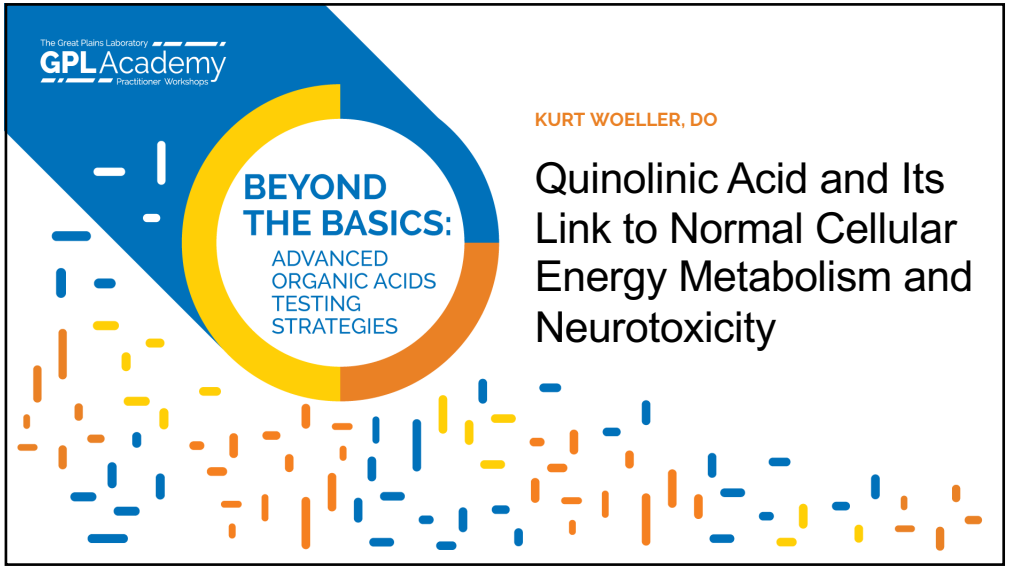
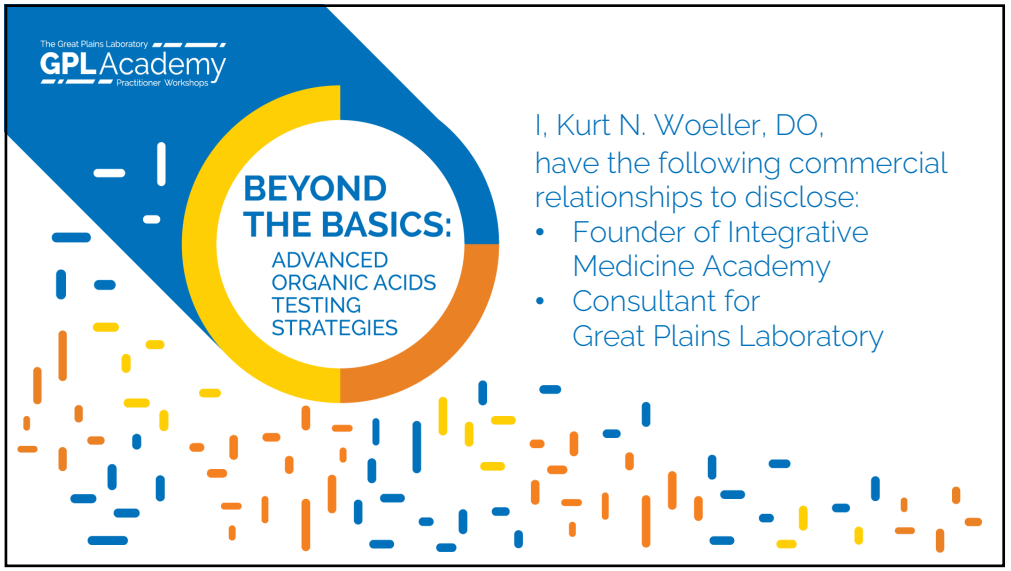

The Great Plains Laboratory
GPL Academy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
KURT WOELLER, DO
Quinolinic Acid and Its Link to Normal Cellular Energy Metabolism and Neurotoxicity

1


The Great Plains Laboratory
GPL Academy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
I, Kurt N. Woeller, DO,
have the following commercial relationships to disclose:
• Founder of Integrative Medicine Academy
• Consultant for Great Plains Laboratory

2

Disclaimer

- The material contained within this presentation is not intended to replace the services and/or medical advice of your personal licensed health care professional.
- This material is for educational purposes only
- This information is not meant to encourage diagnosis and treatment of disease.
- Any application of suggestions set forth in the following portions of this presentation is at the reader's discretion.
- Implementation and/or experimentation with any supplements, herbs, dietary changes, medications, and/or lifestyle changes, etc., is done so at your sole risk and responsibility.

3

3

Lecture Overview

- Tryptophan and competing amino acids
- The biochemical role of Quinolinic Acid (QA)
- Various adverse effects of elevated QA
- Microglia activation, neurological inflammation and QA toxicity.
- The role of glutathione
- Treatment options for elevated QA

4

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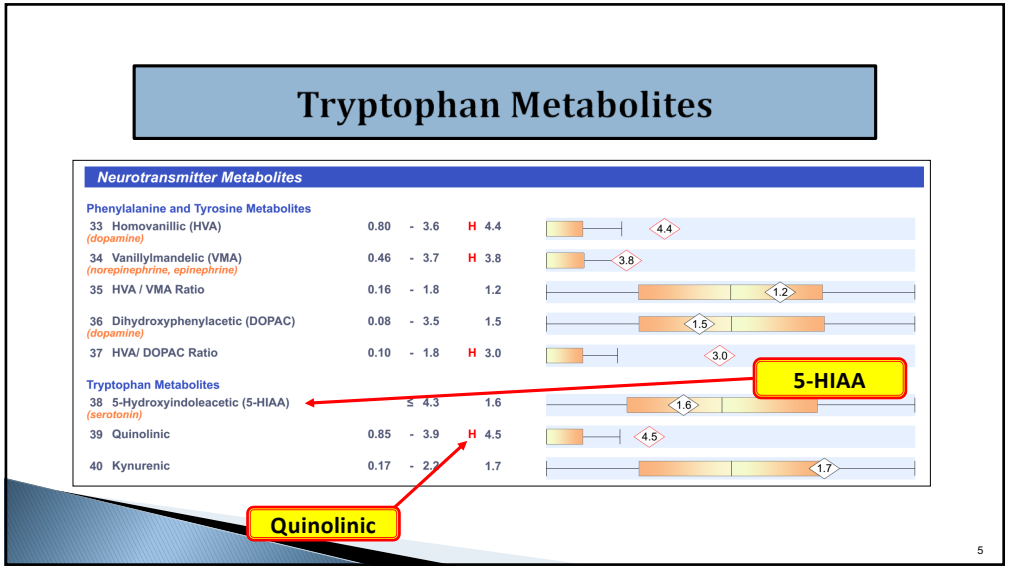
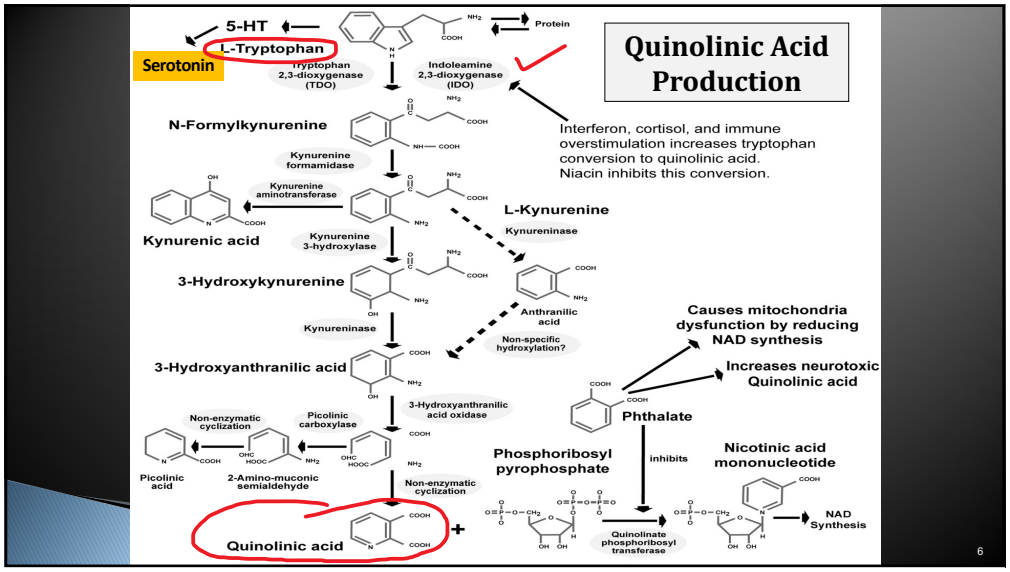
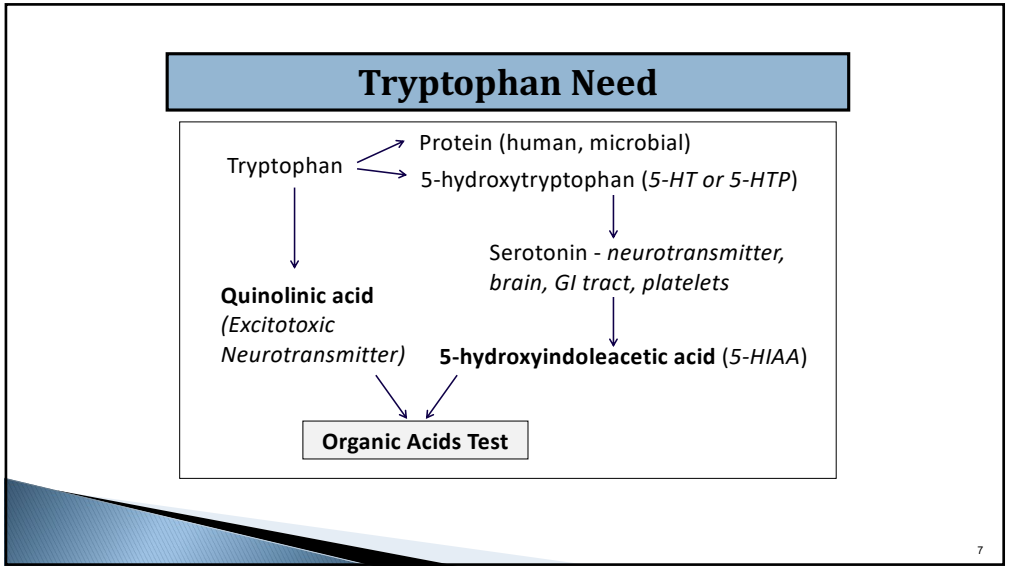

Tryptophan Metabolites
Neurotransmitter Metabolites
Phenylalanine and Tyrosine Metabolites
33 Homovanillic (HVA) (dopamine) 0.80 - 3.6 H 4.4
34 Vanillylmandelic (VMA) (norepinephrine, epinephrine) 0.46 - 3.7 H 3.8
35 HVA / VMA Ratio 0.16 - 1.8 1.2
36 Dihydroxyphenylacetic (DOPAC) (dopamine) 0.08 - 3.5 1.5
37 HVA/ DOPAC Ratio 0.10 - 1.8 H 3.0
Tryptophan Metabolites
38 5-Hydroxyindoleacetic (5-HIAA) (serotonin) ≤ 4.3 1.6
39 Quinolinic 0.85 - 3.9 H 4.5
40 Kynurenic 0.17 - 2.2 1.7
5-HIAA
Quinolinic
5

