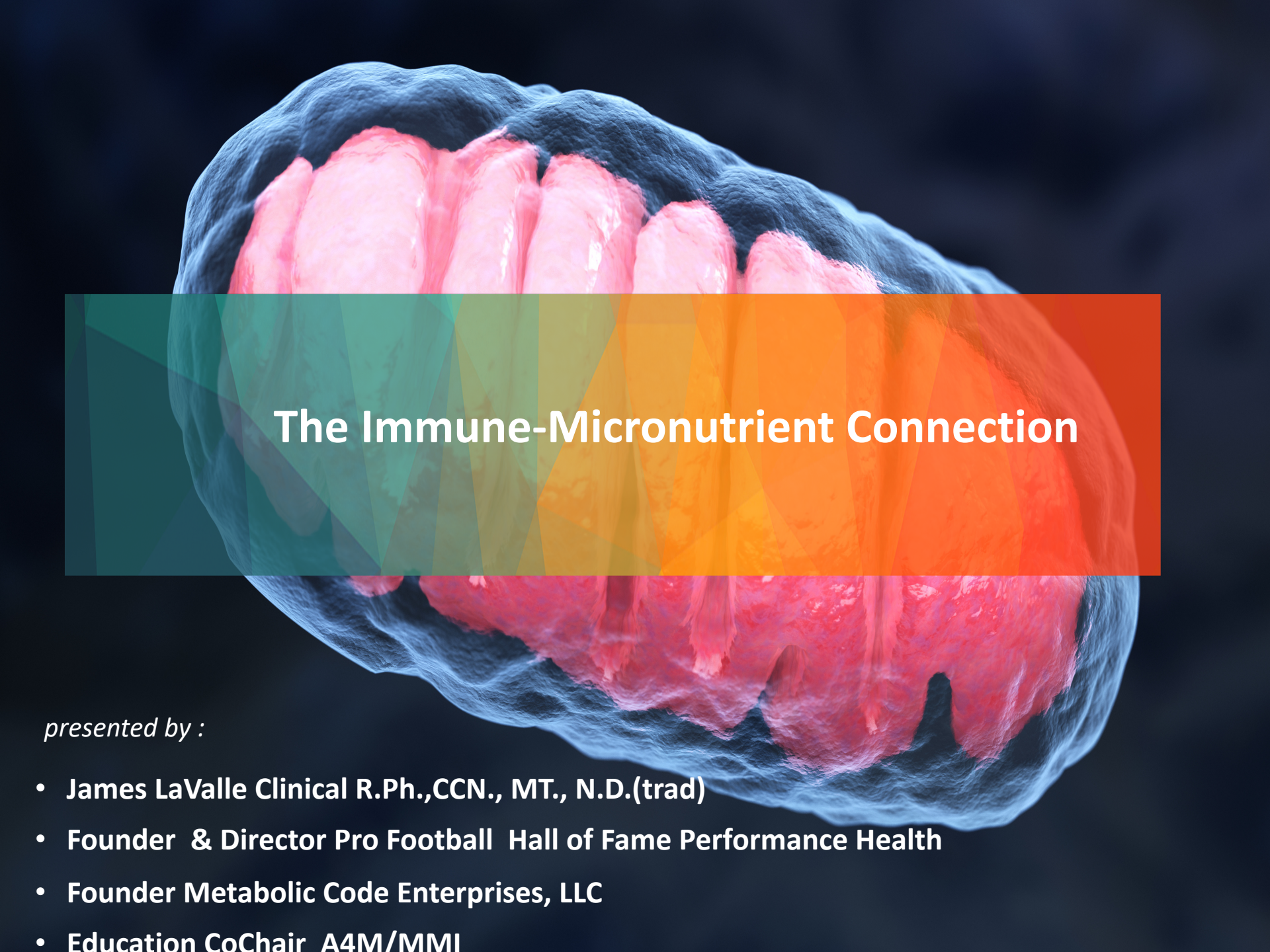


The Immune-Micronutrient Connection

presented by :

- **James LaValle Clinical R.Ph.,CCN., MT., N.D.(trad)**
- **Founder & Director Pro Football Hall of Fame Performance Health**
- **Founder Metabolic Code Enterprises, LLC**
- **Education CoChair A4M/MMI**

This material is provided for educational and informational purposes only to licensed health care professionals. This information is obtained from sources believed to be reliable, but its accuracy cannot be guaranteed. Herbs and other natural substances are very powerful and can occasionally cause dangerous allergic reactions in a small percentage of the population. Licensed health care professionals should rely on sound professional judgment when recommending herbs and natural medicines to specific individuals. Individual use of herbs and natural medicines should be supervised by an appropriate health care professional. The use of any specific product should always be in accordance with the manufacturer's directions.

