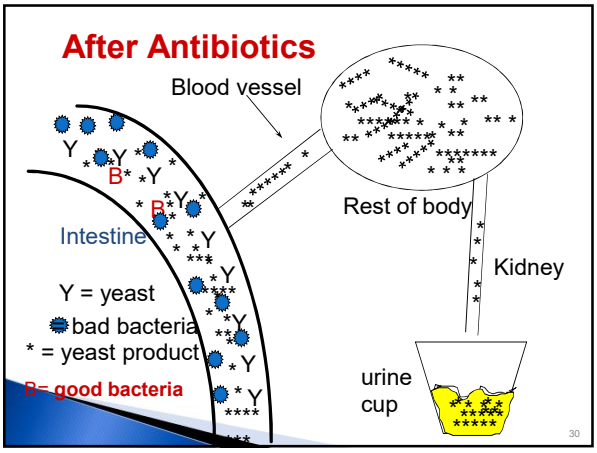


Shaw, W., et al Assessment of antifungal drug therapy in autism by measurement of suspected microbial metabolites in urine with GC/MS. Clinical Practice of Alternative Medicine: 15-26,2000







Conditions In Which Candida May Be a Factor

- Schizophrenia
- Alzheimer's disease
- Systemic lupus erythematosus (SLE)
- Fibromyalgia
- Chronic fatigue syndrome & CFIDS
- HIV infection
- Colitis
- Depression
- PMS
- Vaginal yeast infection
- Multiple sclerosis
- Interstitial cystitis
- Seizures
- Irritable bowel
- Cancer

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Family Practice
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Vol. 18, No. 3
Printed in Great Britain

Effectiveness of nystatin in polysymptomatic patients. A randomized, double-blind trial with nystatin versus placebo in general practice

Heiko Santelmann, Even Laerum, Joergen Roennevig[a] and Hans E Fagertun[b]

Santelmann H, Laerum E, Roennevig J and Fagertun HE. Effectiveness of nystatin in polysymptomatic patients. A randomized, double-blind trial with nystatin versus placebo in general practice. *Family Practice* 2001; **18:** 258–265.

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In the 116 patients selected by the FRDQ-7 questionnaire, nystatin therapy reduced overall symptoms significantly as compared with placebo, even after correction for sugar- and yeast-free diet.

Nystatin showed the most striking effect for mental, abdominal and urogenital complaints. Since we did not perform microbiological studies in the patients and the positive effect of nystatin may be due to its effect on other fungi, a connection between *C.albicans* and FRD remains unproved.

Nystatin is well known for its antifungal effect on *C.albicans* which is found in all segments of the gastrointestinal tract in 10–80% of humans,[14,15,19] as well as on other yeasts and moulds.

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95% Predicative of Positive Response to Nystatin

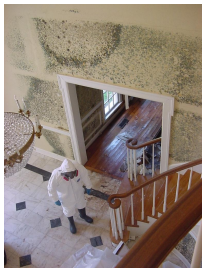
SCORE:

0 = none
1 = occasional or mild
2 = frequent or moderately severe
3 = severe or disabling

1. Have you, at any time in your life, taken broad spectrum antibiotics? (0 or 3)
2. Have you taken tetracycline or other broad-spectrum antibiotics for one month or longer? (0 or 3)
3. Are your symptoms worse on damp, muggy days or in moldy places? (0 or 3)
4. Do you crave sugar? (0 or 3)
5. Do you have a feeling of being "drained?" (0, 1, 2 or 3)
6. WOMEN: Are you bothered with vaginal burning, itching or discharge? (0, 1, 2 or 3)
   MEN: Do you have burning, itching or discharge from the penis? (0, 1, 2 or 3)
7. Are you bothered by burning, itching or tearing of your eyes? (0, 1, 2 or 3)

TOTAL SCORE FOR FRDQ-7:

Score 0–3 = FRD unlikely
Score 4–9 = FRD probable
Score 10–21 = FRD almost certain



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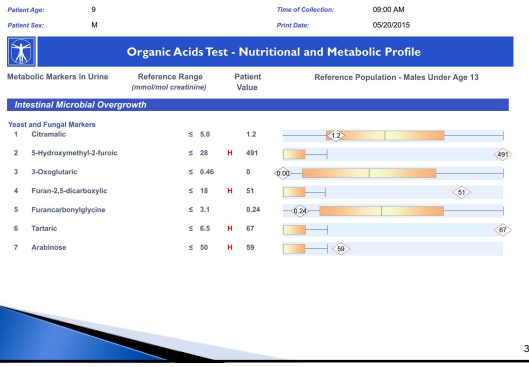

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Clostridia Toxin Assessment

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Lessa FC, Mu Y, Bamberg WM, et al. *Burden of Clostridium difficile infection in the United States.* N Engl J Med. 2015;372:825-834

- The Centers for Disease Control estimates that there were 453,000 documented cases of Clostridium difficile infection (CDI) in the United States in 2011 leading to 29,300 deaths.
- **Between 10% and 30%** of people who have an initial episode of CDI will develop at least one recurrence.
- On the basis of the number of incident cases found in 2011, 45,300-135,900 people developed recurrent CDI.

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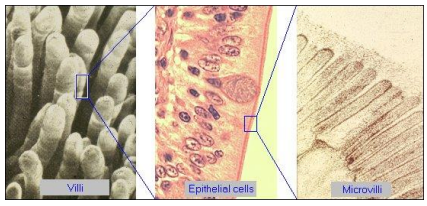
Brush Border, Villi and Tight Junction Damage



Toxin A

Toxin B

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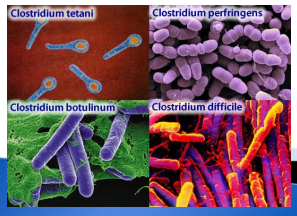
Toxin A & Toxin B

- These two toxins are the main virulence factors related to mucosal damage from C. difficile.
- Toxins A & B lead to digestive tract inflammation, e.g. Pseudomembranous colitis or clostridia difficile associated diarrhea (CDAD).
- Toxin A & Toxin B are both capable of causing mucosal damage (Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP; October 2010. "The role of toxin A and toxin B in Clostridium difficile infection". *Nature* 467 (7316): 711–3).

Other Toxins of Clostridia Bacteria



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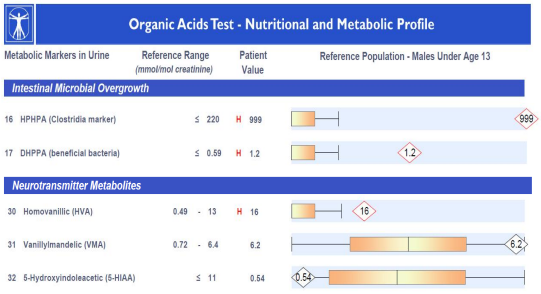
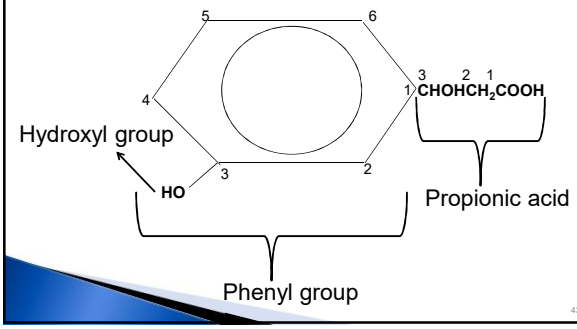
HPHPA Toxicity