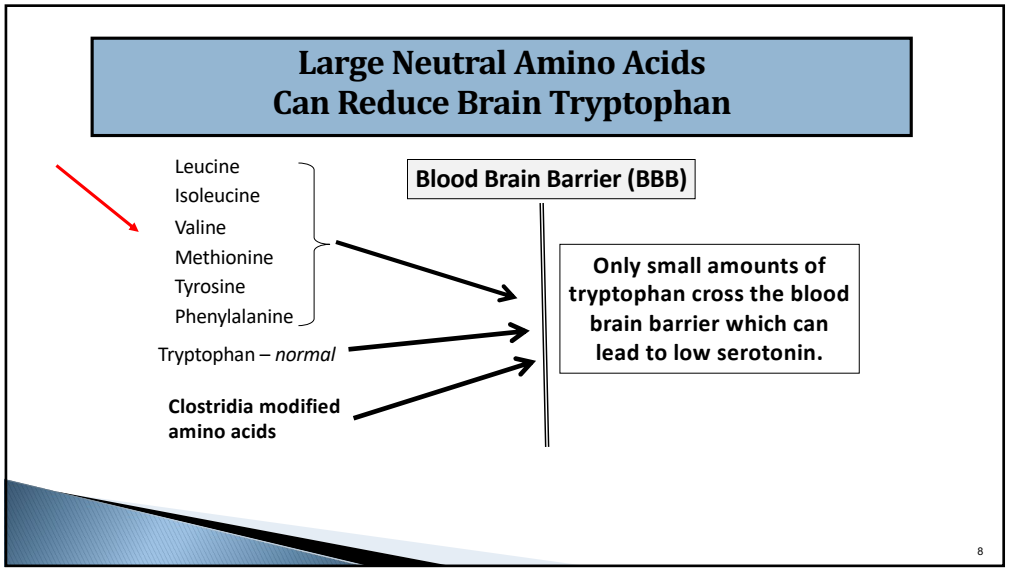
5


Quinolinic Acid Production
5-HT
L-Tryptophan
Serotonin
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
3-Hydroxyanthranilic acid
Causes mitochondria dysfunction by reducing NAD synthesis
Increases neurotoxic Quinolinic acid
Phthalate
3-Hydroxyanthranilic acid oxidase
Non-enzymatic cyclization
Picolinic carboxylase
Picolinic acid
2-Amino-muconic semialdehyde
Non-enzymatic cyclization
Phosphoribosyl pyrophosphate
inhibits
Nicotinic acid mononucleotide
Quinolinic acid
Quinolinate phosphoribosyl transferase
NAD Synthesis
6

6

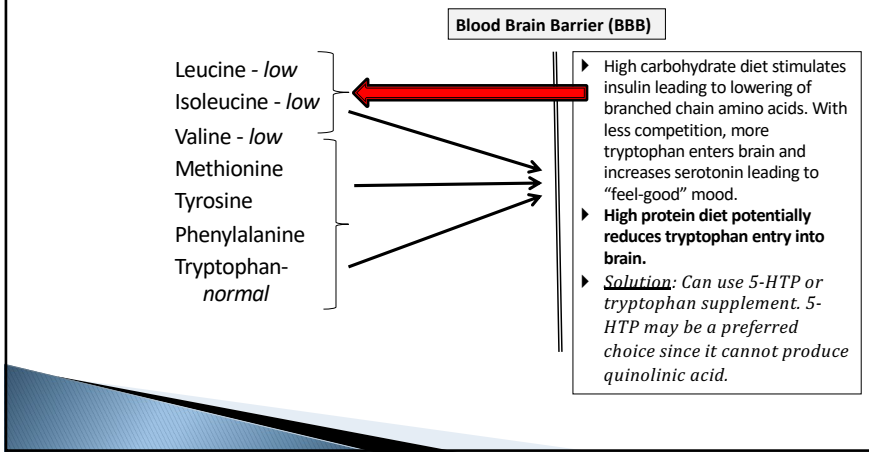

Tryptophan Need
Tryptophan
Protein (human, microbial)
5-hydroxytryptophan (5-HT or 5-HTP)
Serotonin - neurotransmitter, brain, GI tract, platelets
Quinolinic acid (Excitotoxic Neurotransmitter)
5-hydroxyindoleacetic acid (5-HIAA)
Organic Acids Test
7

7


Large Neutral Amino Acids Can Reduce Brain Tryptophan
Leucine
Isoleucine
Valine
Methionine
Tyrosine
Phenylalanine
Blood Brain Barrier (BBB)
Only small amounts of tryptophan cross the blood brain barrier which can lead to low serotonin.
Tryptophan – normal
Clostridia modified amino acids
8

8

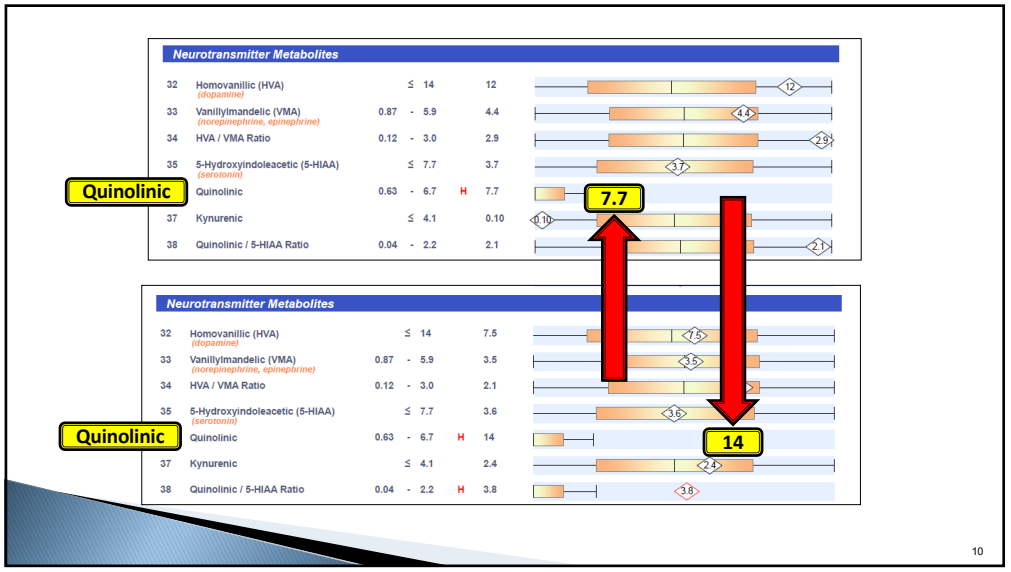
Carbohydrates Increase Brain Tryptophan Passage Across BBB



- High carbohydrate diet stimulates insulin leading to lowering of branched chain amino acids. With less competition, more tryptophan enters brain and increases serotonin leading to "feel-good" mood.
- **High protein diet potentially reduces tryptophan entry into brain.**
- *Solution: Can use 5-HTP or tryptophan supplement. 5-HTP may be a preferred choice since it cannot produce quinolinic acid.*

9

9



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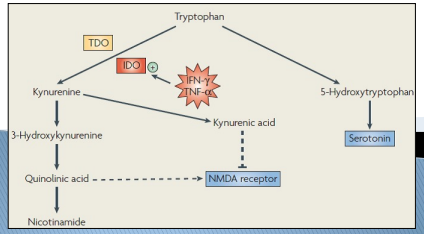
Various Adverse Effects of Elevated Quinolinic Acid

…and the link to Microglial Activation, Brain Inflammation and NMDA receptor activation.



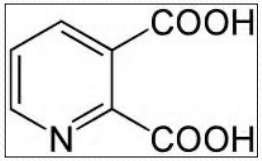
11

11

Quinolinic Acid

Quinolinic Acid

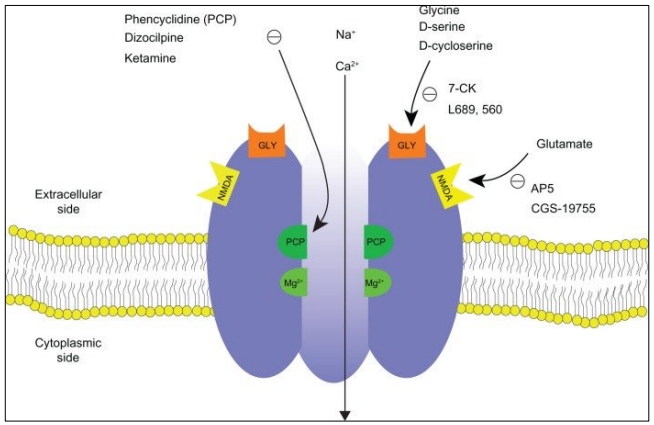
- Byproduct of tryptophan conversion after passing through kynurenine pathway.
- **NMDA receptor agonist**
- Potent neurotoxin
- Involved in neurodegeneration (e.g., Huntington's & Alzheimer's disease), psychiatric disorders (e.g., depression, mood disorders, schizophrenia), body pain, suicidal ideation…



Dicarboxylic acid

12

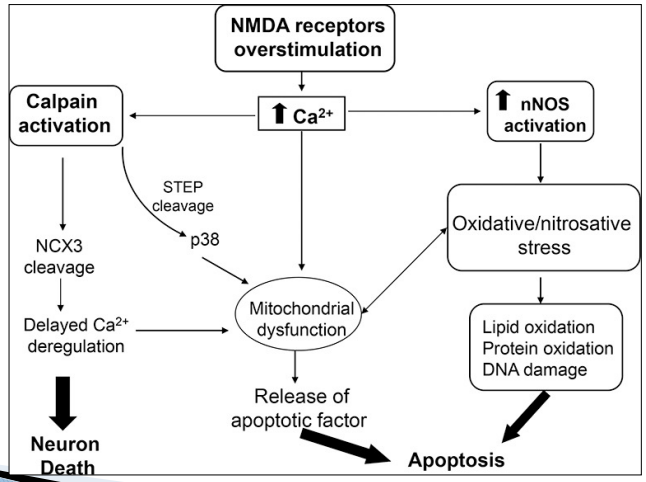
12



Source: ***An update on licit and illicit ketamine use - https://www.researchgate.net/figure/Schematic-diagram-of-NMDA-receptor-complex-The-NMDA-receptor-is-an-ionotropic-glutamate_fig1_259961077.***

13

13



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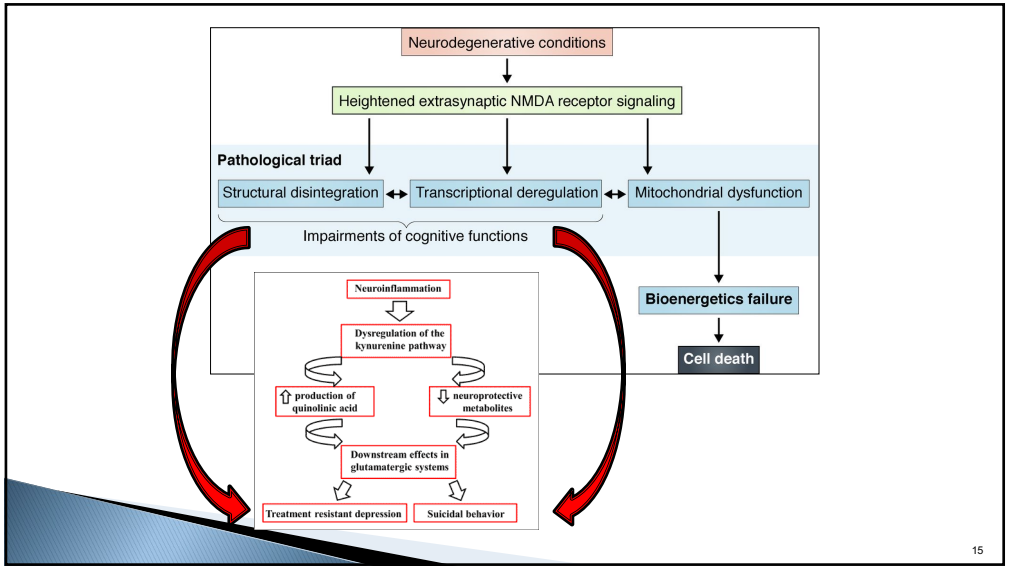
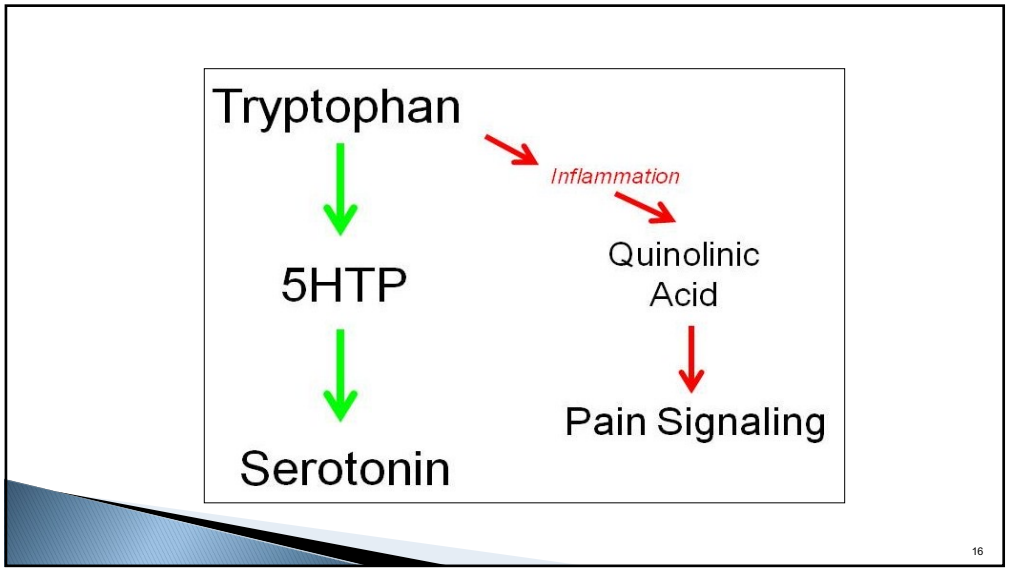
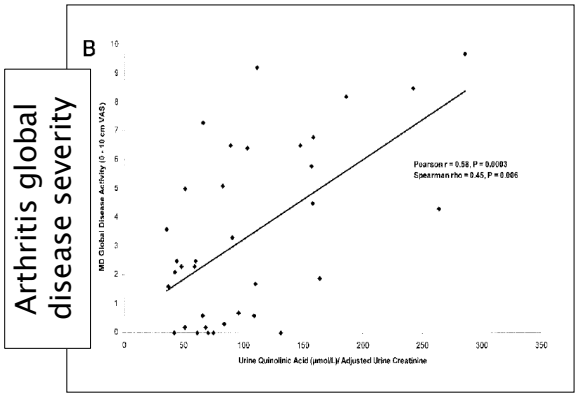