Disclosures

- Nothing to Disclose

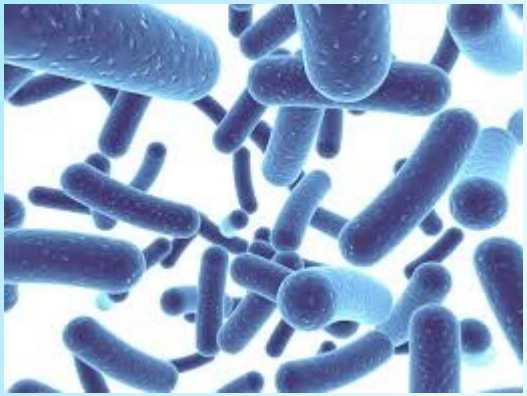
Objectives

- Identify the key nutrients in the immune defense system
- Explore the micronutrient inadequacies leading to immune dysfunction
- Discuss the pharmacists' opportunities to assess nutritional status

ImmunoSupportive Nutrients

- At no other time during our lifetime has immune support been so important
- Recommend defensively/preventative or acutely
- Remember the GUT is the largest organ of immunity – the entire GUT-IMMUNE axis should be supported

Probiotics



- Probiotic flora consists of over 400 species of bacteria
- Enhance immunity – strengthens GUT-IMMUNE interface
- Interact with mucosal cells of GUT to provide a barrier against pathogens
- Microfloral disruption
  - Overtraining; intense physical exercise
  - Poor food choices – high sugar, pesticides, additives, preservatives, antibiotics, hormones, red meats
  - Chronic stress
  - Infections
  - Impure water
  - Drugs
- **20 billion CFU daily**
- **Also add Saccharomyces boulardii 3 billion CFU daily**
- **100 Billion CFU caps also available**

Probiotic General Use
Meta-Analysis

- Meta- analysis 1970-2011
- 79 randomized, controlled trials in 10,351 patients comparing probiotic to placebo
- 11 probiotic strains
- The Question - Are probiotics beneficial in treating gastrointestinal diseases, including
  - Infectious diarrhea
  - IBS
  - *H. pylori* infections
  - *C. Difficile*
  - Antibiotic Associated Diarrhea
  - Traveler's Diarrhea
  - Necrotizing entereocolitis
  - Pouchitis
- The results: YES for all (statistically significant positive outcomes over placebo)

Probiotic General Use
Cochrane Database Review

- 2018 Cochrane Collaboration Overview of 14 Cochrane Database Systematic Reviews from 2006-2015
- Focused on probiotic supplementation and GI related medical conditions
- RESULTS:
  - Probiotic use does have a beneficial effect on diarrheal conditions and related GI symptoms