Structure of 3-(3-hydroxyphenyl)-3-hydroxypropionic acid



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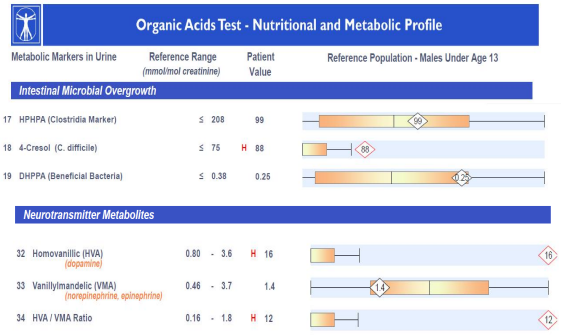
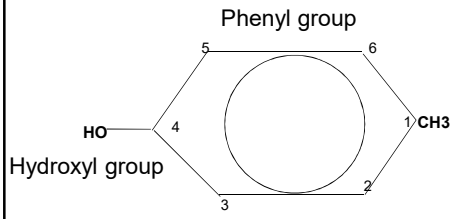
4-Cresol Toxicity

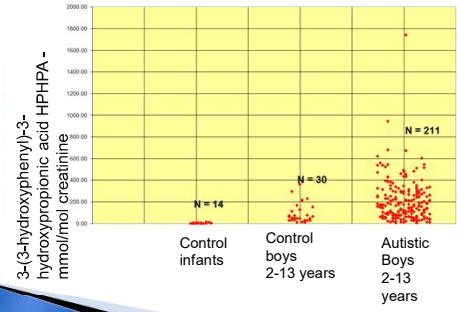


Structure of 4-cresol (methylphenol)



45

Distribution of values for HPHPA Clostridia metabolite in urine samples of male infants, control boys, and boys with autism



46

Research article

Acute Schizophrenia

Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of *Clostridia* spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia

William Shaw

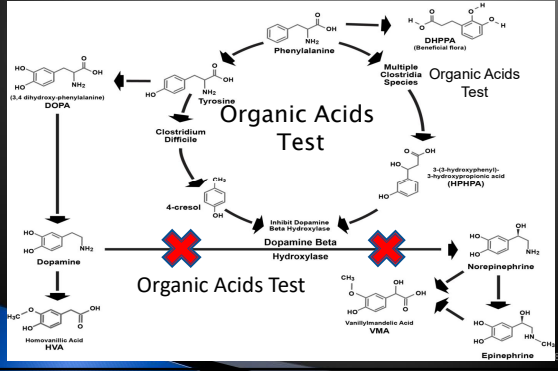
The Great Plains Laboratory, Inc., Lenexa, Kansas, USA

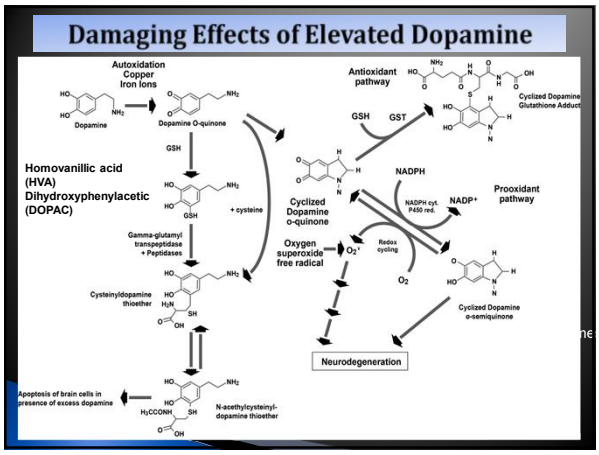
Nutritional Neuroscience 2010 Vol 13 No 3: 1-10

A compound identified as 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) was found in higher concentrations in urine samples of children with autism compared to age and sex appropriate controls and in an adult with recurrent diarrhea due to *Clostridium difficile* infections. The highest value measured in urine samples was 7500 mmol/mol creatinine, a value 300 times the median normal adult value, in a patient with acute schizophrenia during an acute psychotic episode. The psychosis remitted after treatment with oral vancomycin with a concomitant marked decrease in HPHPA. The source of this compound appears to be multiple species of anaerobic bacteria of the *Clostridium* genus. The significance of this compound is that it is a probable metabolite of *m*-tyrosine (3-hydroxyphenylalanine), a tyrosine analog which depletes brain catecholamines and causes symptoms of autism (stereotypical behavior, hyperactivity, and hyper-reactivity) in experimental animals.

47

Effect Of Certain Intestinal Bacteria Toxins On Neurotransmitters





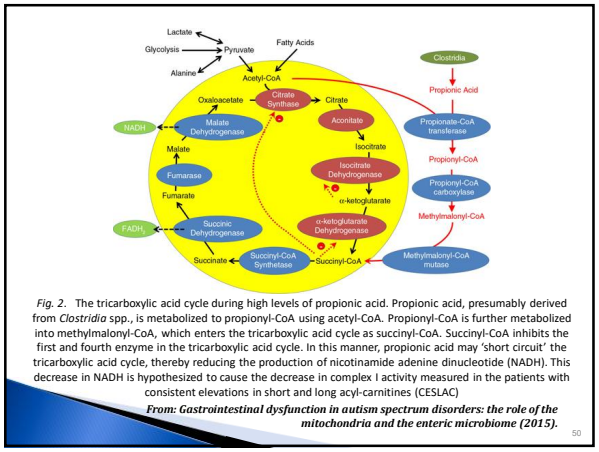
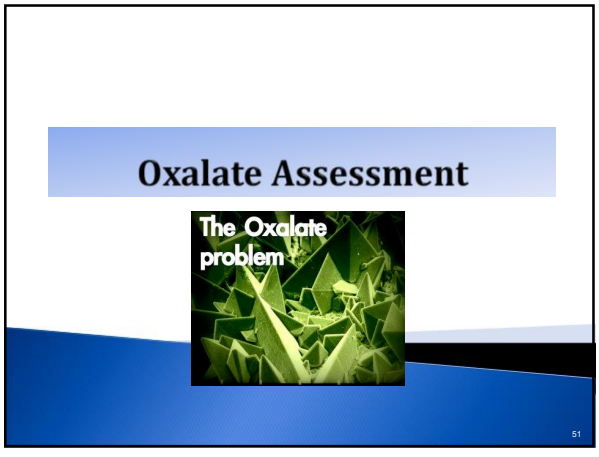


Fig. 2. The tricarboxylic acid cycle during high levels of propionic acid. Propionic acid, presumably derived from *Clostridia* spp., is metabolized to propionyl-CoA using acetyl-CoA. Propionyl-CoA is further metabolized into methylmalonyl-CoA, which enters the tricarboxylic acid cycle as succinyl-CoA. Succinyl-CoA inhibits the first and fourth enzyme in the tricarboxylic acid cycle. In this manner, propionic acid may 'short circuit' the tricarboxylic acid cycle, thereby reducing the production of nicotinamide adenine dinucleotide (NADH). This decrease in NADH is hypothesized to cause the decrease in complex I activity measured in the patients with consistent elevations in short and long acyl-carnitines (CESLAC)

From: Gastrointestinal dysfunction in autism spectrum disorders: the role of the mitochondria and the enteric microbiome (2015).