Neurodegenerative conditions
Heightened extrasynaptic NMDA receptor signaling
Pathological triad
Structural disintegration
Transcriptional deregulation
Mitochondrial dysfunction
Impairments of cognitive functions
Bioenergetics failure
Cell death
Neuroinflammation
Dysregulation of the kynurenine pathway
production of quinolinic acid
neuroprotective metabolites
Downstream effects in glutamatergic systems
Treatment resistant depression
Suicidal behavior
15

15


Tryptophan
Inflammation
5HTP
Quinolinic Acid
Pain Signaling
Serotonin
16

16

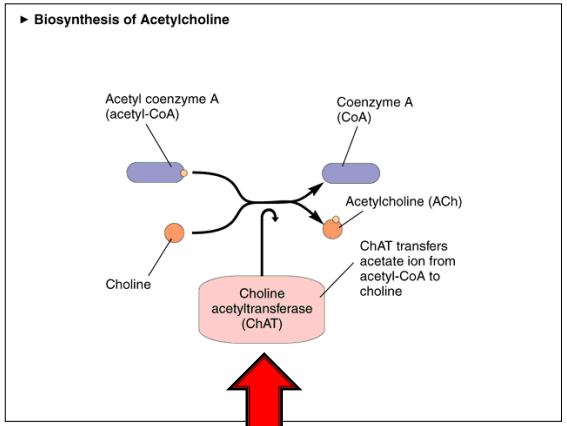
Clinical Chemistry 48, No. 10, 2002



Urine Quinolinic acid/creatinine

17

17



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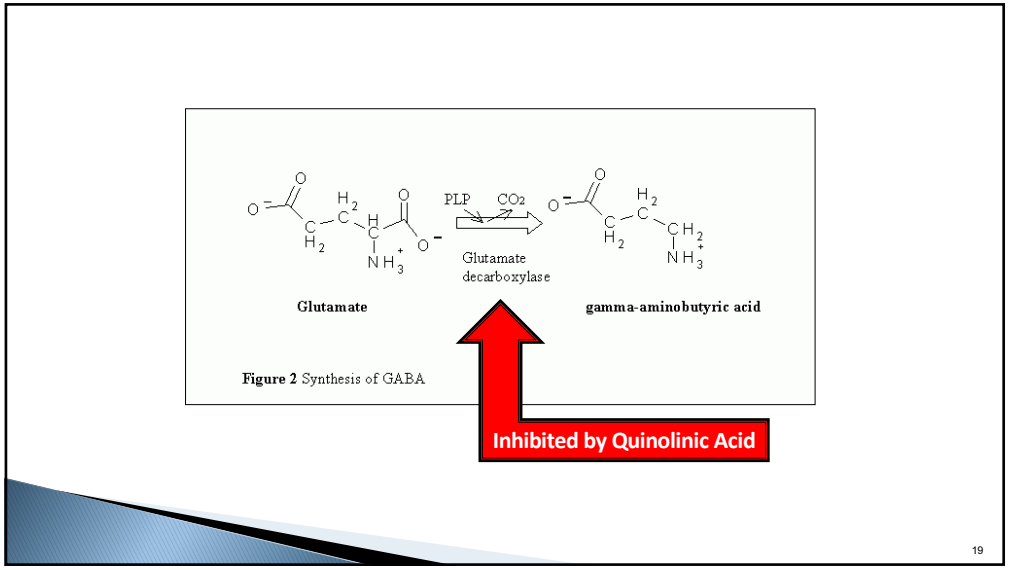
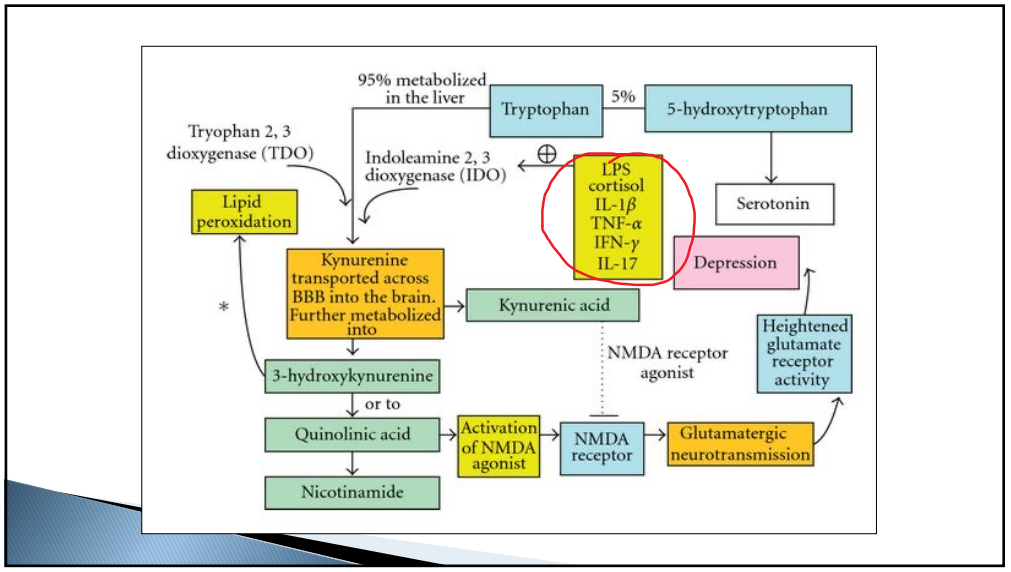
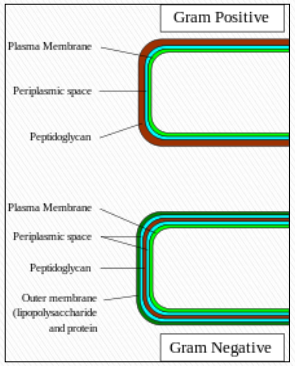

PLP
CO2
Glutamate decarboxylase
Glutamate
gamma-aminobutyric acid
Figure 2 Synthesis of GABA
Inhibited by Quinolinic Acid
19

19


95% metabolized in the liver
Tryptophan
5%
5-hydroxytryptophan
Tryophan 2, 3 dioxygenase (TDO)
Indoleamine 2, 3 dioxygenase (IDO)
LPS
cortisol
IL-1β
TNF-α
IFN-γ
IL-17
Serotonin
Lipid peroxidation
Depression
Kynurenine transported across BBB into the brain. Further metabolized into
Kynurenic acid
*
NMDA receptor agonist
Heightened glutamate receptor activity
3-hydroxykynurenine
or to
Quinolinic acid
Activation of NMDA agonist
NMDA receptor
Glutamatergic neurotransmission
Nicotinamide

20

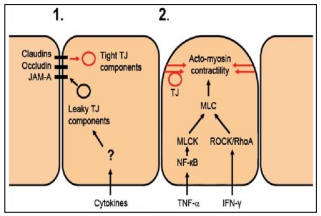
Gram Negative Bacteria and Lipopolysaccharide (LPS)

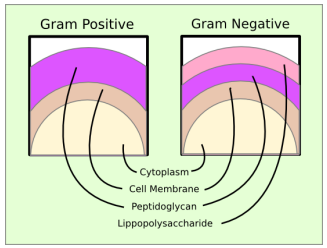


- Gram-Negative Bacteria:
  - Group of bacteria that do not retain the crystal violet stain.
  - Ex: *E. coli, Enterobacter, Campylobacter, Helicobacter, Klebsiella, Proteus, Pseudomonas.*
- Lipopolysaccharide (LPS):
  - Strong stimulator of various cytokines, some of which can have pro-inflammatory effects.
  - Insulin resistance
  - Microglia activation

21

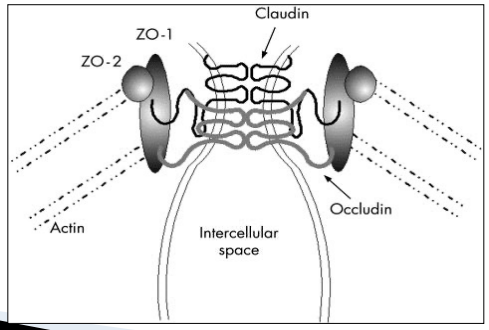
LPS can also be generated by prolonged exercise, alcohol use, severe infection, inflammatory bowel disease, high fat diets and protracted chronic illness.





22

The proteins occludin and zonulin (ZO-1 and ZO-2) are linked to actin to form the tight junction.



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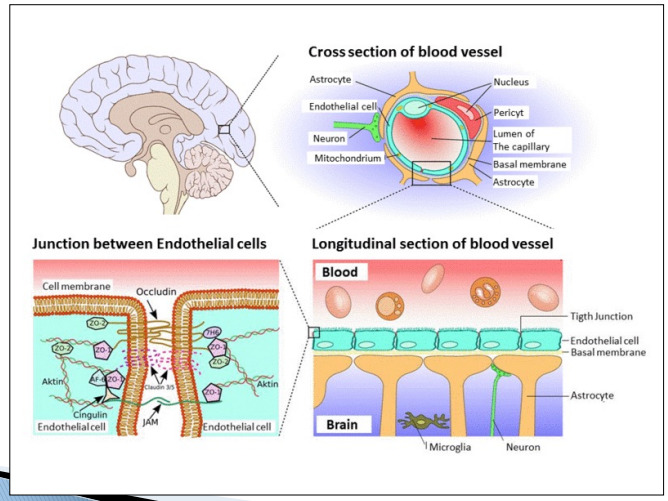

24

24

Neurological Inflammation

Neuroglial activation and neuroinflammation in the brain of patients with autism.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA.

Department of Neurology, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287, USA.

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained at autopsy from 11 patients with autism **(age 5 to 44 years old)** were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an **active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients**. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1.

"Our findings indicate that innate neuro-immune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain."

Ann Neurol. 2005 Feb;57(2):304

25

25

Vargas DL., et.al. Study (John Hopkins)

- Examined tissue from 3 different regions of the brain in 11 deceased individuals with autism – ages 5 to 44 (who died of accidents or injuries).
- Measured cytokine & chemokine from cerebrospinal fluid in 6 living individuals with autism – ages 5 to 12.

Findings:

- Active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients.
- Marked activation of microglia and astroglia
- Macrophage chemoattractant protein 1 (MCP-1) and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues.
- CSF showed a unique pro-inflammatory profile of cytokines, including a marked increase in MCP-1.

"Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain."

