Glutathione System Optimization for Detoxification and Immune Balance

Christopher W. Shade, Ph.D.
Introduction - Framing the Issue

1. Toxicity is Not Toxin Load; Toxicity is *Response* to given Toxic Load
2. Inflammation is major mediator of toxic response to load
   1. Antithetical to detoxification
   2. Thus infectious load inversely proportional to detoxification effectiveness
   3. Yet Toxic load also inversely proportional to immune effectiveness
3. Successful Detoxification needs multiple pathways aligned
   1. Phases 1-3 of biochemical detoxification; specific Phase 1 and 2 reactions vary for different toxins
   2. Cellular excretion to blood/lymph coupled to drainage to urine, feces, sweat
4. Understanding this mechanistic framework allows design of effective detox programs that can then be tuned according to clinical experience
Half-life times - Iraq Poisoning

1) Genetic Polymorphisms

2) Functional Expression (inflammation and Oxid Stress)
The Naturopathic View: Detoxification has 2 Basic Parts:

1. Cellular Detoxification - Conjugation and Excretion from the Cell to the blood and Lymph

2. Drainage - filtration from the blood and lymph
   a) Kidney
   b) Liver
   c) GI
   d) Skin
Drainage

* Also called Lavage in Europe
* _Turning the wheels of the Filters - Kidney, Liver, Lymph the Triune_
* _Great to start with this before cellular toxin mobilization!_
* Herbal - single herb, but mostly herbal formulas (“Bitters”)
* Homeopathic - Heel, Professional Choice, MHP, Unda
* Spagyric - low potency or pure herbal extracts with mineral components (often derived from calcination of the same plant)
  * Soluna Labs
  * Pekana
  * Energetix
Drainage

* Kidney - diuretics
* Liver - mostly bitter herbs, cholegogues
* Lymph/Blood - blood cleaners like echinacea and burdock
* GI - laxatives and fibers (Acacia gum, pectin, alginates)
* Skin! - saunas for sweating; far IR best
The Biochemical View: The Human Detoxification System

* Detoxification *Phases I, II, III*
  * **Phase I** is an activation,
  * **Phase II** is conjugation (release from tissue binding)
  * **Phase III** is transport (recently delineated; control point; **Drainage**)

The View: The same processes are repeated at cellular/microscopic and organ/macroscopic levels; Phase 3 may work at cellular level but be damaged at organ level.
The Human Detoxification System

* **Phase III** is the transport out!
  * Several transport proteins (cMOAT, OAT, MRP1, MRP2, GS-X)
    * Organic Anion Transporters
  * Same transporters for many pathways (glucuronide, sulfate, glycinate, GSH)
  * In cells, liver, intestines, kidneys - biggest in liver then intestines
What is Metals Resistance

MRP proteins as potential mediators of heavy metal resistance in zebrafish cells.

Long Y, Li Q, Wang Y, Cui Z.

Abstract

Acquired resistance of mammalian cells to heavy metals is closely relevant to enhanced expression of several multidrug resistance-associated proteins (MRP), but it remains unclear whether MRP proteins confer resistance to heavy metals in zebrafish. In this study, we obtained zebrafish (Danio rerio) fibroblast-like ZF4 cells with resistance to toxic heavy metals after chronic cadmium exposure and selection for 6 months. These cadmium-resistant cells (ZF4-Cd) were maintained in 5μM cadmium and displayed cross-resistance to cadmium, mercury, arsenite and arsenate. ZF4-Cd cells remained the resistance to heavy metals after protracted culture in cadmium-free medium. In comparison with ZF4-WT cells, ZF4-Cd cells exhibited accelerated rate of cadmium excretion, enhanced activity of MRP-like transport, elevated expression of abcc2, abcc4 and mt2 genes, and increased content of cellular GSH. Inhibition of MRP-like transport activity, GSH biosynthesis and GST activity significantly attenuated the resistance of ZF4-Cd cells to heavy metals. The results indicate that some of MRP transporters are involved in the efflux of heavy metals conjugated with cellular GSH and thus play crucial roles in heavy metal detoxification of zebrafish cells.
AntiOxidant–Detoxification–Protein Repair SuperSystem