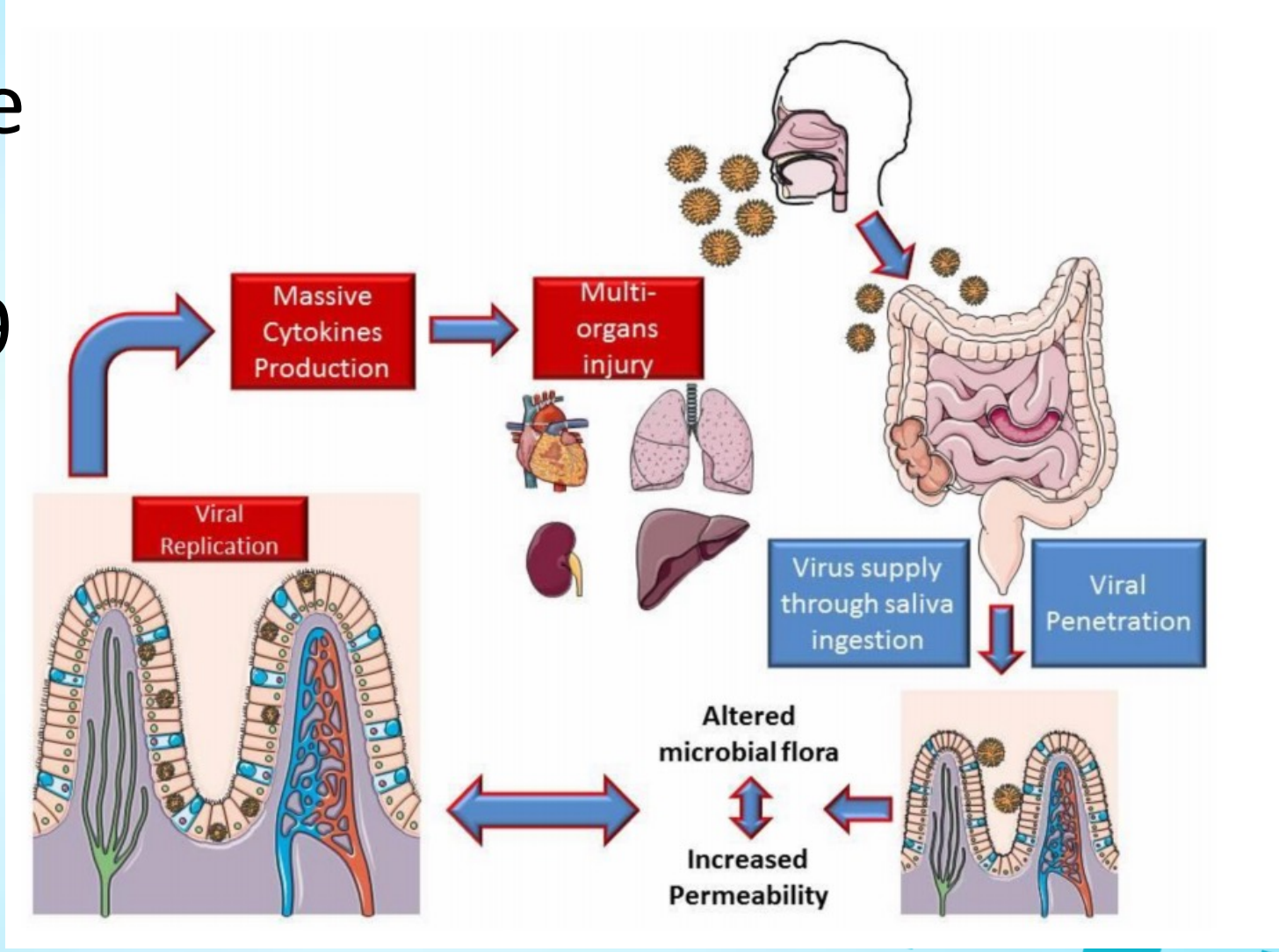
Parker EA, et al. Probiotics and gastrointestinal conditions: an overview of

Probiotics and COVID-19 Symptoms

- Orally administered probiotic strains can reduce the incidence and severity of viral RTIs – “flatten the curve”
- Supports GUT microbiome
- Produce natural bacteriocins – AMP (antimicrobial peptides 30-60 amino acids)
- Improves GUT-Immune connection
- Control Drug and viral associated diarrhea
- GI infections
- Improves mucin production – decreases viral replication in intestines - antiviral
- Respiratory tract infections – improves GUT-LUNG crosstalk
- Study in 4,230 youngsters w/ URTI given probiotics
  - 2x decrease risk of upper respiratory tract infections
  - Decreased severity of disease