50



51

Requisition #: Physician Name:

Patient Name: Date of Collection:

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Females Under Age 13
Oxalate Metabolites			
18 Glyceric	0.71 - 9.5	H 18	18
19 Glycolic	20 - 202	100	100
20 Oxalic	15 - 174	H 483	483
Glycolytic Cycle Metabolites			
21 Lactic	0.18 - 44	H 301	
22 Pyruvic	0.88 - 9.1	9.0	
23 2-Hydroxybutyric	≤ 2.2	H 3.7	3.7

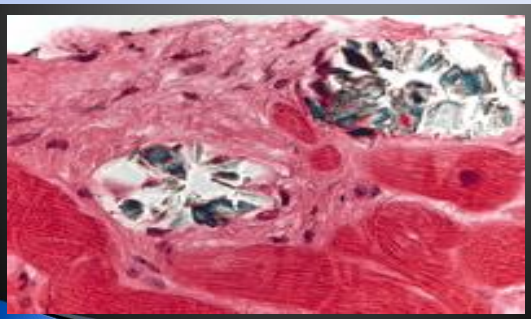
52

Oxalate Staghorn in Kidney (X-Ray)

75-90% of kidney stones are oxalates. 10-15 percent of adults will be diagnosed with a kidney stone in their lifetime.

53

Oxalate Crystals in the Heart

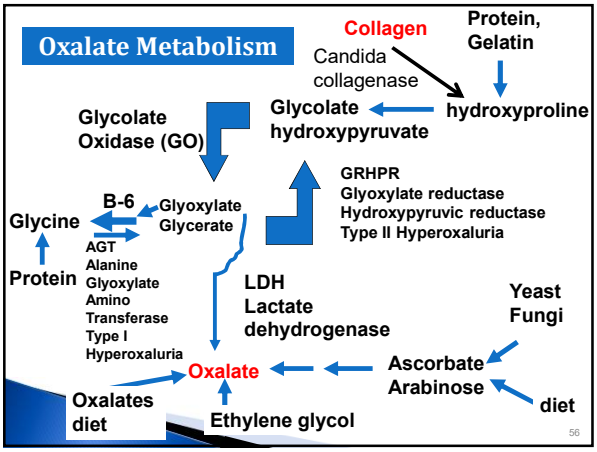


54

Oxalate Crystals in Leg Lesions

55

Oxalate Metabolism



56

"Isolation and characteristics of collagenolytic enzyme produced by Candida albicans"

Infect Immun., H. Kaminishi, et.al, 1986 August; 53(2): 312-316.

Collagen makes up a significant percentage (approx. 30%) of all protein in the body.

57

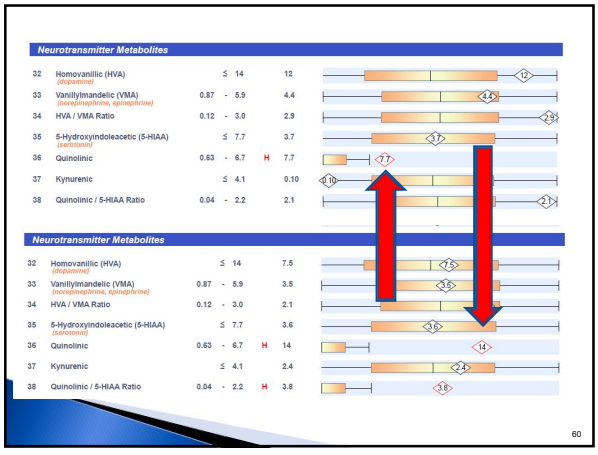
Other Diseases/Disorders in Which Oxalate May Play a Role

- Arthritis
- Behavior problems in children
- Interstitial cystitis
- Joint pain
- Fibromyalgia
- Heart disease (*atherosclerosis, conduction issues)*
- Heavy metal toxicity
- Osteoporosis
- Thyroid problems

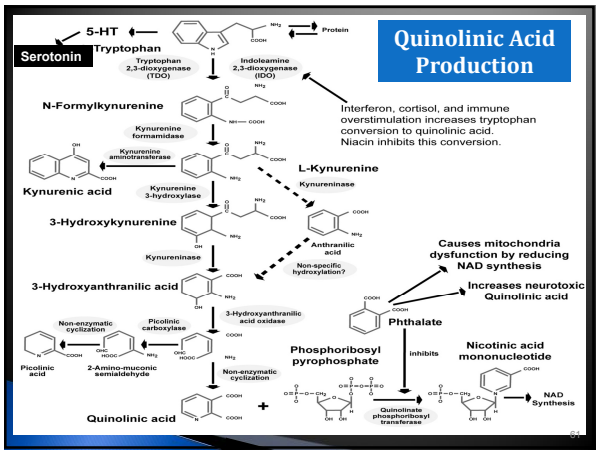
58

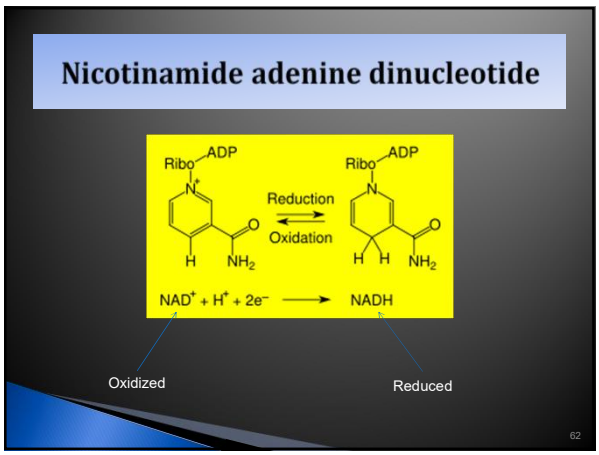
Quinolinic Acid & Neurochemical Assessment