- AntiOxidant
  - GPx, TPx, APx

- Detoxification
  - MRP1, MRP2, OATP

- Protein Repair & Regulatory
  - GR, TrR, LADH, DHAR
  - GRx, Trx, R-LA
  - Vit C&E CoQ10

- RS-SG Signaling
  - GST, UGT, SULT1&2

- Signaling
Biochemical Hg Removal Requirements

1. Intracellular Glutathione Sufficiency

2. Effective GST Activity (Phase II-Mobilization)

3. Effective Phase III Clearance including intestinal binding and Elimination
Breakdown of the defense system

* Glutathione deficiency
  * Genetic
  * Environmental
* Glutathione S-Transferase (Phase II) problems
  * Genetic
  * Environmental
* Phase III can get blocked and then downregulates Phase II enzymes
  * Can stop multiple detoxification pathways and control the expression of the Glutathione system!
Biggest Reason for *Phase III* Dysfunction

**Inflammation!**

*Especially in Gut!*

- Hallmark of Autism cases
- Easily caused by heavy metal induced oxidative damage
- Also Leaky Gut contributes to LPS load
Phase I

Phase II

Glutathione Conjugation
Sulfation
Glucuronidation

Oxidative Activation

Cellular MRP1

Blood

LIVER

OATP

MRP2

Normal Small Intestine
Autism Recovery Telesummit

**Phase I**

**Phase II**

**Phase III**

Oxidative Activation

Glutathione Conjugation

Sulfation

Glucuronidation

Inflamed Small Intestine

Cellular MRP1

Blood

Inflammation causes Downregulation of MRP2

Negative Feedback – Inhibition of Phase II

OATP

MRP2

LIVER

QuickSilver Scientific

Autism Recovery Telesummit
Autism Recovery Telesummit

**Oxidative Activation**

- Oxidative Stress From Phase I/Phase II mismatch

**Phase I**

**Phase II**

**Phase III**

Glutathione Conjugation

- Build-up of both cellular and blood-borne toxins

Sulfation

- Free-radicals Create Membrane Stress

Glucuronidation

- Inflammation causes Downregulation of MRP2

Cellular MRP1

- Inflamed Small Intestine

Blood

LIVER

OATP

MRP2

Autism Recovery Telesummit
Synergistic Toxicities - LPS

Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents

Patricia E. Ganey, Robert A. Roth

Department of Pharmacology and Toxicology, Institute for Environmental Toxicology, B346 Life Sciences Bldg.,
Michigan State University, East Lansing, MI 48824, USA

Received 26 January 2001; received in revised form 10 September 2001; accepted 14 September 2001

Diet
Alcohol
GI disease, trauma or ischemia
Reye's syndrome
Liver disease
b.d. obstruction, cirrhosis, etc.
Surgery, anesthesia
Xenobiotic agents

GI Tract
Locus of infection
LPS
Liver
Systemic Circulation

LPS via Portal Vein

LPS in Plasma
GI Disturbance
Infection
Altered Diet
Alcohol
Surgery

Time
Period of increased sensitivity

Toxicology 169 (2001) 195–208
www.elsevier.com/locate/toxicol
Synergistic Toxicities - LPS

Augmentation of mercury-induced nephrotoxicity by endotoxin in the mouse

Wilson K. Rumbeiha a,⁎, Scott D. Fitzgerald a, W. Emmett Braselton b, Robert A. Roth b, James J. Pestka c, John B. Kaneene d

[Graphs showing changes in SUN, Mercury, and Urine Mercury levels across different groups.]
Symptoms of Mercury Toxicity

*Immune Dysregulation Th2 Shift*

*Depletion of glutathione causes immune shift from Th1 to Th2 dominance (decrease Interferon and increase IL-4), causing susceptibility to chronic infections.*
Glutathione Balance Crucial To Proper Inflammatory Response

Biochemical and Molecular Roles of Nutrients

Protein-Deficient Pigs Cannot Maintain Reduced Glutathione Homeostasis When Subjected to the Stress of Inflammation

FAROOK JAHOOQ, LINDA J. WYKES, PETER J. REEDS, JOSEPH F. HENRY, MELANIE P. DEL ROSARIO AND MARGARET E. FRAZER

USDA/Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030
Symptoms of Mercury Toxicity