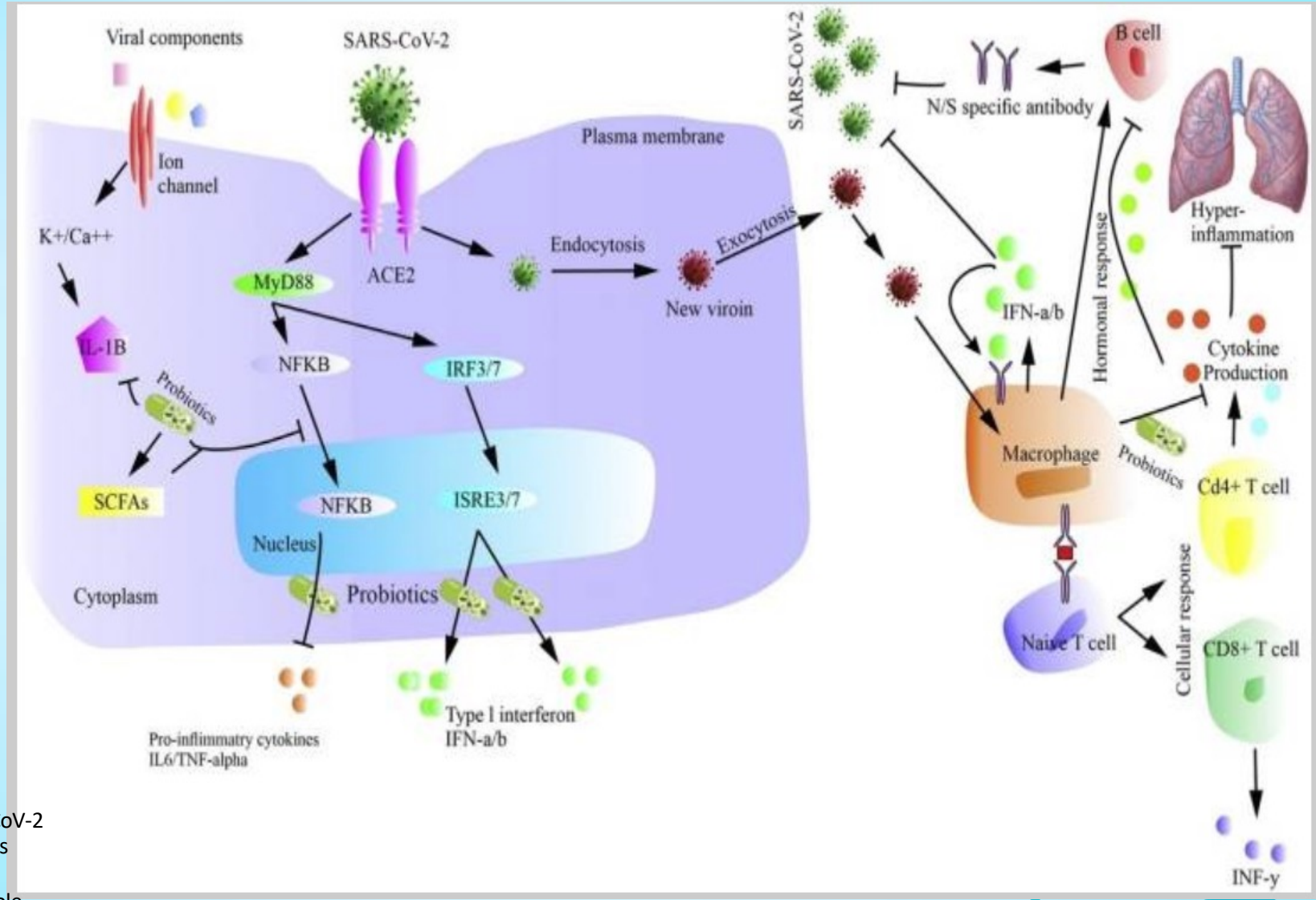
Sundararaman A, et al. Role of probiotics to combat viral infections with emphasis on COVID-19. Appl Microbiol Biotechnol. 2020;1-16.