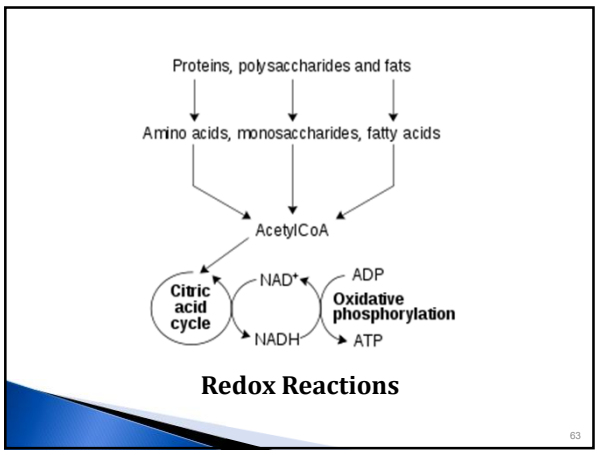
59

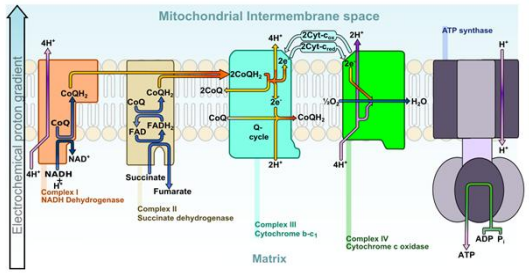


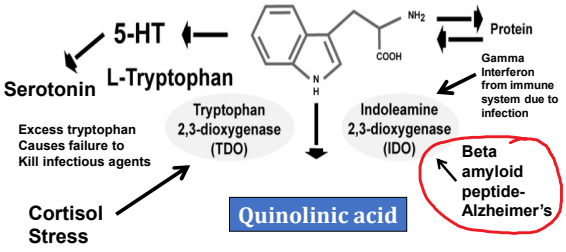
60


Quinolinic Acid Production
5-HT
Serotonin
Tryptophan
Protein
Tryptophan 2,3-dioxygenase (TDO)
Indoleamine 2,3-dioxygenase (IDO)
N-Formylkynurenine
Kynurenine formamidase
Interferon, cortisol, and immune overstimulation increases tryptophan conversion to quinolinic acid. Niacin inhibits this conversion.
Kynurenine aminotransferase
L-Kynurenine
Kynurenic acid
Kynurenine 3-hydroxylase
Kynureninase
3-Hydroxykynurenine
Anthranilic acid
Non-specific hydroxylation?
Causes mitochondria dysfunction by reducing NAD synthesis
Increases neurotoxic Quinolinic acid
3-Hydroxyanthranilic acid
3-Hydroxyanthranilic acid oxidase
Phthalate
Non-enzymatic cyclization
Picolinic carboxylase
Phosphoribosyl pyrophosphate
inhibits
Nicotinic acid mononucleotide
Picolinic acid
2-Amino-muconic semialdehyde
Non-enzymatic cyclization
NAD Synthesis
Quinolinic acid
Quinolinate phosphoribosyl transferase
61


Nicotinamide adenine dinucleotide
Ribo
ADP
Reduction
Oxidation
NAD+ + H+ + 2e−
NADH
Oxidized
Reduced
62


Proteins, polysaccharides and fats
Amino acids, monosaccharides, fatty acids
AcetylCoA
Citric acid cycle
NAD+
NADH
ADP
ATP
Oxidative phosphorylation
Redox Reactions
63





Kills cells containing bacteria, viruses, parasites. May also damage infectious organisms themselves. IDO causes drastic reduction in tryptophan for protein synthesis needed by infected cells and infectious organisms - tryptophan at very low levels.

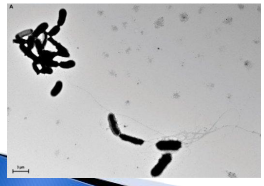
65

Human amyloid-beta acts as natural antibiotic in the brain: Alzheimer's-associated amyloid plaques may trap microbes

Date: May 25, 2016

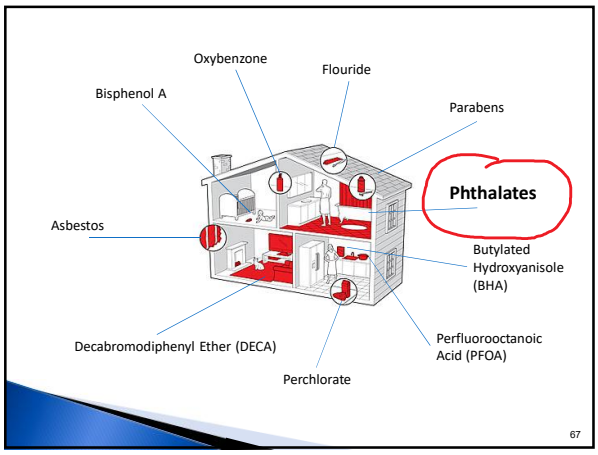
Source: Massachusetts General Hospital

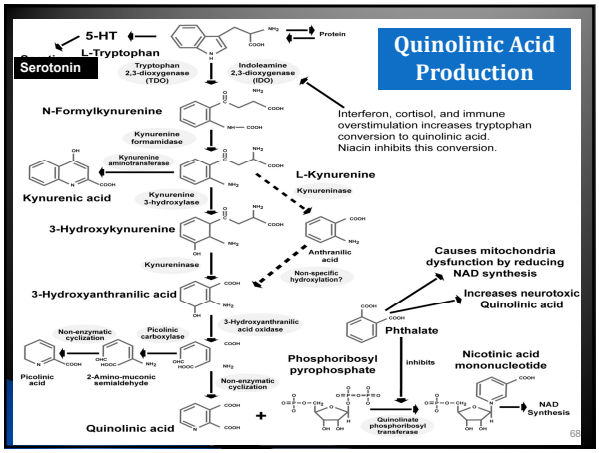
Summary: A new study provides additional evidence that amyloid-beta protein -- which is deposited in the form of beta-amyloid plaques in the brains of patients with Alzheimer's disease -- is a normal part of the innate immune system, the body's first-line defense against infection.



Amyloid fibrils propagate from yeast surfaces and capture Candida albicans in culture medium.
Credit: D.K.V. Kumar et al. / Science Translational Medicine (2016)

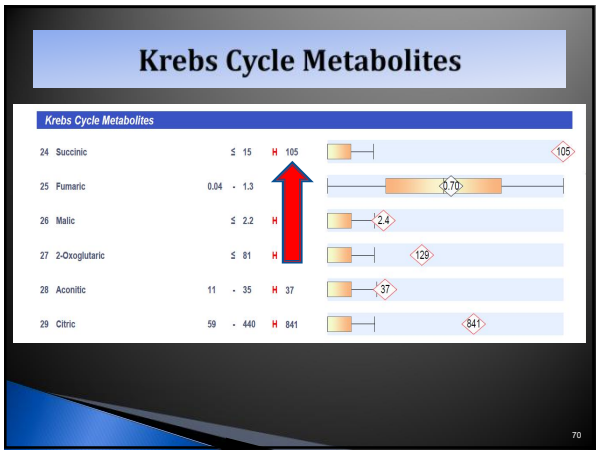
66


Oxybenzone
Flouride
Bisphenol A
Parabens
Phthalates
Asbestos
Butylated Hydroxyanisole (BHA)
Perfluorooctanoic Acid (PFOA)
Decabromodiphenyl Ether (DECA)
Perchlorate
67

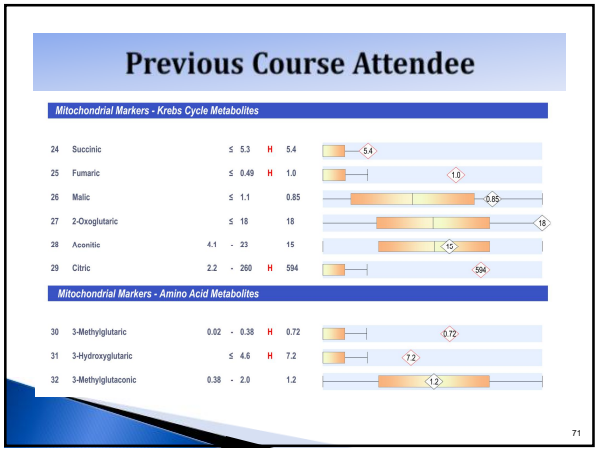

Quinolinic Acid Production
5-HT
Serotonin
L-Tryptophan
Protein
Tryptophan 2,3-dioxygenase (TDO)
Indoleamine 2,3-dioxygenase (IDO)
Interferon, cortisol, and immune overstimulation increases tryptophan conversion to quinolinic acid. Niacin inhibits this conversion.
N-Formylkynurenine
Kynurenine formamidase
Kynurenine aminotransferase
Kynurenic acid
L-Kynurenine
Kynureninase
Kynurenine 3-hydroxylase
3-Hydroxykynurenine
Anthranilic acid
Non-specific hydroxylation?
Kynureninase
Causes mitochondria dysfunction by reducing NAD synthesis
Increases neurotoxic Quinolinic acid
3-Hydroxyanthranilic acid
3-Hydroxyanthranilic acid oxidase
Phthalate
Non-enzymatic cyclization
Picolinic carboxylase
Phosphoribosyl pyrophosphate
Inhibits
Nicotinic acid mononucleotide
Picolinic acid
2-Amino-muconic semialdehyde
Non-enzymatic cyclization
NAD Synthesis
Quinolinic acid
Quinolinate phosphoribosyl transferase
68