**Toxicity-Infection-Inflammation**

- Chronic Inflammation
- Depressed Detoxification
- Immune Dysregulation
- Vascular Permeability
- Food Allergies/ Hypersensitivites
- Raised Th2
- Lowered Th1

**NO/ONOO^-/Glutamate**
**Neuro-Excitotoxicity/MCS**
**Alzheimers/Parkinsons**
**CV Disease/Diabetes**
**Hashimotos, etc.**

*LPS from CI*
Therapy Approach
1) Block Removal - Restore Integrity of the Membrane/Matrix System and Support Detox Phases

* Phosphatidyl Choline (PC) - Membrane/transporter rebuilding
* Drainage Remedies - filtering blood and lymph
* Vitamin C - Matrix re-ording (fibroblasts), free radical control, and gentle detox
  * Use high quality Liposomal Vitamin C for both PC and C
* DIM - epigenetic repair of Nrf2
* CoQ10 - Restore mitochondrial integrity
* Liposomal Glutathione
Detox Support

1) Drainage of the ExtraCellular Matrix (Drainage Bitters)  
   **Phase III**

2) Rebuilding of the Membranes (PC)  
   **Phase III**

3) Support Intracellular Detoxification (B-Complex, Bitters, GSH)  
   **Phase II and GSH**
Phosphatidyl Choline Therapy - Hepatocytes

www.bioscience.org
Phosphatidyl Choline Therapy - Heptocytes

Cholestasis
physrev.physiology.org
Healthy Detoxification

Cellular Phase I
- Oxidative Activation
- Glutathione Conjugation
- Sulfation
- Glucuronidation

Cellular Phase II
- Glutathione Conjugation
- Sulfation
- Glucuronidation

Phase III
- OATP
- Blood
- Liver
- MRP1

Inflamed Small Intestine
- Cellular MRP1
- Oxidative Activation
- Glutathione Conjugation
- Sulfation
- Glucuronidation
- Oxidative Stress From Phase I/Phase II Mismatch
- Free-radicals
- Create Membrane Stress
- Negative Feedback – Inhibition of Phase II

Inflamed Small Intestine
- MRP2
- Inflammation causes Downregulation of MRP2
- Normal Small Intestine
- Cellular MRP1
- Healthy Detoxification
- Impaired Detoxification

Autism Recovery Telesummit
Phosphatidyl Choline Therapy - Proximal Tubules

www.solvobiotech.com
Mitochondrial Stress via Membrane Deterioration

Need PC and well-delivered CoQ10 to repair
- Free radical control from Ascorbate and restoration of Nrf2

Endoplasmic Reticulum Stress via Membrane Deterioration

Need PC to repair

- Free radical control from Ascorbate and restoration of Nrf2
Golgi Apparatus - Constructs the Matrix
Golgi is a big Membrane!
Phospholipid Encapsulation

- Phospholipids are the basic building blocks of cell membranes and are have both oil-soluble (hydrophobic) and water-soluble (hydrophilic) ends. They can be used to either create solutions that greatly enhance absorption of nutrient compounds.
Biochemical Hg Removal Requirements

1. Effective Phase III Clearance including intestinal binding and Elimination

2. Effective GST Activity (Phase II-Mobilization)

3. Intracellular Glutathione Sufficiency
Product/System for Intestinal Detoxification

1. Intestinal Binders - for Phase III
   - Thiol resins, chlorella, clays/zeolite, pectins/alginate

2. Phytonutrients - *For Phase II*
   - *polyphenols, sulfur-based phytonutrients*

3. Glutathione Supplementation
   - *liposomal, acetyl, precursors, phytonutrients*
Product/System for Intestinal Detoxification

1. Intestinal Binders - for Phase III
   - Thiol resins - QS IMD most specific heavy metal binder
   - Chlorella - multi mode, some thiol, cation exchange, anion exchange; metals, biotoxins
   - Activated Charcoal - multi-specific with hydrophobic sorption large; POP’s, VOC’s, pesticides, herbicides, LPS, mold toxins
   - clays/zeolite - not heavy metal specific; cation exchange, sorption; aflatoxin (mold), some pesticides and herbicides, bacteriostatic, clay healing to GI lining
Product/System for Intestinal Detoxification