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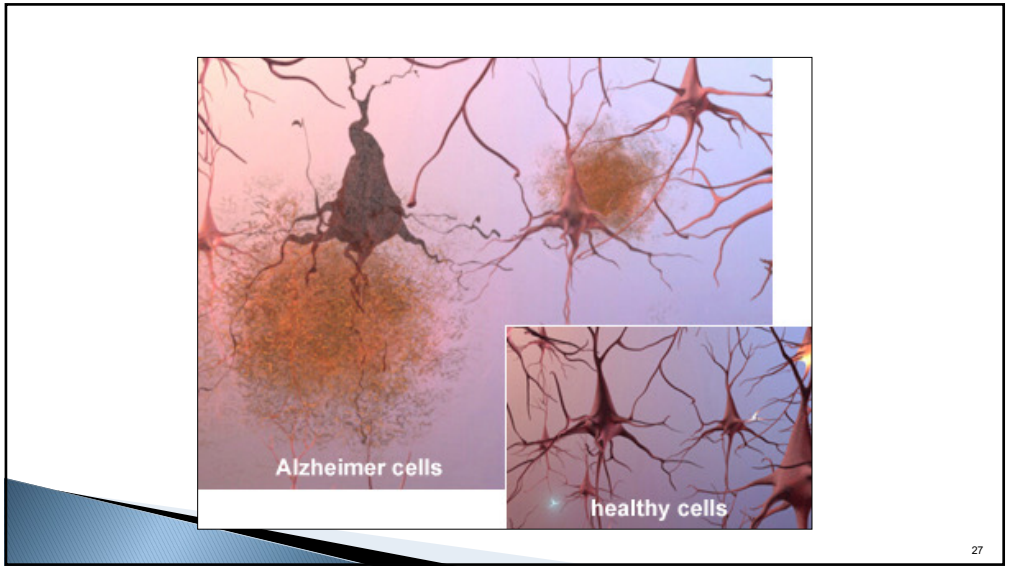
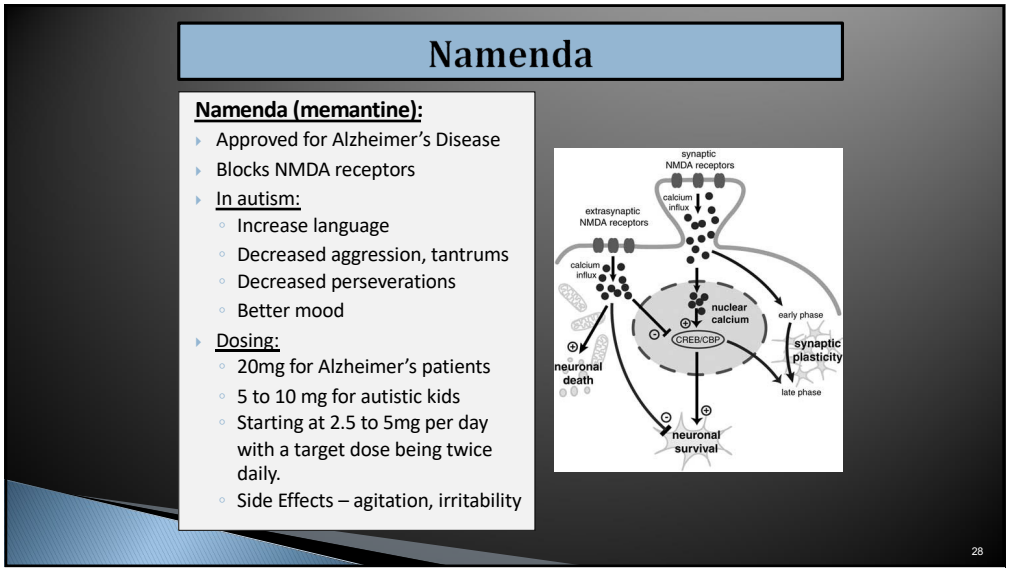
26


Alzheimer cells
healthy cells
27

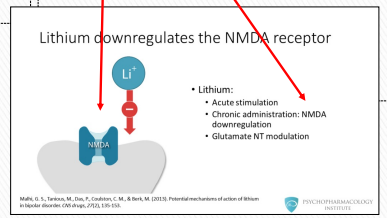
27


Namenda
Namenda (memantine):
Approved for Alzheimer's Disease
Blocks NMDA receptors
In autism:
Increase language
Decreased aggression, tantrums
Decreased perseverations
Better mood
Dosing:
20mg for Alzheimer's patients
5 to 10 mg for autistic kids
Starting at 2.5 to 5mg per day with a target dose being twice daily.
Side Effects – agitation, irritability
synaptic NMDA receptors
calcium influx
extrasynaptic NMDA receptors
calcium influx
nuclear calcium
CREB/CBP
early phase
synaptic plasticity
late phase
neuronal death
neuronal survival
28

28

L-Theanine and Nutritional Lithium

Theanine	Lithium
• Amino Acid found in Green Tea • Increases GABA, as well as Serotonin and Dopamine. • Affinity for NMDA, Kainate and AMPA receptors. Helps block glutamate excitotoxicity. • 100mg to 400mg daily	• Regulates the NMDA receptor • Improves mood, cognitive performance, mental sharpness • High Doses used for Bipolar Disorder • Elemental or Orotate Lithium – between 5mg to 20mg helpful for overall brain health.



29

29

Lithium Chloride *(liquid)*

Supplement Facts		
Serving size	10 Drops (0.421) ml	
Servings per container	118	%DV
Lithium (Chloride)	500 mcg	*
Other Ingredients: Ultra Pure water. *Daily Value not established.		

30

30

Lithium Orotate *(capsules)*

Supplement Facts		
Serving size	**1 Vegetarian Capsule**	**%DV**
Servings per container	60	
Lithium (as Orotate)	10 mg	*
Other Ingredients: Natural Vegetable Capsules,Microcrystalline Cellulose, Magnesium Stearate, and Silicon Dioxide.		

10mg

Supplement Facts		
Serving Size:	1 Vegetarian Capsule	%DV
Servings per container	120	
Lithium (as Orotate)	4.8 mg	*
Other Ingredients: Monocrystalline Cellulose and vegetarian capsule (hydroxypropyl methylcellulose, water)		

4.8mg

31

31

Hair Analysis – *Low Lithium*

Potassium	(K)	160	12- 200
Copper	(Cu)	9	11- 18
Zinc	(Zn)	38	100- 190
Manganese	(Mn)	0.30	0.10- 0.50
Chromium	(Cr)	0.56	43- 0.80
Vanadium	(V)	0.12	0.10
Molybdenum	(Mo)	0.095	0.13
Boron	(B)	3.0	0.7
Iodine	(I)	0.57	0.25-
Lithium	(Li)	0.004	0.007-
Phosphorus	(P)	145	150- 220
Selenium	(Se)	0.68	0.70- 1.1
Strontium	(Sr)	0.22	0.16- 1.0
Sulfur	(S)	47800	45500- 53000
Cobalt	(Co)	0.026	0.004- 0.020
Iron	(Fe)	14	7.0- 16
Germanium	(Ge)	0.019	0.030- 0.040

Available from Great Plains Laboratory

32

32

"Argumentative, disrespectful, withdrawn, distant, sullen and depressed" teenager

ESSENTIAL AND OTHER ELEMENTS		RESULT µg/g	REFERENCE INTERVAL
Calcium	(Ca)	473	200- 700
Magnesium	(Mg)	69	18- 70
Sodium	(Na)	230	20- 200
Potassium	(K)	45	9- 80
Copper	(Cu)	11	11- 32
Zinc	(Zn)	190	150- 230
Manganese	(Mn)	0.07	0.08- 0.50
Chromium	(Cr)	0.36	0.40- 0.70
Vanadium	(V)	0.023	0.020- 0.075
Molybdenum	(Mo)	0.032	0.030- 0.060
Boron	(B)	2.4	0.34- 3.0
Iodine	(I)	2.2	0.25- 1.3
Lithium	(Li)	0.004	0.007- 0.020
Phosphorus	(P)	173	150- 220
Selenium	(Se)	0.82	0.70- 1.2
Strontium	(Sr)	16	0.30- 3.2
Sulfur	(S)	48700	44000- 50000
Cobalt	(Co)	0.003	0.004- 0.020
Iron	(Fe)	4.8	7.0- 16
Germanium	(Ge)	0.035	0.030- 0.040
Rubidium	(Rb)	0.041	0.009- 0.090
Zirconium	(Zr)	0.036	0.047- 0.70

PERCENTILE: 2.5th, 16th, 50th, 84th, 97.5th

33

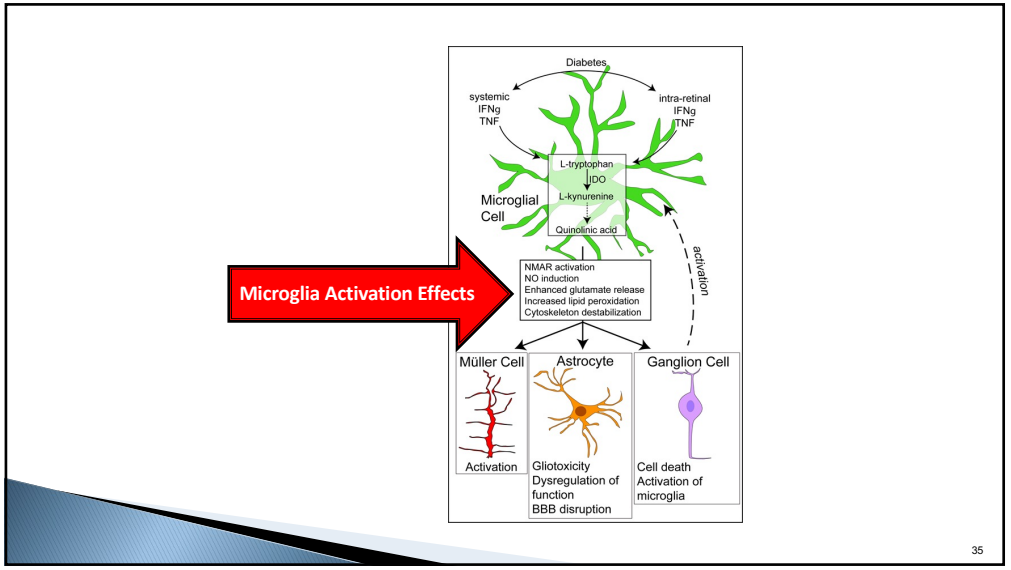
33

I just wanted to give you an update on how has been doing. I ordered all the supplements you recommended below, and am slowly transitioning into taking them all. On April 4th I started him on what I thought were the most important supplement, Lithium Orotate. Kurt, honestly I still don't believe the results my wife and I are seeing!!! It was literally overnight that changed. He was argumentative, disrespectful, withdrawn, distant, sullen, and depressed. He hated every aspect of school. He would only respond to questions with one or two words (and then it was with a hostile attitude). He has done a 180 degree turn. His attitude is great. He's talking, I mean he's really talking to us. For the first time. He's never done that before. Ever. He jokes!! He laughs!! He's doing ALL his school work ahead of the due date, and he's getting A's on everything!! We have our fingers crossed that this will also be reflected on his test scores, as he gets very anxious during tests, and as a rule of thumb he fails most of his tests.

My thanks pale in comparison to what I believe you have given my son. He has the opportunity now to succeed. We know he has more to overcome, but now with his current changes we feel it is possible. I can't express how profound the changes in him are. It's like he's turning into the best version of himself.

34

34



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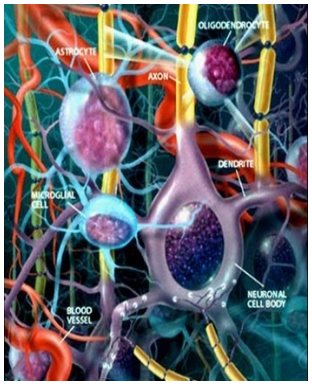
Astroglia & Microglia

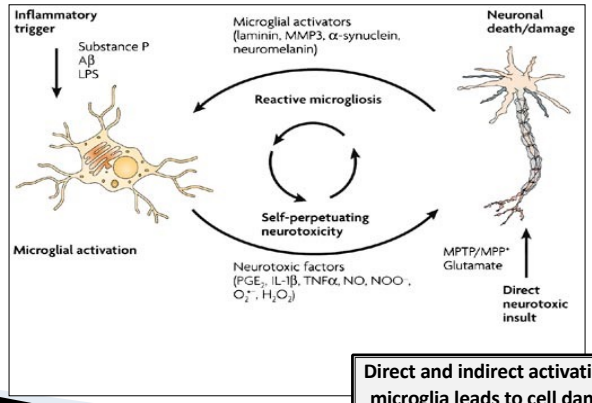
- **Astroglia:**
  - Physical support of brain
  - Support blood brain barrier
  - Brain nutrient support
  - Brain repair and scarring
- **Microglia:**
  - **Main immune defense in the brain**.
  - 20% of glial cells in the brain
  - Microglia cells are constantly on the move looking for invading pathogens.
  - They initiate the inflammatory response
  - Present foreign virus, bacteria, etc. to immune system.
  - **Can become chronically activated and not turn off.**



36

36

Neuroinflammation



Direct and indirect activation of microglia leads to cell damage

37

37

Amyloid Activation of Microglia

J Biol Chem. 2011 Feb 4;286(5):3693-706. Epub 2010 Oct 22.

Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity.

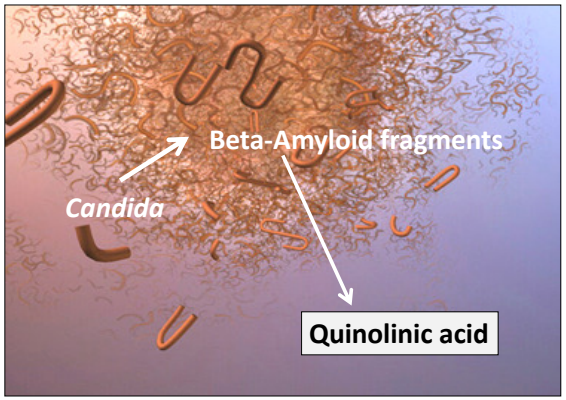
Maezawa I, Zimin PI, Wulff H, Jin LW.

Abstract

Neuroinflammation and associated neuronal dysfunction mediated by activated microglia play an important role in the pathogenesis of Alzheimer disease (AD). Microglia are activated by aggregated forms of amyloid-β protein (Aβ), usually demonstrated in vitro by stimulating microglia with micromolar concentrations of fibrillar Aβ, a major component of amyloid plaques in AD brains. Here we report that amyloid-β oligomer (AβO), at 5-50 nm, induces a unique pattern of microglia activation that requires the activity of the scavenger receptor A and the Ca(2+)-activated potassium channel KCa3.1. AβO treatment induced an activated morphological and biochemical profile of microglia, including activation of p38 MAPK and nuclear factor κB. Interestingly, although increasing nitric oxide (NO) production, AβO did not increase several proinflammatory mediators commonly induced by lipopolyliposaccharides or fibrillar Aβ, suggesting that AβO stimulates both common and divergent pathways of microglia activation. AβO at low nanomolar concentrations, although not neurotoxic, induced indirect, microglia-mediated damage to neurons in dissociated cultures and in organotypic hippocampal slices. The indirect neurotoxicity was prevented by (i) doxycycline, an inhibitor of microglia activation; (ii) TRAM-34, a selective KCa3.1 blocker; and (iii) two inhibitors of inducible NO synthase, indicating that KCa3.1 activity and excessive NO release are required for AβO-induced microglial neurotoxicity. Our results suggest that AβO, generally considered a neurotoxin, may more potently cause neuronal damage indirectly by activating microglia in AD.

38

38



39

39

OPEN ACCESS Freely available online

PLoS one

The Alzheimer's Disease-Associated Amyloid β-Protein Is an Antimicrobial Peptide March 2010

Stephanie J. Soscia[1,2], James E. Kirby[3], Kevin J. Washicosky[1], Stephanie M. Tucker[1], Martin Ingelsson[4], Bradley Hyman[1,5], Mark A. Burton[6,7], Lee E. Goldstein[6,7], Scott Duong[3], Rudolph E. Tanzi[1,5]*, Robert D. Moir[1,5]

1 Genetics and Aging Research Unit, Mass General Institute for Neurodegenerative Disease and Department of Neurology, Massachusetts General Hospital, Charlestown, Massachusetts, United States of America, **2** Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts, United States of America, **3** Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States of America, **4** Department of Public Health/Geriatrics, Uppsala University, Uppsala, Sweden, **5** Harvard Medical School, Boston, Massachusetts, United States of America, **6** Molecular Aging and Developmental Laboratory, Photonics Center, College of Engineering, Boston University School of Medicine, Boston University, Boston, Massachusetts, United States of America, **7** Boston University Alzheimer's Disease Center, Boston University, Boston, Massachusetts, United States of America

Abstract

Background: The amyloid β-protein (Aβ) is believed to be the key mediator of Alzheimer's disease (AD) pathology. Aβ is most often characterized as an incidental catabolic byproduct that lacks a normal physiological role. However, Aβ has been shown to be a specific ligand for a number of different receptors and other molecules, transported by complex trafficking pathways, modulated in response to a variety of environmental stressors, and able to induce pro-inflammatory activities.

Methodology/Principal Findings: Here, we provide data supporting an *in vivo* function for Aβ as an antimicrobial peptide (AMP). Experiments used established *in vitro* assays to compare antimicrobial activities of Aβ and LL-37, an archetypical human AMP. Findings reveal that Aβ exerts antimicrobial activity against eight common and clinically relevant microorganisms with a potency equivalent to, and in some cases greater than, LL-37. Furthermore, we show that AD whole brain homogenates have significantly higher antimicrobial activity than aged matched non-AD samples and that AMP action correlates with tissue Aβ levels. Consistent with Aβ-mediated activity, the increased antimicrobial action was ablated by immunodepletion of AD brain homogenates with anti-Aβ antibodies.

Conclusions/Significance: Our findings suggest Aβ is a hitherto unrecognized AMP that may normally function in the innate immune system. This finding stands in stark contrast to current models of Aβ-mediated pathology and has important implications for ongoing and future AD treatment strategies.

Citation: Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, et al. (2010) The Alzheimer's Disease-Associated Amyloid β-Protein Is an Antimicrobial Peptide. PLoS ONE 5(3): e9505. doi:10.1371/journal.pone.0009505

40

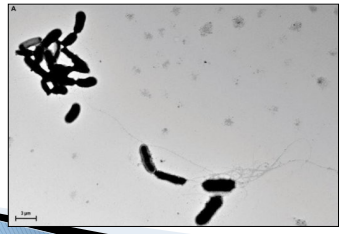
40

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.

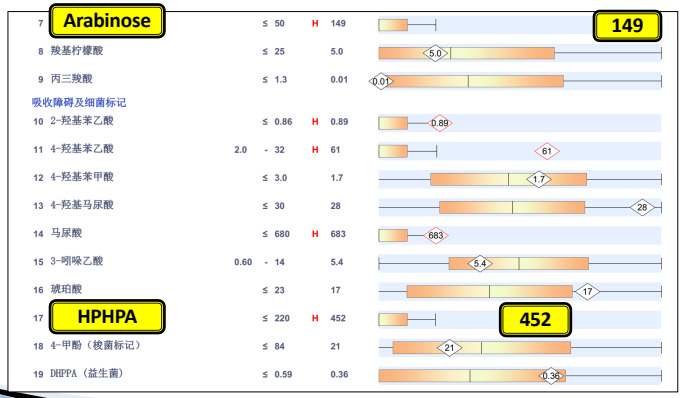


Amyloid fibrils propagate from yeast surfaces and capture Candida albicans in culture medium.
Credit: D.K.V. Kumar et al. / Science Translational Medicine (2016)

41

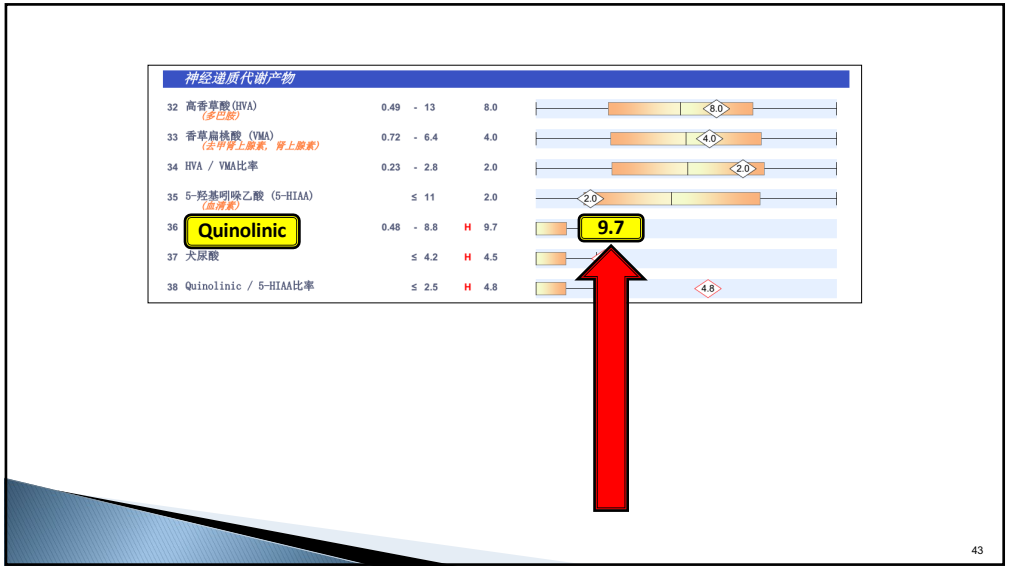
41

Multiple Tests - Same Person

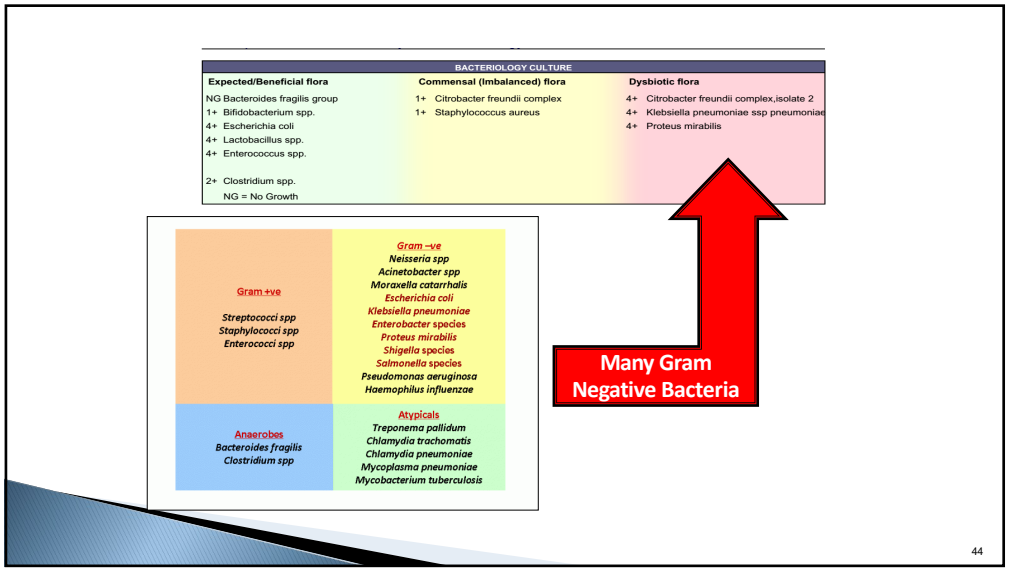


42

42


神经递质代谢产物
32 高香草酸(HVA) (多巴胺) 0.49 - 13 8.0
33 香草扁桃酸 (VMA) (去甲肾上腺素，肾上腺素) 0.72 - 6.4 4.0
34 HVA / VMA比率 0.23 - 2.8 2.0
35 5-羟基吲哚乙酸 (5-HIAA) (血清素) ≤ 11 2.0
36 Quinolinic 0.48 - 8.8 H 9.7
37 尿酸 ≤ 4.2 H 4.5
38 Quinolinic / 5-HIAA比率 ≤ 2.5 H 4.8
8.0
4.0
2.0
2.0
9.7
4.8
43