Mitochondrial Dysfunction Assessment
69

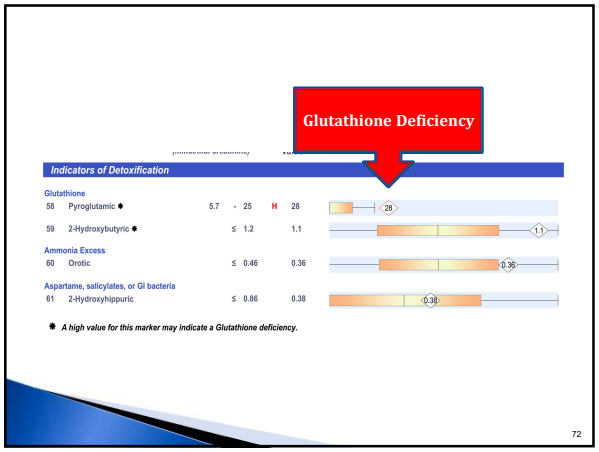
Krebs Cycle Metabolites

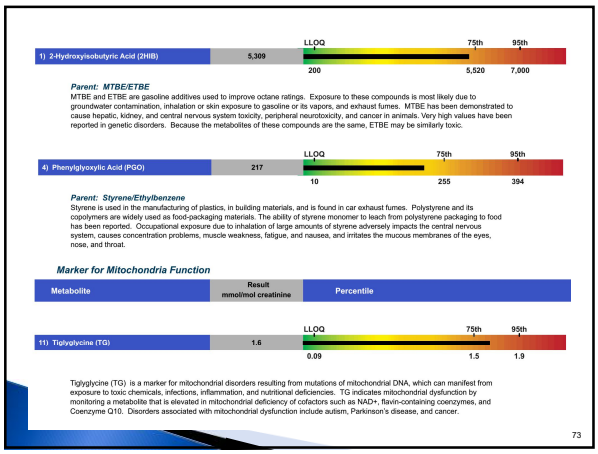


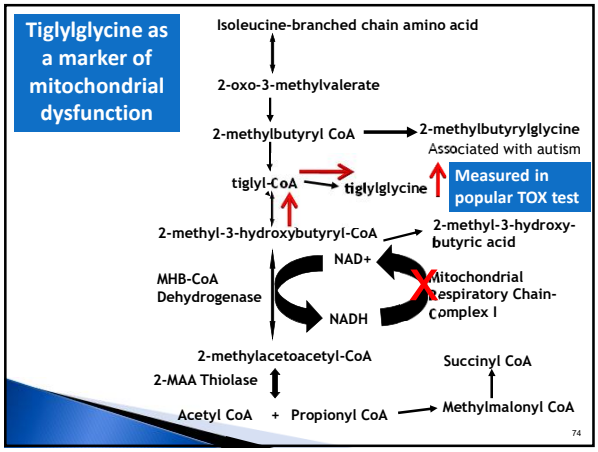
Previous Course Attendee

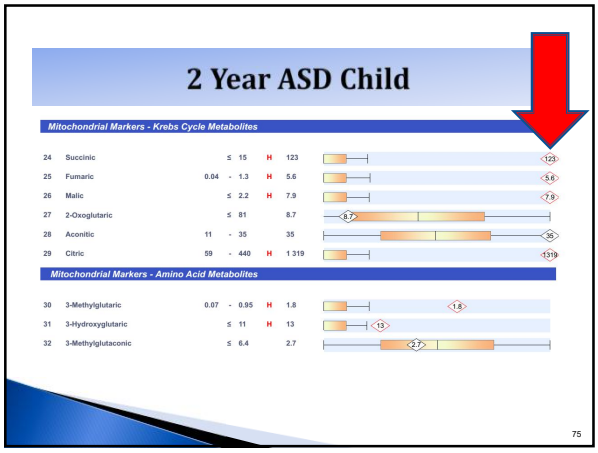


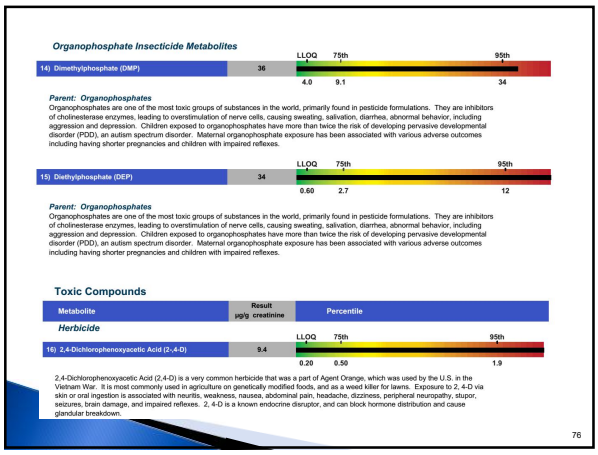
Glutathione Deficiency

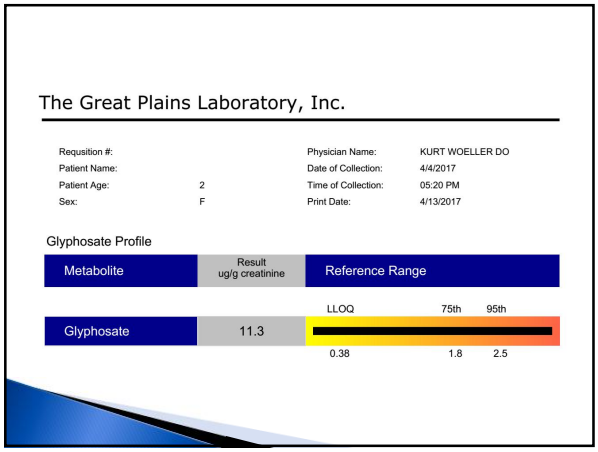


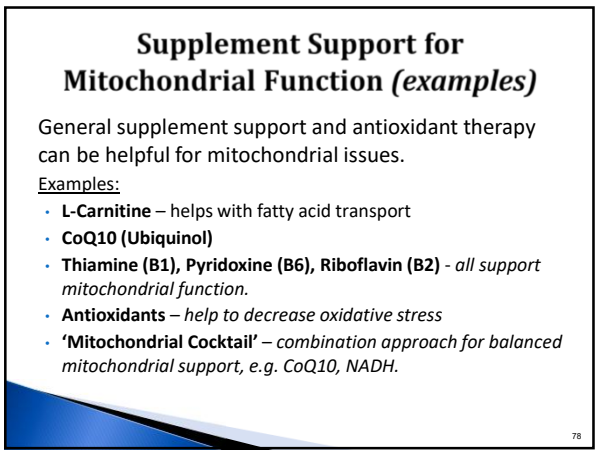

LLOQ
75th
95th
1) 2-Hydroxyisobutyric Acid (2HIB)
5,309
200
5,520
7,000
Parent: MTBE/ETBE
MTBE and ETBE are gasoline additives used to improve octane ratings. Exposure to these compounds is most likely due to groundwater contamination, inhalation or skin exposure to gasoline or its vapors, and exhaust fumes. MTBE has been demonstrated to cause hepatic, kidney, and central nervous system toxicity, peripheral neurotoxicity, and cancer in animals. Very high values have been reported in genetic disorders. Because the metabolites of these compounds are the same, ETBE may be similarly toxic.
LLOQ
75th
95th
4) Phenylglyoxylic Acid (PGO)
217
10
255
394
Parent: Styrene/Ethylbenzene
Styrene is used in the manufacturing of plastics, in building materials, and is found in car exhaust fumes. Polystyrene and its copolymers are widely used as food-packaging materials. The ability of styrene monomer to leach from polystyrene packaging to food has been reported. Occupational exposure due to inhalation of large amounts of styrene adversely impacts the central nervous system, causes concentration problems, muscle weakness, fatigue, and nausea, and irritates the mucous membranes of the eyes, nose, and throat.
Marker for Mitochondria Function
Metabolite
Result mmol/mol creatinine
Percentile
LLOQ
75th
95th
11) Tiglylglycine (TG)
1.6
0.09
1.5
1.9
Tiglylglycine (TG) is a marker for mitochondrial disorders resulting from mutations of mitochondrial DNA, which can manifest from exposure to toxic chemicals, infections, inflammation, and nutritional deficiencies. TG indicates mitochondrial dysfunction by monitoring a metabolite that is elevated in mitochondrial deficiency of cofactors such as NAD+, flavin-containing coenzymes, and Coenzyme Q10. Disorders associated with mitochondrial dysfunction include autism, Parkinson's disease, and cancer.
73


Tiglylglycine as a marker of mitochondrial dysfunction
Isoleucine-branched chain amino acid
2-oxo-3-methylvalerate
2-methylbutyryl CoA
2-methylbutyrylglycine
Associated with autism
tiglyl-CoA
tiglylglycine
Measured in popular TOX test
2-methyl-3-hydroxybutyryl-CoA
2-methyl-3-hydroxy-butyric acid
NAD+
MHB-CoA Dehydrogenase
Mitochondrial Respiratory Chain-Complex I
NADH
2-methylacetoacetyl-CoA
Succinyl CoA
2-MAA Thiolase
Acetyl CoA + Propionyl CoA
Methylmalonyl CoA
74


2 Year ASD Child
Mitochondrial Markers - Krebs Cycle Metabolites
24 Succinic ≤ 15 H 123
25 Fumaric 0.04 - 1.3 H 5.6
26 Malic ≤ 2.2 H 7.9
27 2-Oxoglutaric ≤ 81 8.7
28 Aconitic 11 - 35 35
29 Citric 59 - 440 H 1 319
Mitochondrial Markers - Amino Acid Metabolites
30 3-Methylglutaric 0.07 - 0.95 H 1.8
31 3-Hydroxyglutaric ≤ 11 H 13
32 3-Methylglutaconic ≤ 6.4 2.7
75


Organophosphate Insecticide Metabolites
LLOQ 75th 95th
14) Dimethylphosphate (DMP) 36
4.0 9.1 34
Parent: Organophosphates