1. Intestinal Binders - for Phase III (cont.)
   - Cholestyramine - strong anion exchange for bile salt binding and biotoxin binding; potentially all toxin conjugates
   - Chitosan - weak anion exchange functionality (like Cholystyramine, but less electrostatic charge, more like Welchol); biotoxins, potentially all toxin conjugates
   - Pectins/Alginates - some lead binding, no Hg binding; immunomodulatory action lowers inflammation; possibly other binding
Correct Phase III Elimination

- Phase I
  - Oxidative Activation
  - Glutathione Conjugation
  - Sulfation
  - Glucuronidation
  - OATP
  - Blood
  - LIVER
  - MRP2

- Phase II
  - Oxidative Activation
  - Glutathione Conjugation
  - Sulfation
  - Glucuronidation
  - Cellular MRP1
  - Oxidative Stress From Phase I/Phase II mismatch

- Phase III
  - Oxidative Activation
  - Glutathione Conjugation
  - Sulfation
  - Glucuronidation
  - OATP
  - Blood
  - LIVER
  - MRP2

- Normal Small Intestine
  - Inflammation causes Downregulation of MRP2
  - Build-up of both cellular and blood-borne toxins

- Inflamed Small Intestine
  - Negative Feedback – Inhibition of Phase II
  - Oxidative Stress From Phase I/Phase II mismatch
  - Build-up of both cellular and blood-borne toxins

- Correct Phase III Elimination
Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents

Patricia E. Ganey, Robert A. Roth *

Department of Pharmacology and Toxicology, Institute for Environmental Toxicology, B346 Life Sciences Bldg., Michigan State University, East Lansing, MI 48824, USA

Received 26 January 2001; received in revised form 10 September 2001; accepted 14 September 2001

Diet
Alcohol
GI disease, trauma or ischemia
Reye's syndrome
Liver disease
b.d. obstruction, cirrhosis, etc.
Surgery, anesthesia
Xenobiotic agents

GI Tract
Locus of Infection

+ LPS

via Portal Vein

LPS

Liver

LPS in Plasma

GI Disturbance
Infection
Altered Diet
Surgery

Period of increased sensitivity

www.elsevier.com/locate/toxicol
Product/System for Intestinal Detoxification

1. Intestinal Binders - for Phase III
   - Thiol resins, chlorella, clays/zeolite, pectins/alginites

2. Phytonutrients - For Phase II
   - polyphenols, sulfur-based phytonutrients

3. Glutathione Supplementation
   - liposomal, acetyl, precursors, phytonutrients
“Phytogenomics”

* Certain Phytochemicals upregulate Phase II enzymes as well as GSH, SOD (cellular antioxidants)

* The Anti-Inflammatory Cascade

* Polyphenolic Antioxidants

* Sulfur compounds
  * Alpha Lipoic Acid (R-Lipoic)
  * Crucifers
  * Garlic oil
Chemoprevention by Keap1-Nrf2 Signaling pathway by Phase II Inducers

- Phytochemicals (and their Radicals!)
- R-Lipoate
- ......

Kwak et al., 2004, Mutation Research, 555:133-148
Hartaki – *Terminalia Chebula*

*Chebulic myrobalan fruit (Terminalia chebula)*
Decay in GSH levels and Enzyme Activity with Age