Intestinal Involvement of COVID-19 Infection

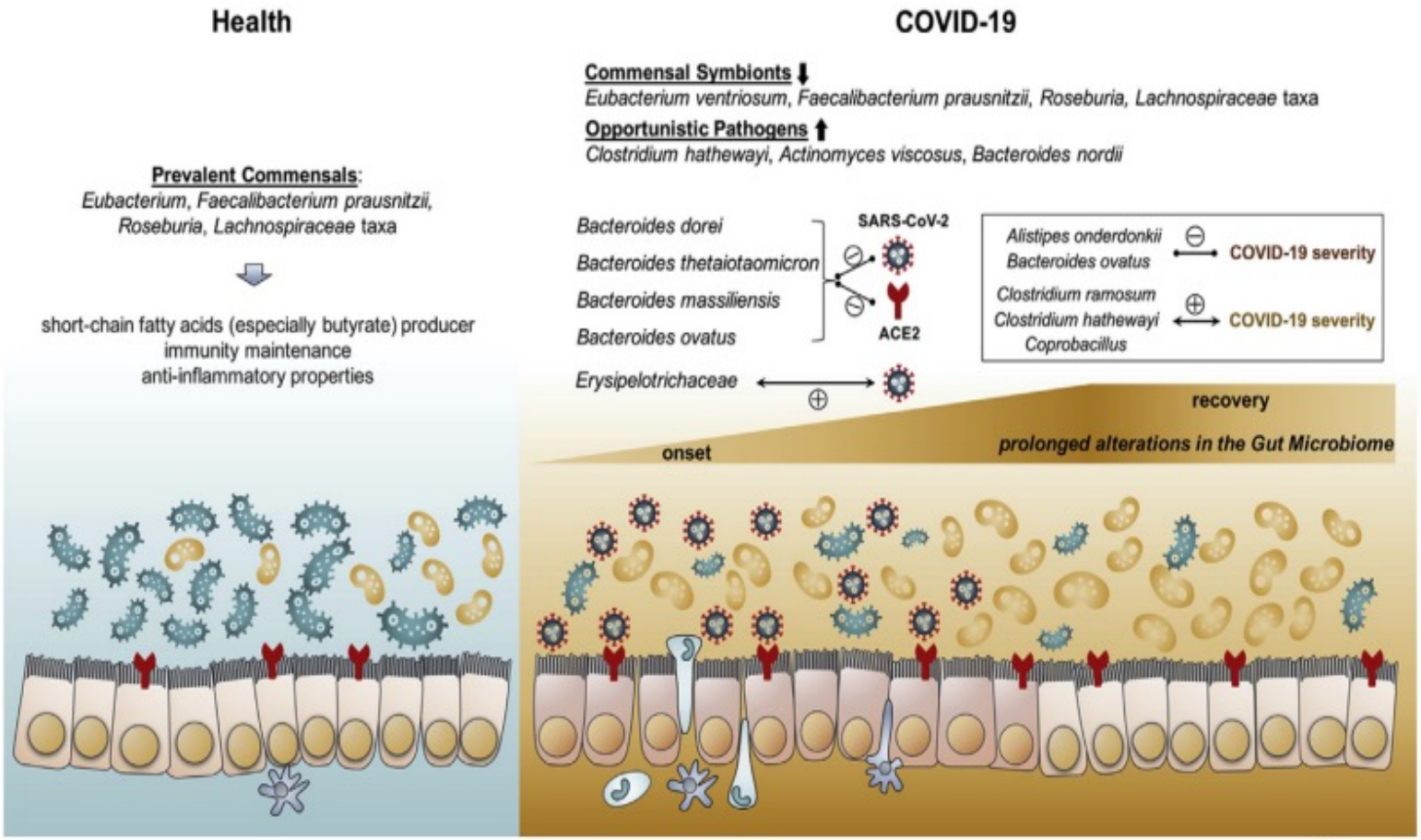


Infusino F, et al. Diet Supplementation, Probiotics, and Nutraceuticals in SARS-CoV-2 Infection: A Scoping Review. Nutrients 2020;12:1718.

Probiotic effects on SARS-CoV-2



Din AU, et al. SARS-CoV-2 microbiome dysbiosis linked disorders and possible probiotics role. Biomed Pharmacol. 2021;133:110947



Zuo T, et al. Alterations in GUT microbiota of patients with COVID-19 during time of hospitalization. Gatroenterol. 2020;159:944-55.

Gastroenterology

GUT-Immune Dysfunction and COVID-19 Infection

- GUT Dysbiosis reported to increase risk of infections
- Up to 20% of COVID-19 patients report overt gastrointestinal issues
- Studies have detected SARS-CoV-2 virus in anal swabs and stool samples in almost 50% of patients with COVID-19
- Suggests the digestive tract is an extrapulmonary site for virus replication and activity

Sencio V, et al. Gut Dysbiosis during Influenza Contributes to Pulmonary Pneumococcal Superinfection through Altered Short-Chain Fatty Acid Production. Cell Reports. 2020;30(9):2934-47.

GUT-Immune Dysfunction and COVID-19 Infection

- Virus reported to alter production of SCFAs by the gut microbiota
- Dysbiotic microbiota transfers susceptibility to respiratory bacterial infection
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects gastrointestinal tissues

Sencio V, et al. Gut Dysbiosis during Influenza Contributes to Pulmonary Pneumococcal Superinfection through Altered Short-Chain Fatty Acid Production. Cell Reports. 2020;30(9):2934-47.

GUT-Immune Dysfunction and COVID-19

- Fecal microbiomes from patients with SARS-CoV-2 19 report depletion of symbionts and enrichment of opportunistic pathogens
- Dysbiosis persists after clearance of SARSCoV-2
- Multiple species from the *Bacteroidetes phylum* correlated inversely with fecal shedding of SARS-CoV-2

Zuo T, et al. Alterations in GUT microbiota of patients with COVID-19 during time of hospitalization. Gatroenterol. 2020;159:944-55.