Organophosphates are one of the most toxic groups of substances in the world, primarily found in pesticide formulations. They are inhibitors of cholinesterase enzymes, leading to overstimulation of nerve cells, causing sweating, salivation, diarrhea, abnormal behavior, including aggression and depression. Children exposed to organophosphates have more than twice the risk of developing pervasive developmental disorder (PDD), an autism spectrum disorder. Maternal organophosphate exposure has been associated with various adverse outcomes including having shorter pregnancies and children with impaired reflexes.
LLOQ 75th 95th
15) Diethylphosphate (DEP) 34
0.60 2.7 12
Parent: Organophosphates
Organophosphates are one of the most toxic groups of substances in the world, primarily found in pesticide formulations. They are inhibitors of cholinesterase enzymes, leading to overstimulation of nerve cells, causing sweating, salivation, diarrhea, abnormal behavior, including aggression and depression. Children exposed to organophosphates have more than twice the risk of developing pervasive developmental disorder (PDD), an autism spectrum disorder. Maternal organophosphate exposure has been associated with various adverse outcomes including having shorter pregnancies and children with impaired reflexes.
Toxic Compounds
Metabolite
Result ug/g creatinine
Percentile
Herbicide
LLOQ 75th 95th
16) 2,4-Dichlorophenoxyacetic Acid (2,4-D) 9.4
0.20 0.50 1.9
2,4-Dichlorophenoxyacetic Acid (2,4-D) is a very common herbicide that was a part of Agent Orange, which was used by the U.S. in the Vietnam War. It is most commonly used in agriculture on genetically modified foods, and as a weed killer for lawns. Exposure to 2, 4-D via skin or oral ingestion is associated with neuritis, weakness, nausea, abdominal pain, headache, dizziness, peripheral neuropathy, stupor, seizures, brain damage, and impaired reflexes. 2, 4-D is a known endocrine disruptor, and can block hormone distribution and cause glandular breakdown.
76


The Great Plains Laboratory, Inc.
Requsition #:
Patient Name:
Patient Age: 2
Sex: F
Physician Name: KURT WOELLER DO
Date of Collection: 4/4/2017
Time of Collection: 05:20 PM
Print Date: 4/13/2017
Glyphosate Profile
Metabolite
Result ug/g creatinine
Reference Range
LLOQ 75th 95th
Glyphosate 11.3
0.38 1.8 2.5


Supplement Support for Mitochondrial Function (examples)
General supplement support and antioxidant therapy can be helpful for mitochondrial issues.
Examples:
• L-Carnitine – helps with fatty acid transport
• CoQ10 (Ubiquinol)
• Thiamine (B1), Pyridoxine (B6), Riboflavin (B2) - all support mitochondrial function.
• Antioxidants – help to decrease oxidative stress
• 'Mitochondrial Cocktail' – combination approach for balanced mitochondrial support, e.g. CoQ10, NADH.
78

MitoSpectra

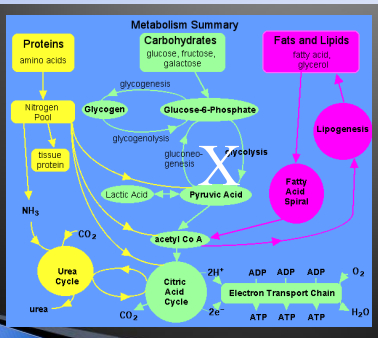
Requisition #: *Physician Name:*

Patient Name: *Date of Collection:*

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Females Under Age 13
Oxalate Metabolites			
18 Glyceric	0.71 - 9.5	H 18	18
19 Glycolic	20 - 202	100	100
20 Oxalic	15 - 174	H 483	483
Glycolytic Cycle Metabolites			
21 Lactic	0.18 - 44	H 301	301
22 Pyruvic	0.88 - 9.1	9.0	9.0
23 2-Hydroxybutyric	≤ 2.2	H 3.7	3.7

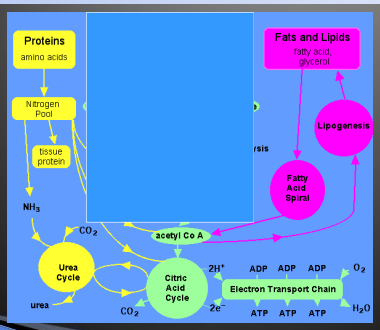
80

Oxalate Disruption of Pyruvic Acid in Metabolism



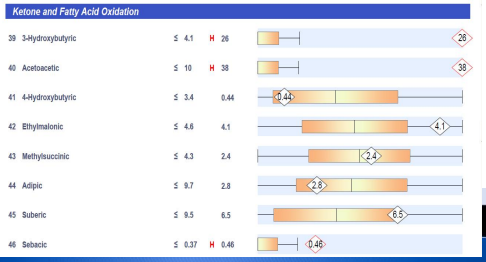
81

What Happens When Pyruvic Acid Formation is Blocked?