Table 2. Effect of T. chebula aqueous extract on enzymatic antioxidants in young and aged rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Young</th>
<th>Old</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSOD (μmol H2O2 consumed/min/mg protein)</td>
<td>3.37 ± 0.20</td>
<td>2.11 ± 0.24</td>
<td>-37%</td>
</tr>
<tr>
<td>GPx (μmol GSH utilized/min/mg protein)</td>
<td>4.57 ± 0.24</td>
<td>6.44 ± 0.24</td>
<td>7.96 ± 0.26 *1</td>
</tr>
<tr>
<td>GR (μmol NADPH oxidized/min/mg protein)</td>
<td>5.61 ± 0.28</td>
<td>3.61 ± 0.31</td>
<td>5.28 ± 0.26 *1</td>
</tr>
<tr>
<td>GST (μmol of CDNB-GSH conjugated/min/mg protein)</td>
<td>0.96 ± 0.02</td>
<td>0.64 ± 0.03</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>GSHOH (Units/min/mg protein)</td>
<td>3.25 ± 0.18</td>
<td>3.54 ± 0.14 *6</td>
<td>1.64 ± 0.16 *6</td>
</tr>
<tr>
<td>Kidney MnSOD (50% reduction of NBT/min/mg protein)</td>
<td>3.35 ± 0.18</td>
<td>3.42 ± 0.21</td>
<td>2.06 ± 0.18 *1</td>
</tr>
<tr>
<td>CAT (μmol H2O2 consumed/min/mg protein)</td>
<td>4.14 ± 0.20</td>
<td>4.20 ± 0.15</td>
<td>4.02 ± 0.22 *1</td>
</tr>
<tr>
<td>GPx (μmol GSH utilized/min/mg protein)</td>
<td>5.18 ± 0.27</td>
<td>5.07 ± 0.31</td>
<td>6.03 ± 0.21 *1</td>
</tr>
<tr>
<td>GR (μmol NADPH oxidized/min/mg protein)</td>
<td>4.85 ± 0.34</td>
<td>4.68 ± 0.34</td>
<td>2.86 ± 0.26 *1</td>
</tr>
<tr>
<td>GST (μmol of CDNB-GSH conjugated/min/mg protein)</td>
<td>1.32 ± 0.06</td>
<td>1.41 ± 0.03</td>
<td>0.92 ± 0.04 *1</td>
</tr>
<tr>
<td>GSHOH (Units/min/mg protein)</td>
<td>1.80 ± 0.08</td>
<td>1.88 ± 0.06</td>
<td>1.11 ± 0.08 *1</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD for six rats in each group. Superscript letters represent *p < 0.05 (Tukey-Kramer Multiple comparisons Test).

*1 As compared with Young control.
*6 As compared with Aged control.
*9 p < 0.05; *10 p < 0.01; *11 p < 0.001.
### Decay in GSH levels and Enzyme Activity with Age

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Young</th>
<th>Old</th>
<th>% Change</th>
<th>Old Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSOD (50% reduction of NBT/min/mg protein)</td>
<td>3.37 ± 0.20</td>
<td>2.11 ± 0.18</td>
<td>-37%</td>
<td>3.13 ± 0.23</td>
</tr>
<tr>
<td>CAT (μmol H₂O₂ consumed/min/mg protein)</td>
<td>4.59 ± 0.31</td>
<td>4.51 ± 0.22</td>
<td>-37%</td>
<td>4.54 ± 0.25</td>
</tr>
<tr>
<td>GPx (μmol GSH utilized/min/mg protein)</td>
<td>6.41 ± 0.24</td>
<td>6.34 ± 0.20</td>
<td>-50%</td>
<td>6.67 ± 0.23</td>
</tr>
<tr>
<td>GR (μmol NADPH oxidized/min/mg protein)</td>
<td>5.61 ± 0.28</td>
<td>5.73 ± 0.31</td>
<td>-35%</td>
<td>5.46 ± 0.25</td>
</tr>
<tr>
<td>GST (μmoles of CDNB-GSH conjugated/min/mg protein)</td>
<td>0.98 ± 0.02</td>
<td>1.03 ± 0.01</td>
<td>-37%</td>
<td>0.99 ± 0.04</td>
</tr>
<tr>
<td>GSTD (Unit/mg protein)</td>
<td>3.29 ± 0.18</td>
<td>3.54 ± 0.14</td>
<td>-25%</td>
<td>3.14 ± 0.19</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD for six rats in each group. Superscript letters represent p < 0.05 (Tukey–Kramer Multiple comparisons Test).

*As compared with Young control.
*As compared with Aged control.
*p < 0.05; p < 0.01; p < 0.001.
BUT WHAT IF MECHANISMS ARE NOT WORKING???
Reversing Epigenetic Blocks???