43


BACTERIOLOGY CULTURE
Expected/Beneficial flora
NG Bacteroides fragilis group
1+ Bifidobacterium spp.
4+ Escherichia coli
4+ Lactobacillus spp.
4+ Enterococcus spp.
2+ Clostridium spp.
NG = No Growth
Commensal (Imbalanced) flora
1+ Citrobacter freundii complex
1+ Staphylococcus aureus
Dysbiotic flora
4+ Citrobacter freundii complex,isolate 2
4+ Klebsiella pneumoniae ssp pneumoniae
4+ Proteus mirabilis
Gram +ve
Streptococci spp
Staphylococci spp
Enterococci spp
Gram –ve
Neisseria spp
Acinetobacter spp
Moraxella catarrhalis
Escherichia coli
Klebsiella pneumoniae
Enterobacter species
Proteus mirabilis
Shigella species
Salmonella species
Pseudomonas aeruginosa
Haemophilus influenzae
Anaerobes
Bacteroides fragilis
Clostridium spp
Atypicals
Treponema pallidum
Chlamydia trachomatis
Chlamydia pneumoniae
Mycoplasma pneumoniae
Mycobacterium tuberculosis
Many Gram Negative Bacteria
44

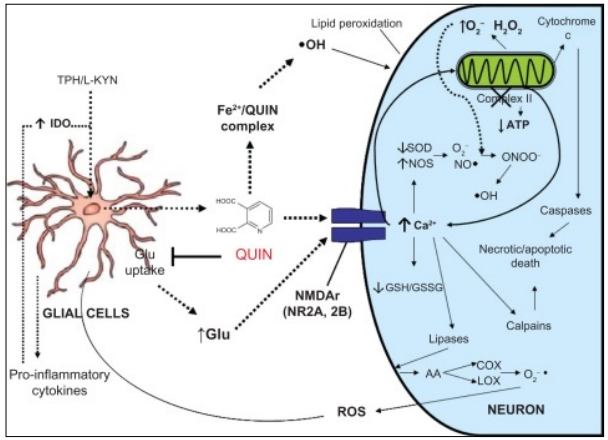
44

Low Hair Lithium

Zinc	(Zn)	120	100- 190
Manganese	(Mn)	0.14	0.10- 0.50
Chromium	(Cr)	0.44	0.43- 0.80
Vanadium	(V)	0.077	0.030- 0.10
Molybdenum	(Mo)	0.22	0.050- 0.13
Boron	(B)	2.6	0.70- 5.0
Iodine	(I)	0.61	0.25- 1.3
Lithium	(Li)	< 0.004	0.007- 0.020
Phosphorus	(P)	154	150- 220
[illegible]	(Se)	0.65	0.70- 1.1
[illegible]	(Sr)	0.34	0.16- 1.0
[illegible]	(S)	46300	45500- 53000
[illegible]	(Co)	0.005	0.004- 0.020
[illegible]	(Fe)	8.5	7.0- 16

45

45



Source: ***Quinolinic Acid, an Endogenous Molecule Combining Excitotoxicity, Oxidative Stress and Other Toxic Mechanisms - https://www.researchgate.net/figure/Schematic-representation-of-the-classical-proposed-mechanisms-by-which-quinolinic-acid_fig1_221694336***

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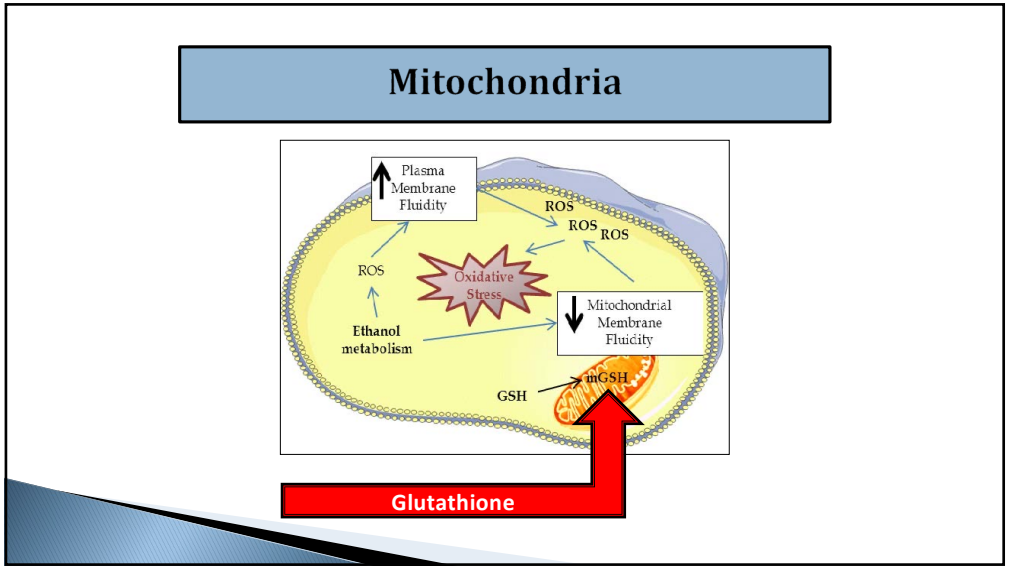
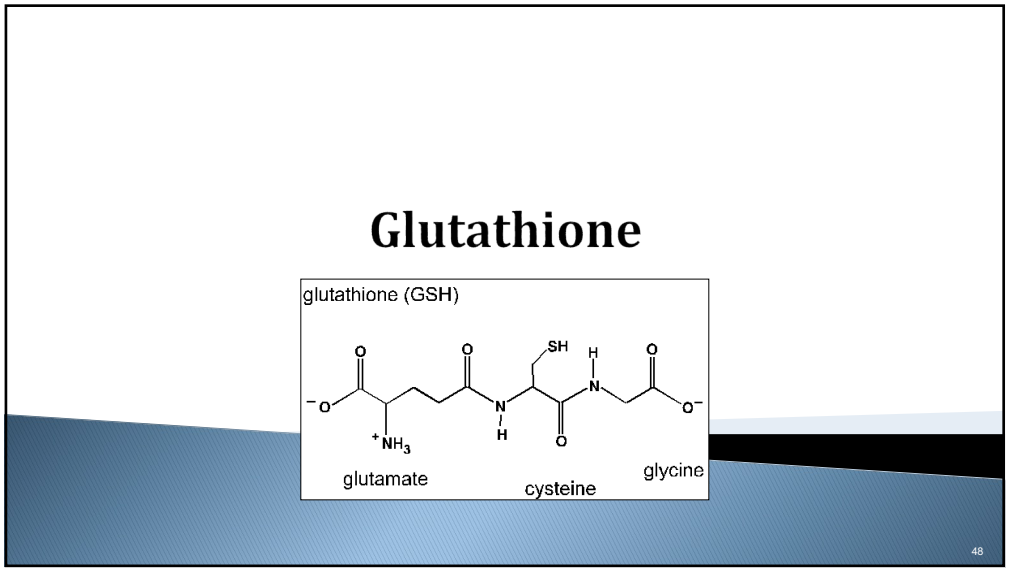
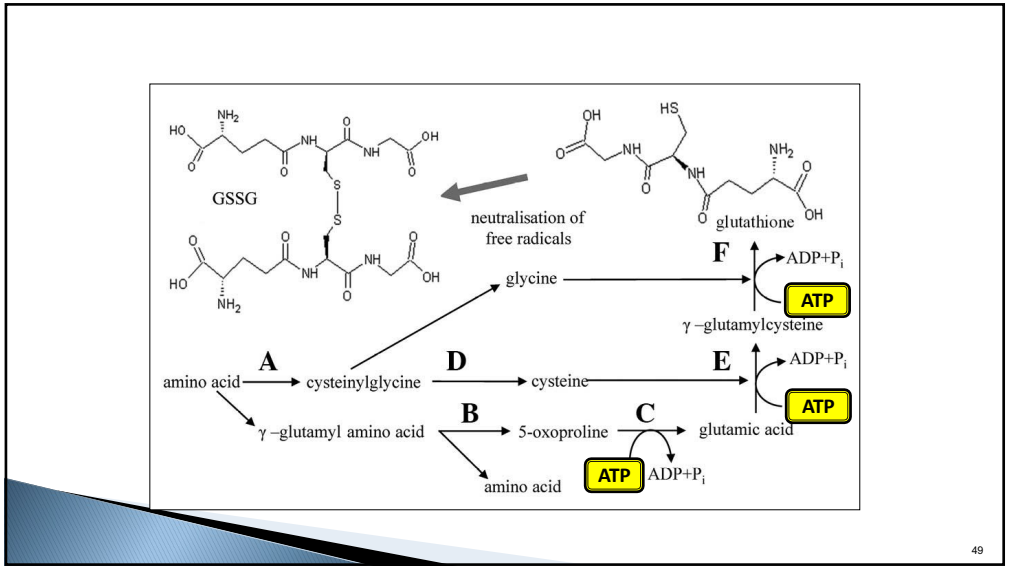

Mitochondria
Plasma Membrane Fluidity
ROS
ROS
ROS
ROS
Oxidative Stress
Ethanol metabolism
Mitochondrial Membrane Fluidity
GSH
mGSH
Glutathione

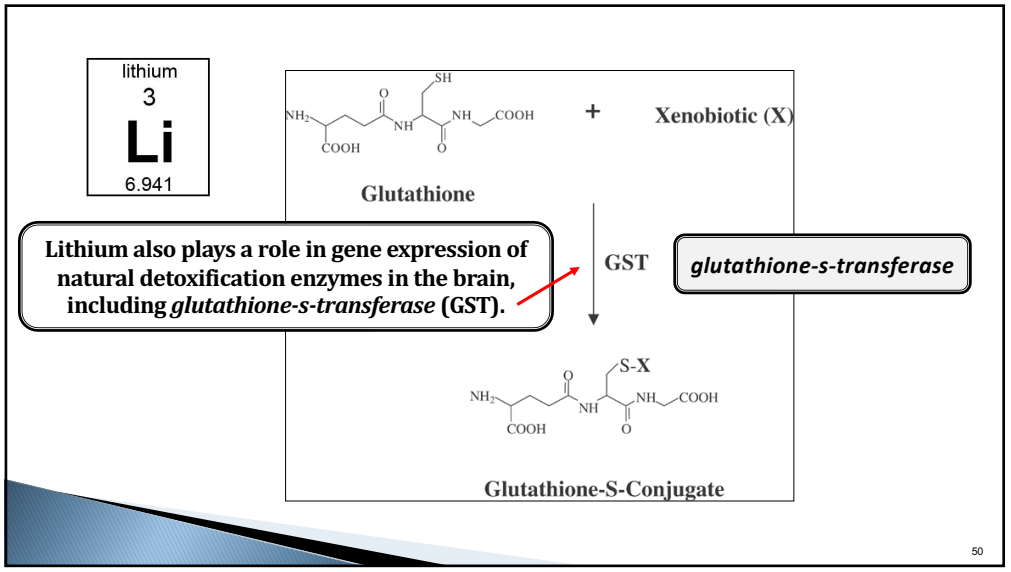
47


Glutathione
glutathione (GSH)
glutamate
cysteine
glycine
48

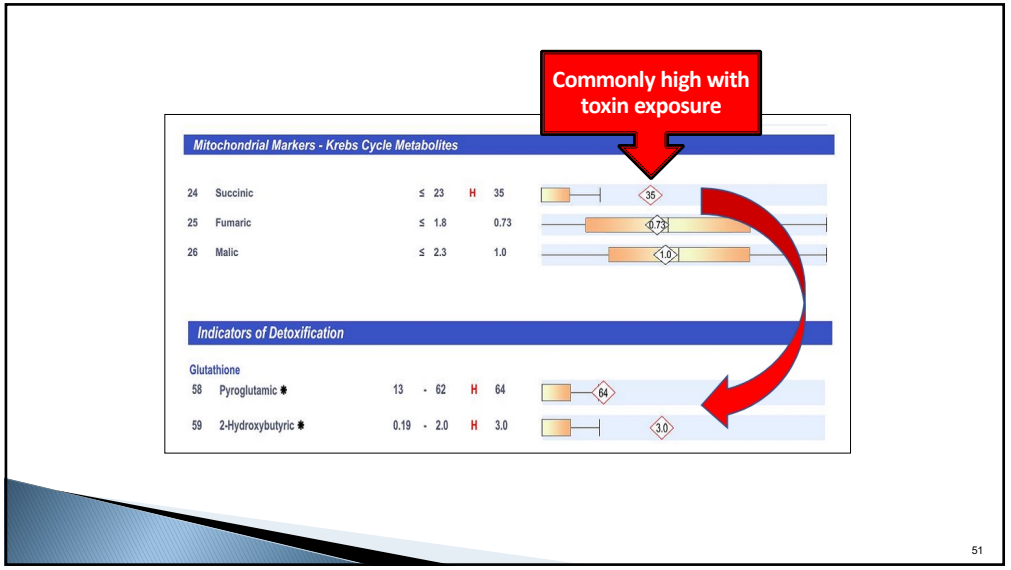
48


GSSG
neutralisation of free radicals
glutathione
glycine
F
ADP+Pi
ATP
γ –glutamylcysteine
amino acid
A
cysteinylglycine
D
cysteine
E
ADP+Pi
ATP
γ –glutamyl amino acid
B
5-oxoproline
C
glutamic acid
amino acid
ATP
ADP+Pi
49