82

Fatty Acid Metabolites



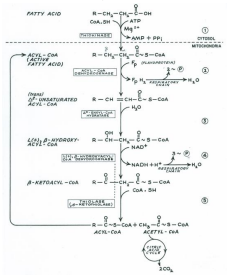
83

Beta-Oxidation

- **Beta-oxidation** is the process by which fatty acid are broken down in the mitochondria to generate acetyl-CoA. The acetyl-CoA then enters citric acid cycle generating NADH which is used by the electron transport chain.

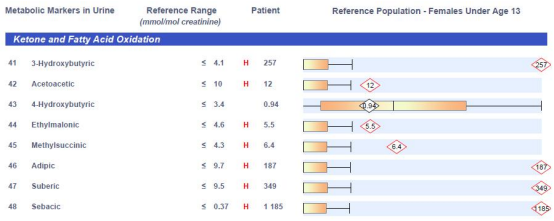
***#44 - #48** are specific indicators of oxidation of fatty acids in the cytoplasm of the cell:*

- *Fatty acid oxidation disorders*
- *Carnitine deficiency*
- *Fasting*
- *Large intake of Medium Chain Triglycerides, i.e. baby formulas*
- ***Supplementation of 500mg to 1000mg of L-Carnitine may be beneficial.***



84

2 year old girl from China



	Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient
	Ketone and Fatty Acid Oxidation			
41	3-Hydroxybutyric	≤ 4.1	H	257
42	Acetoacetic	≤ 10	H	12
43	4-Hydroxybutyric	≤ 3.4		0.94
44	Ethylmalonic	≤ 4.6	H	5.5
45	Methylsuccinic	≤ 4.3	H	6.4
46	Adipic	≤ 9.7	H	187
47	Suberic	≤ 9.5	H	349
48	Sebacic	≤ 0.37	H	1 185

Reference Population - Females Under Age 13

High Dose *Medium Chain Triglycerides (MCT Oil)*

85

Nutritional Markers & Remaining Sections

86

Nutritional Markers

Indirect:

- Methylmalonic acid - *vitamin B-12*
- Methylcitric acid - *biotin*
- Glutaric and 3-hydroxy-3-methylglutaric - *indicators of riboflavin and coenzyme Q-10 deficiency, respectively.*

	Vitamin Q10 (CoQ10)			
55	3-Hydroxy-3-methylglutaric ♦	≤ 88	H	313

Direct:

- Ascorbic acid - *vitamin C*
- Pantothenic acid - *B vitamin*
- Pyridoxic acid - *metabolite of vitamin B-6*

87

Pyrimidines

Elevated in cancer, genetic disease, folate issues

Pyrimidines

39	Uracil	≤ 16	H 32	32	
40	Thymine	≤ 0.91	H 0.92	0.92	

88

Bone Metabolites

72 Phosphoric 1 439 - 9 732 H 12 408 12408

High values

- High intake
- Hyperparathyroidism
- Vitamin D-resistant rickets
- Immobilization following paraplegia or fracture
- Vitamin D intoxication
- Renal tubular damage, heavy metal toxicity
- Familial hypophosphatemia
- Metabolic acidosis

Low values

- Low intake
- Hypoparathyroidism
- Pseudohypoparathyroidism
- Parathyroidectomy
- **Vitamin D deficiency**

89

骨质代谢

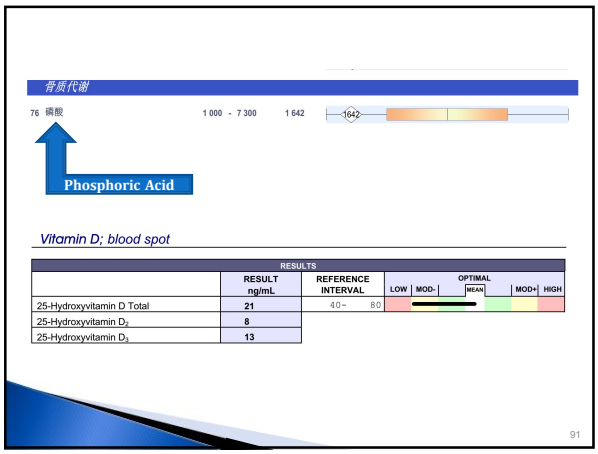
76 磷酸 1 000 - 7 300 1 940 1940

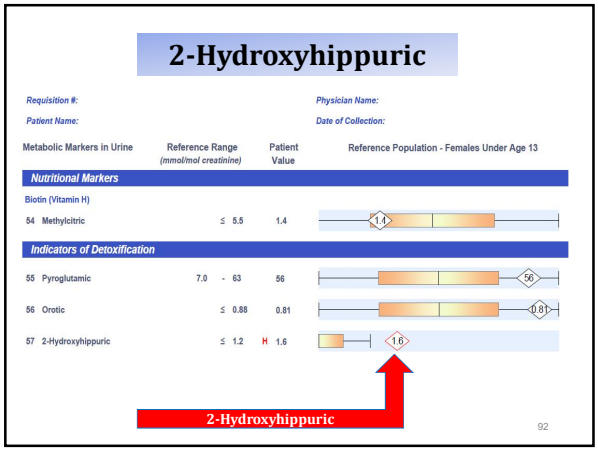
Phosphoric Acid

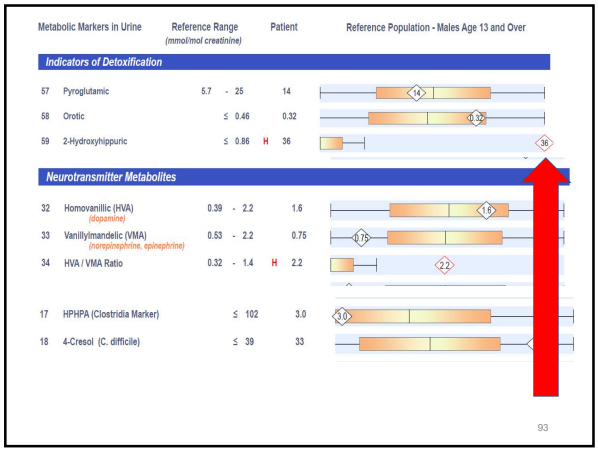
Vitamin D; blood spot

RESULTS	RESULT ng/mL	REFERENCE INTERVAL	OPTIMAL (LOW | MOD- | MEAN | MOD+ | HIGH)
25-Hydroxyvitamin D Total	28	40- 80	
25-Hydroxyvitamin D_2	< 1.5		
25-Hydroxyvitamin D_3	28		