Epigenetic Modifications of Nrf2 by 3,3′-diindolylmethane *In Vitro* in TRAMP C1 Cell Line and *In Vivo* TRAMP Prostate Tumors

Tien-Yuan Wu,1 Tin Oo Khor,1 Zheng-Yuan Su,1 Constance Lay-Lay Saw,1 Limin Shu,1 Ka-Lung Cheung,1 Ying Huang,1 Siwang Yu,2 and Ah-Ng Tony Kong1,3

Received 20 January 2013; accepted 17 April 2013; published online 9 May 2013

Abstract. 3,3′-diindolylmethane (DIM) is currently being investigated in many clinical trials including prostate, breast, and cervical cancers and has been shown to possess anticancer effects in several *in vivo* and *in vitro* models. Previously, DIM has been reported to possess cancer chemopreventive effects in prostate carcinogenesis in TRAMP mice; however, the *in vivo* mechanism is unclear. The present study aims to investigate the *in vitro* and *in vivo* epigenetic modulation of DIM in TRAMP-C1 cells and in TRAMP mouse model. *In vitro* study utilizing TRAMP-C1 cells showed that DIM suppressed DNMT expression and reversed CpG methylation status of Nrf2 resulting in enhanced expression of Nrf2 and Nrf2-target gene NQO1. In *vivo* study, TRAMP mice fed with DIM-supplemented diet showed much lower incidence of tumorigenesis and metastasis than the untreated control group similar to what was reported previously. DIM increased apoptosis, decreased cell proliferation and enhanced Nrf2 and Nrf2-target gene NQO1 expression in prostate tissues. Importantly, immunohistochemical analysis showed that DIM reduced the global CpG 5-methylcytosine methylation. Focusing on one of the early cancer chemopreventive target gene Nrf2, bisulfite genomic sequencing showed that DIM decreased the methylation status of the first five CpGs of the Nrf2 promoter region, corroborating with the results of *in vitro* TRAMP-C1 cells. In summary, our current study shows that DIM is a potent cancer chemopreventive agent for prostate cancer and epigenetic modifications of the CpG including Nrf2 could be a potential mechanism by which DIM exerts its chemopreventive effects.

KEY WORDS: 3,3′-diindolylmethane (DIM); epigenetic; methylation; Nrf2; prostate cancer.

Removes Epigenetic blocks to AND upregulates Nrf2; plus inflammation, immune, and hormone control
Product/System for Intestinal Detoxification

1. Intestinal Binders - for Phase III
   - Thiol resins, chlorella, clays/zeolite, pectins/alginites

2. Phytonutrients - *For Phase II*
   - polyphenols, sulfur-based phytonutrients

3. Glutathione Supplementation
   - liposomal, acetyl, precursors, phytonutrients
Nutritional Glutathione Support

1. **Vitamin C** supports antioxidant system and Glutathione synthesis
2. Antioxidant Phytonutrients (**Polyphenolics**) and **Alpha Lipoic Acid** upregulate Glutathione production
3. Glutathione Precursors feed Glutathione synthesis
   - 1. **N-Acetyl Cysteine**
   - 2. **Whey Protein**
Direct Glutathione Support: when Precursors might not work

1. *IV GSH*
2. Nebulized
3. Transdermal
4. Acetyl GSH
5. Liposomal
Immune Response

* GSH:GSSG Ratios in HIV Pts
* Plus results of infection with Tb

Needed 1000X More NAC to achieve what Lipo GSH did!
Symptoms of Mercury Toxicity

Immune Dysregulation Th2 Shift

*Depletion of glutathione causes immune shift* from Th1 to Th2 dominance (decrease Interferon and increase IL-4), causing susceptibility to chronic infections.
Protocol Development

* Step I (2-8 weeks)
  * PC and drainage remedies (all)
* Step II (3-6 months; titrating and cycling)
  * PC and drainage (all)
  * Intestinal binders (all)
  * Liposomal Vitamin C (80+%)
Protocol Overview

Titration of Dosages

Dosage

IMD 100mg

IMD 300mg

Time (3-12 months)

2 Ways to Fail:
1) Start too high
2) Stay too low
2. Cycling On/Off

- 5-on/2-off multiples; 10/4 deeper; 20/8 deepest
- can go 4/3 if drainage too much
- go with Parasympathetic/Sympathetic dominance
Thank You Luminara Serdar and Autism Recovery Telesummit!