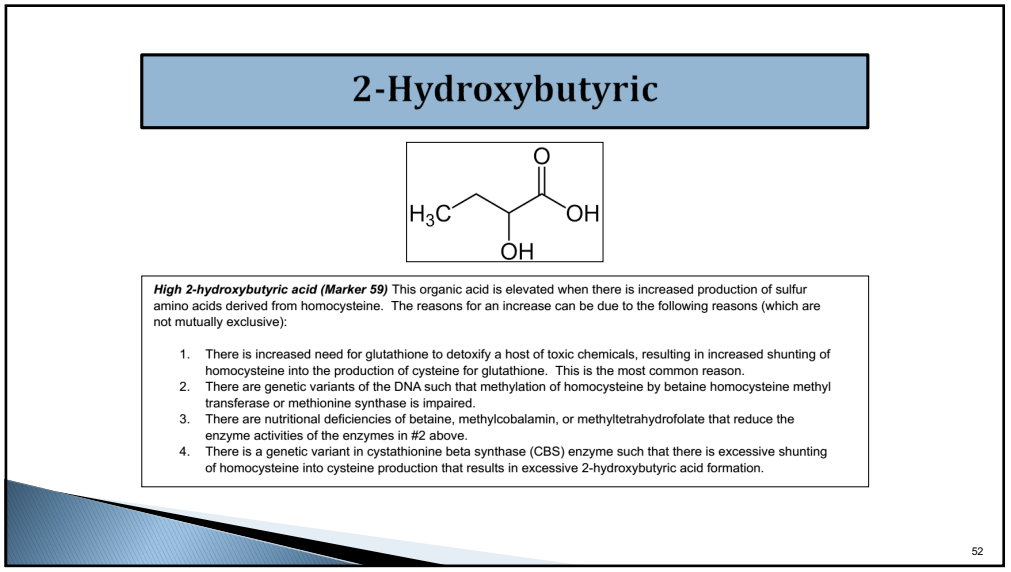
49


lithium
3
Li
6.941
Glutathione
+
Xenobiotic (X)
Lithium also plays a role in gene expression of natural detoxification enzymes in the brain, including *glutathione-s-transferase* (GST).
GST
glutathione-s-transferase
S-X
Glutathione-S-Conjugate
50

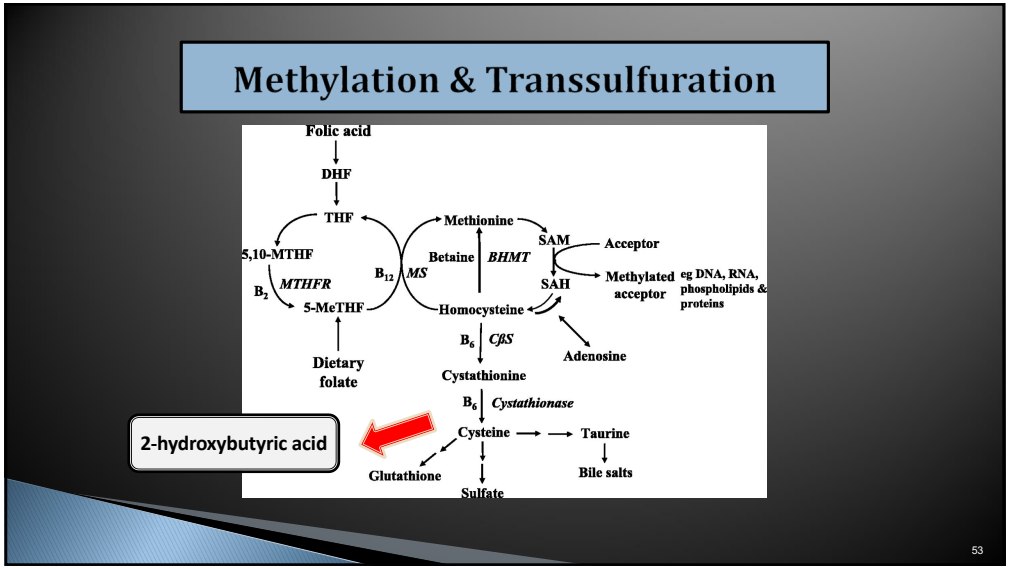
50


Commonly high with toxin exposure
Mitochondrial Markers - Krebs Cycle Metabolites
24 Succinic ≤ 23 H 35
25 Fumaric ≤ 1.8 0.73
26 Malic ≤ 2.3 1.0
Indicators of Detoxification
Glutathione
58 Pyroglutamic ✱ 13 - 62 H 64
59 2-Hydroxybutyric ✱ 0.19 - 2.0 H 3.0
51

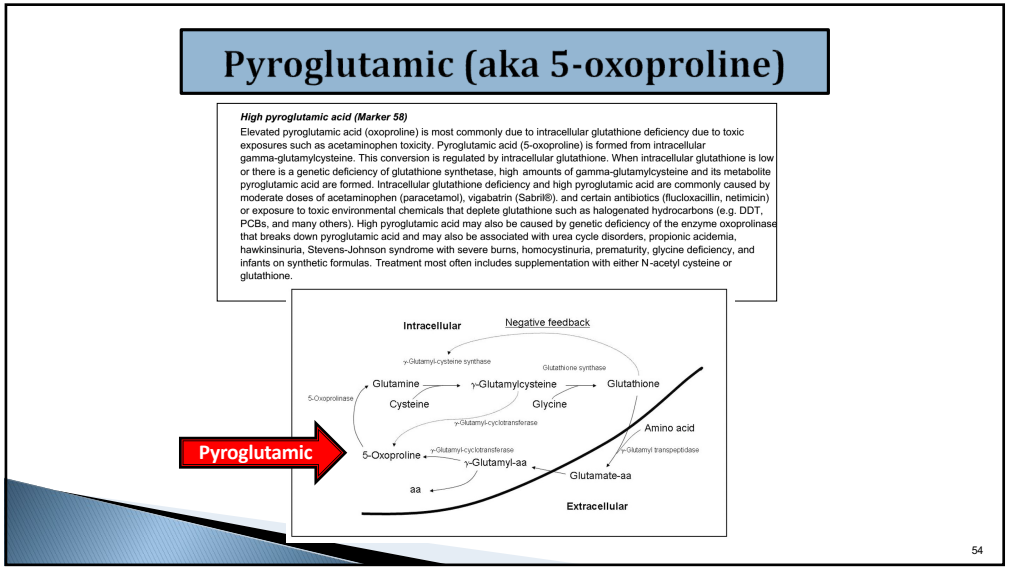
51


2-Hydroxybutyric
O
H3C
OH
OH
High 2-hydroxybutyric acid (Marker 59) This organic acid is elevated when there is increased production of sulfur amino acids derived from homocysteine. The reasons for an increase can be due to the following reasons (which are not mutually exclusive):
1. There is increased need for glutathione to detoxify a host of toxic chemicals, resulting in increased shunting of homocysteine into the production of cysteine for glutathione. This is the most common reason.
2. There are genetic variants of the DNA such that methylation of homocysteine by betaine homocysteine methyl transferase or methionine synthase is impaired.
3. There are nutritional deficiencies of betaine, methylcobalamin, or methyltetrahydrofolate that reduce the enzyme activities of the enzymes in #2 above.
4. There is a genetic variant in cystathionine beta synthase (CBS) enzyme such that there is excessive shunting of homocysteine into cysteine production that results in excessive 2-hydroxybutyric acid formation.
52

52


Methylation & Transsulfuration
Folic acid
DHF
THF
5,10-MTHF
MTHFR
B2
5-MeTHF
Dietary folate
B12
MS
Methionine
Betaine
BHMT
SAM
Acceptor
Methylated acceptor
eg DNA, RNA, phospholipids & proteins
SAH
Homocysteine
Adenosine
B6
CβS
Cystathionine
B6
Cystathionase
Cysteine
Taurine
Bile salts
Glutathione
Sulfate
2-hydroxybutyric acid
53

53


Pyroglutamic (aka 5-oxoproline)
High pyroglutamic acid (Marker 58)
Elevated pyroglutamic acid (oxoproline) is most commonly due to intracellular glutathione deficiency due to toxic exposures such as acetaminophen toxicity. Pyroglutamic acid (5-oxoproline) is formed from intracellular gamma-glutamylcysteine. This conversion is regulated by intracellular glutathione. When intracellular glutathione is low or there is a genetic deficiency of glutathione synthetase, high amounts of gamma-glutamylcysteine and its metabolite pyroglutamic acid are formed. Intracellular glutathione deficiency and high pyroglutamic acid are commonly caused by moderate doses of acetaminophen (paracetamol), vigabatrin (Sabril®), and certain antibiotics (flucloxacillin, netimicin) or exposure to toxic environmental chemicals that deplete glutathione such as halogenated hydrocarbons (e.g. DDT, PCBs, and many others). High pyroglutamic acid may also be caused by genetic deficiency of the enzyme oxoprolinase that breaks down pyroglutamic acid and may also be associated with urea cycle disorders, propionic acidemia, hawkinsinuria, Stevens-Johnson syndrome with severe burns, homocystinuria, prematurity, glycine deficiency, and infants on synthetic formulas. Treatment most often includes supplementation with either N-acetyl cysteine or glutathione.
Intracellular
Negative feedback
γ-Glutamyl-cysteine synthase
Glutathione synthase
Glutamine
γ-Glutamylcysteine
Glutathione
5-Oxoprolinase
Cysteine
Glycine
γ-Glutamyl-cyclotransferase
Amino acid
γ-Glutamyl transpeptidase
Pyroglutamic
5-Oxoproline
γ-Glutamyl-cyclotransferase
γ-Glutamyl-aa
Glutamate-aa
aa
Extracellular
54

54

Cellular Support Supplements (examples)

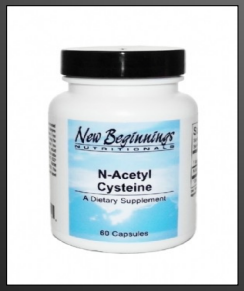
- Vitamin A, C, D, E
- Riboflavin *(vitamin B2)*
- Vitamin B6
- Methyl-Folate
- Methylcobalamin
- Selenium

- Methionine, Cysteine (*sulfur containing amino acids*)
- L-Glutamine, Acetyl-L-Carnitine, CoQ10 (*ubiquinol*)
- N-Acetyl-Cysteine *(NAC)*
- Alpha Lipoic Acid
- Milk Thistle

55

55

N-Acetylcysteine (NAC)



- N-Acetylcysteine (NAC) is a precursor to glutathione.
- Helps boost glutathione levels
- 1 capsule = 500mg
- Contents can be opened into food or drink.
- Equivalent *(approximately)* dosage to oral glutathione.

1 to 2 capsules daily

56

56

Liposomal Glutathione

- Liposome is a microscopic fluid-filled pouch with walls identical to the phospholipids that make up the human cell membranes.
- The outer wall of the liposome is fat soluble, while the inside is water soluble.

Suggested Dosage:

- **For Adults:** *Begin with 1.5 teaspoonfuls (approx. 650 mg) twice daily for 7 to 10 days and can be lowered to one teaspoonful (approx. 430mg) daily as a "maintenance serving."*
- **For Children:** *Begin with 1/8 - ¼ of a teaspoon (approx. 55-108 mg) for every 30lbs of the child's weight, twice a day.*

https://nbnus.com

57

57

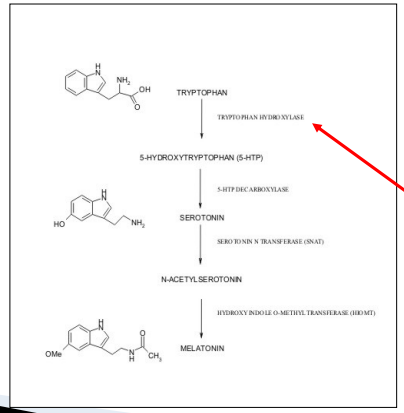
Tri-Fortify (liposomal glutathione)

- **One teaspoon provides 450mg of glutathione and 50mg of Vitamin C**
- **Watermelon and orange flavor**
- **Hold under tongue for 30-60 seconds and then swallow**

58

58

5-HTP is committed towards the Serotonin pathway. It cannot go backwards to Quinolinic Acid.