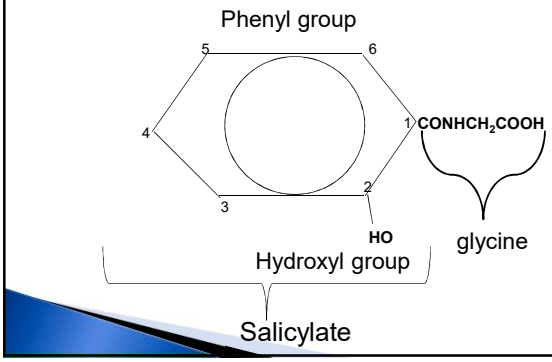
90


肾质代谢
76 磷酸
1 000 - 7 300
1 642
1642
Phosphoric Acid
Vitamin D; blood spot
RESULTS
RESULT ng/mL
REFERENCE INTERVAL
OPTIMAL
LOW
MOD-
MEAN
MOD+
HIGH
25-Hydroxyvitamin D Total
21
40 – 80
25-Hydroxyvitamin D2
8
25-Hydroxyvitamin D3
13
91


2-Hydroxyhippuric
Requisition #:
Physician Name:
Patient Name:
Date of Collection:
Metabolic Markers in Urine
Reference Range (mmol/mol creatinine)
Patient Value
Reference Population - Females Under Age 13
Nutritional Markers
Biotin (Vitamin H)
54 Methylcitric
≤ 5.5
1.4
Indicators of Detoxification
55 Pyroglutamic
7.0 - 63
56
56 Orotic
≤ 0.88
0.81
57 2-Hydroxyhippuric
≤ 1.2
H 1.6
2-Hydroxyhippuric
92


Metabolic Markers in Urine
Reference Range (mmol/mol creatinine)
Patient
Reference Population - Males Age 13 and Over
Indicators of Detoxification
57 Pyroglutamic
5.7 - 25
14
58 Orotic
≤ 0.46
0.32
59 2-Hydroxyhippuric
≤ 0.86
H 36
Neurotransmitter Metabolites
32 Homovanillic (HVA) (dopamine)
0.39 - 2.2
1.6
33 Vanillylmandelic (VMA) (norepinephrine, epinephrine)
0.53 - 2.2
0.75
34 HVA / VMA Ratio
0.32 - 1.4
H 2.2
17 HPHPA (Clostridia Marker)
≤ 102
3.0
18 4-Cresol (C. difficile)
≤ 39
33
93

Structure of 2-Hydroxyhippuric Acid - *metabolite of aspartame (Nutrasweet®), aspirin, foods, additives*



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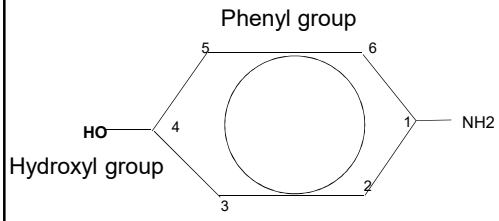
Structure of Aminophenol – *major acetaminophen metabolite*



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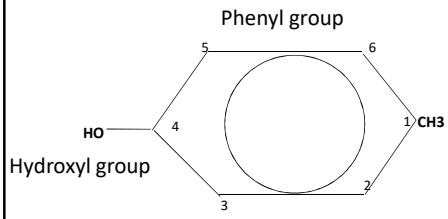
Structure of 4-cresol (methylphenol)



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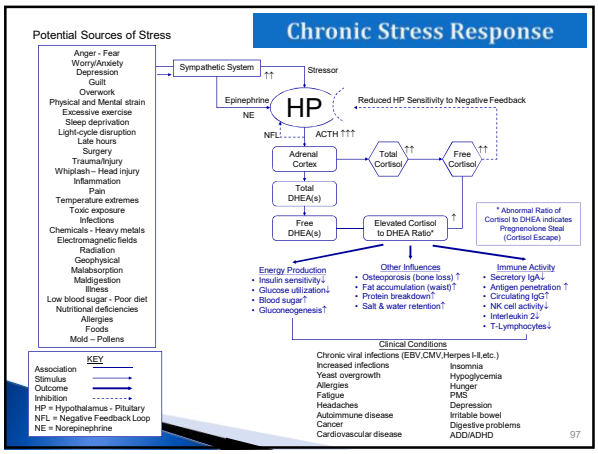
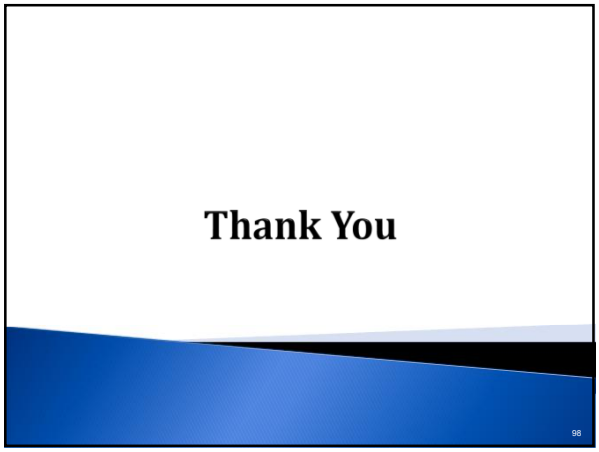

Chronic Stress Response
Potential Sources of Stress
Anger - Fear
Worry/Anxiety
Depression
Guilt
Overwork
Physical and Mental strain
Excessive exercise
Sleep deprivation
Light-cycle disruption
Late hours
Surgery
Trauma/Injury
Whiplash – Head injury
Inflammation
Pain
Temperature extremes
Toxic exposure
Infections
Chemicals - Heavy metals
Electromagnetic fields
Radiation
Geophysical
Malabsorption
Maldigestion
Illness
Low blood sugar - Poor diet
Nutritional deficiencies
Allergies
Foods
Mold – Pollens
Sympathetic System
Stressor
Epinephrine
NE
HP
Reduced HP Sensitivity to Negative Feedback
NFL
ACTH
Adrenal Cortex
Total Cortisol
Free Cortisol
Total DHEA(s)
Free DHEA(s)
Elevated Cortisol to DHEA Ratio*
* Abnormal Ratio of Cortisol to DHEA indicates Pregnenolone Steal (Cortisol Escape)
Energy Production
Insulin sensitivity↓
Glucose utilization↓
Blood sugar↑
Gluconeogenesis↑
Other Influences
Osteoporosis (bone loss) ↑
Fat accumulation (waist)↑
Protein breakdown↑
Salt & water retention↑
Immune Activity
Secretory IgA↓
Antigen penetration ↑
Circulating IgG↑
NK cell activity↓
Interleukin 2↓
T-Lymphocytes↓
Clinical Conditions
Chronic viral infections (EBV,CMV,Herpes I-II,etc.)
Increased infections
Yeast overgrowth
Allergies
Fatigue
Headaches
Autoimmune disease
Cancer
Cardiovascular disease
Insomnia
Hypoglycemia
Hunger
PMS
Depression
Irritable bowel
Digestive problems
ADD/ADHD
KEY
Association
Stimulus
Outcome
Inhibition
HP = Hypothalamus - Pituitary
NFL = Negative Feedback Loop
NE = Norepinephrine
97


Thank You
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