- **BH4**
- **Calcium & Magnesium**
- **Folate**
- **Iron**
- **Vitamin B6**
- **Vitamin D**

59

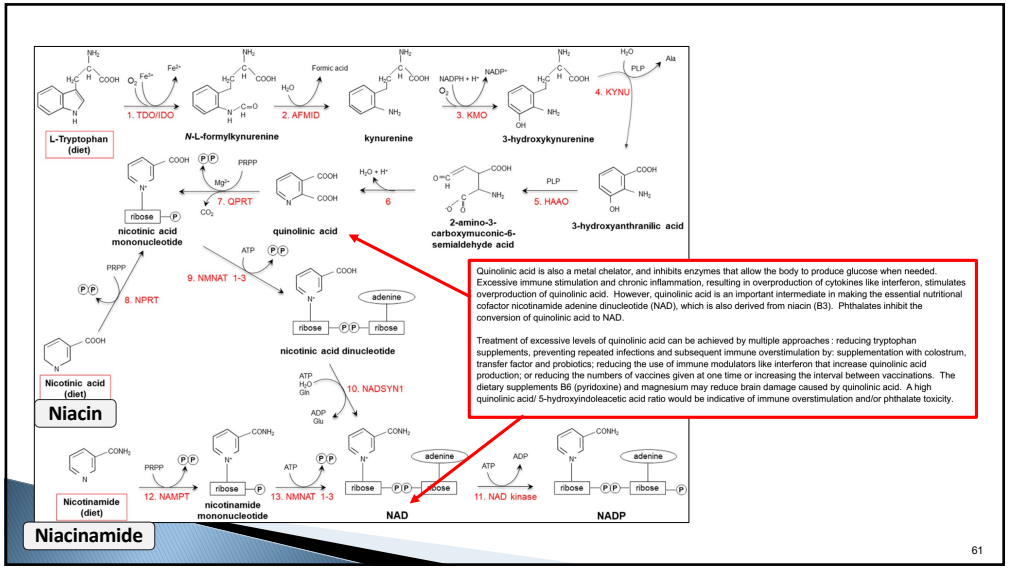
59

Considerations for High Quinolinic Acid

1. Address underlying infections and other stressors as best as possible:
   - *Organic Acids Test*
   - *Stool Pathogen Testing, e.g., CDSA*
   - *Viral Testing – IgG & IgM: herpes viruses, e.g. I, II, VI, CMV, EBV.*
2. Foundational support nutrients (vitamins, minerals, essential fatty acids), including antioxidants:
   - Can add other antioxidants such as curcumin (and other polyphenols such as green tea extract), glutathione (and support products, e.g., broccoli extracts), N-acetylcysteine, vitamin E, Resveratrol, etc.

60

60



61

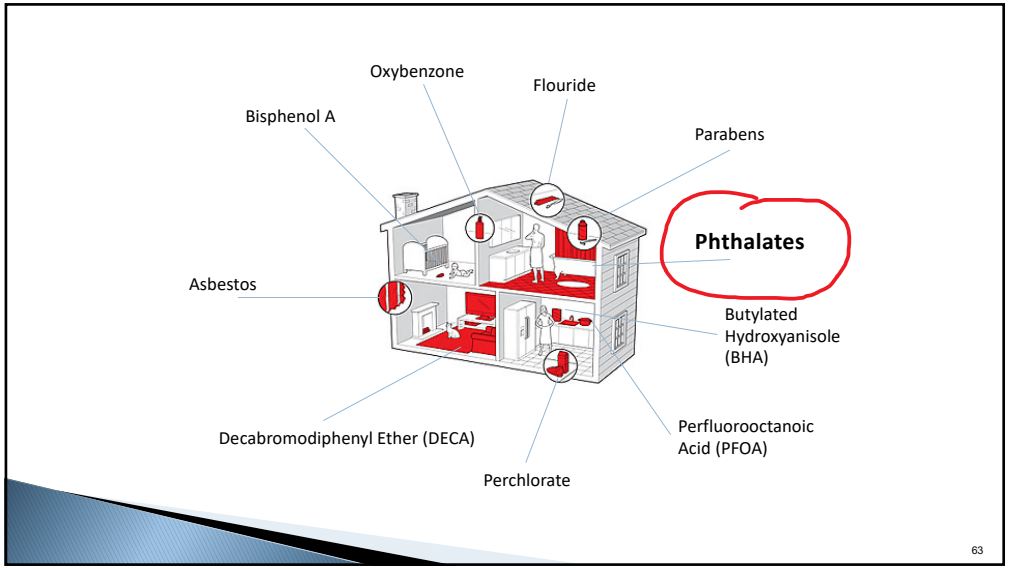
Niacinamide

Niacinamide

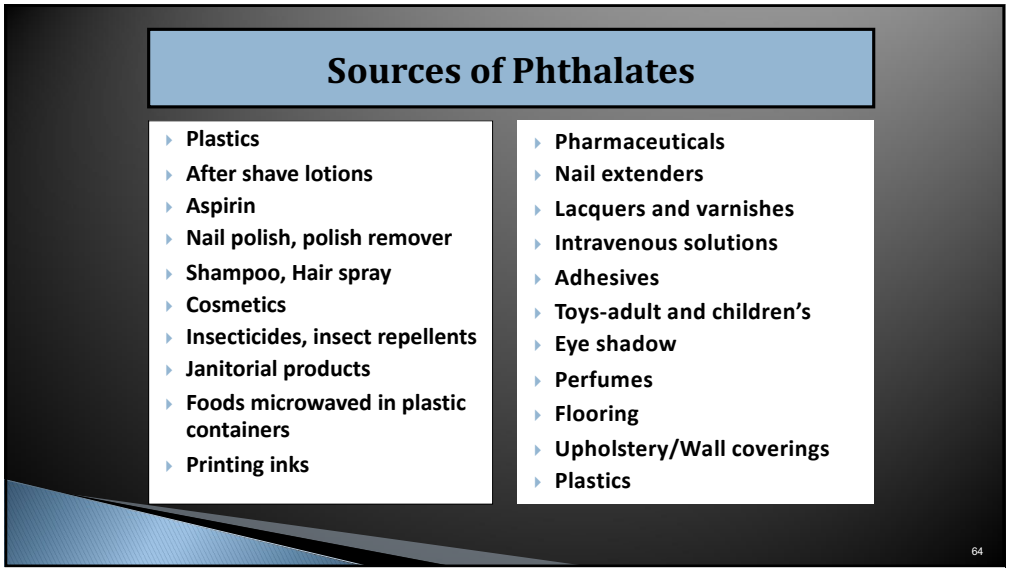
- One of the two principal forms of Vitamin B3 (niacin).
- Niacinamide is the biologically active form of niacin.
- Assists in ATP production
- No flushing
- 500mg to 1000mg+ daily

62

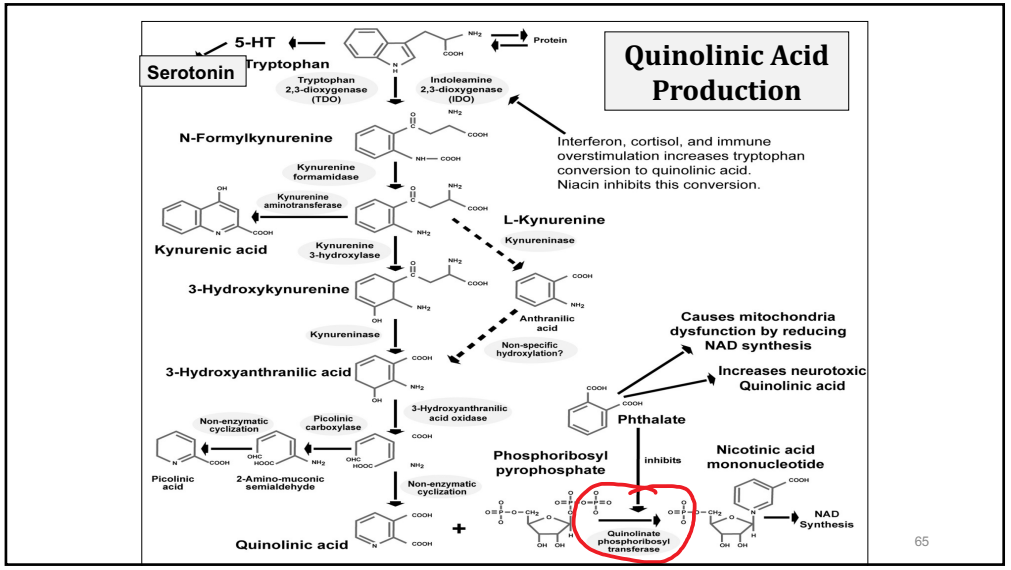
62


Oxybenzone
Flouride
Bisphenol A
Parabens
Phthalates
Asbestos
Butylated Hydroxyanisole (BHA)
Decabromodiphenyl Ether (DECA)
Perfluorooctanoic Acid (PFOA)
Perchlorate
63

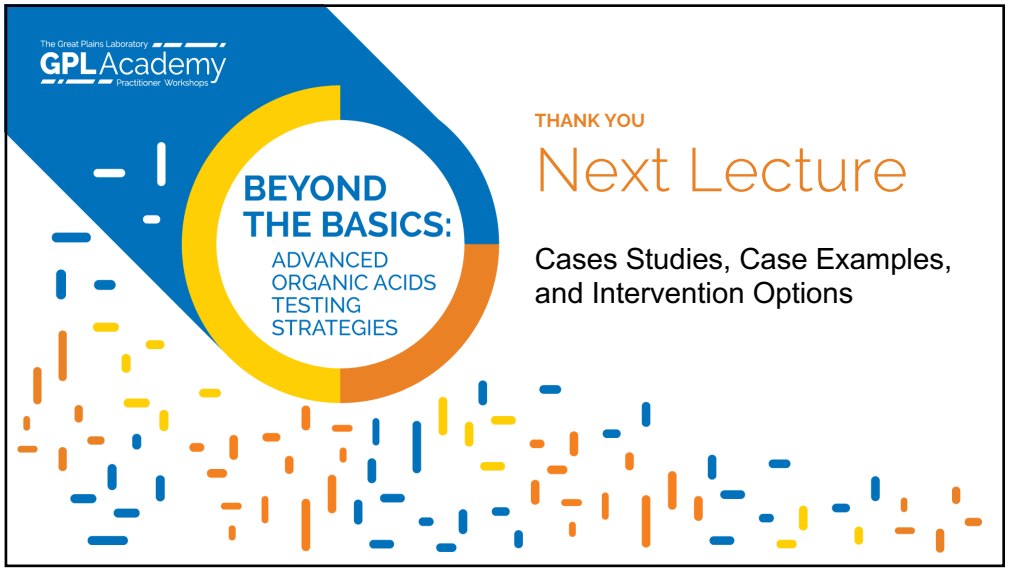
63


Sources of Phthalates
Plastics
After shave lotions
Aspirin
Nail polish, polish remover
Shampoo, Hair spray
Cosmetics
Insecticides, insect repellents
Janitorial products
Foods microwaved in plastic containers
Printing inks
Pharmaceuticals
Nail extenders
Lacquers and varnishes
Intravenous solutions
Adhesives
Toys-adult and children's
Eye shadow
Perfumes
Flooring
Upholstery/Wall coverings
Plastics
64

64


Quinolinic Acid Production
5-HT
Serotonin
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
L-Kynurenine
Kynurenic acid
Kynurenine 3-hydroxylase
Kynureninase
3-Hydroxykynurenine
Anthranilic acid
Kynureninase
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
Quinolinic acid
Quinolinate phosphoribosyl transferase
NAD Synthesis
65

65


The Great Plains Laboratory
GPL Academy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
THANK YOU
Next Lecture
Cases Studies, Case Examples, and Intervention Options

66