GUT-Immune Dysfunction and COVID-19

- Studies report microbiome changes in COVID-19 infection:
  - Baseline abundance of *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* correlated with COVID-19 severity
  - Inverse correlation between abundance of *Faecalibacterium prausnitzii* (an antii
  - *Bacteroides dorei, Bacteroides thetaiotaomicron, Bacteroides massiliensis, and Bacteroides ovatus* overexpressed and related to disease severity over course of disease
    - *Reported to d*ownregulate expression of angiotensin-converting enzyme 2 (ACE2)

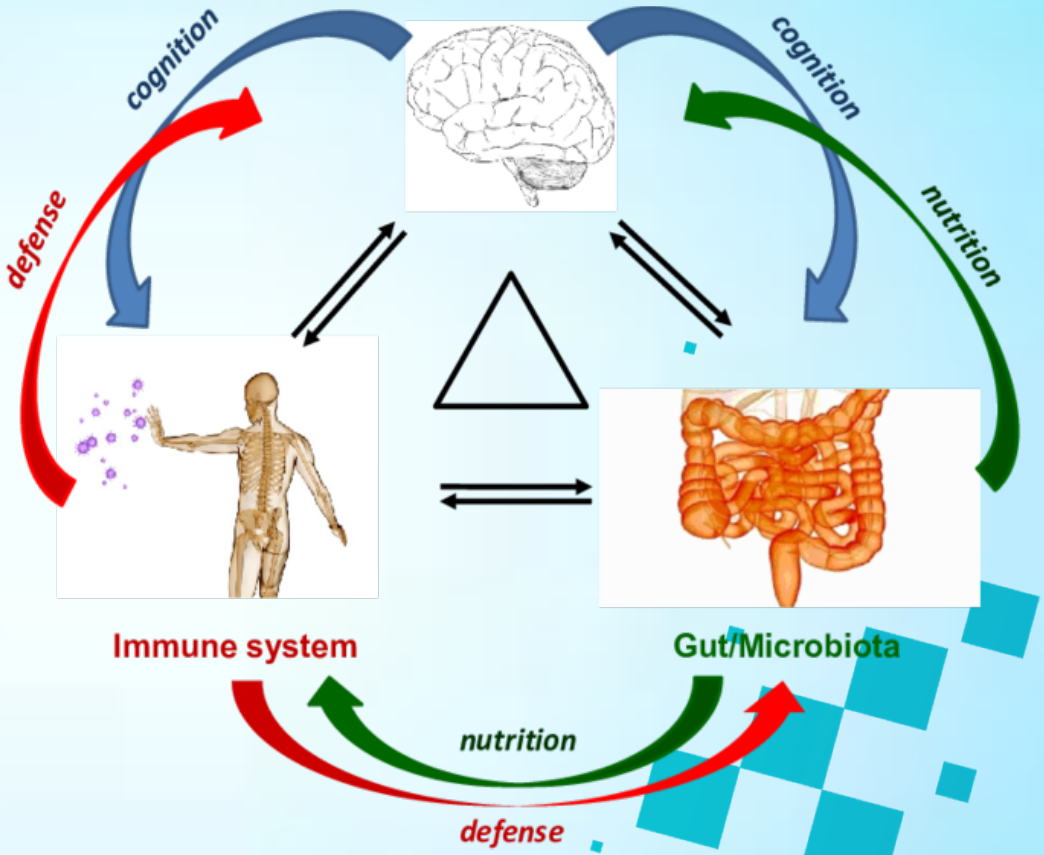
Zuo T, et al. Alterations in GUT microbiota of patients with COVID-19 during time of hospitalization. Gatroenterol. 2020;159:944-55.

Gut - Immune Inflammation

- Over-activation of immunity in GUT leads to increased production of inflammatory cytokines
- Leaky gut allows bacterial and toxins to enter bloodstream
- Leads to peripheral and central inflammation

Viera M, et al. Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science. 2018;359(6380):1156-61.

COVID and Post COVID Systems Biology Approach



Review > Respir Care. 2020 May;65(5):673-685. doi: 10.4187/respcare.07097.
Epub 2020 Mar 3.

Probiotics for the Prevention of Ventilator-Associated Pneumonia: A Meta-Analysis of Randomized Controlled Trials

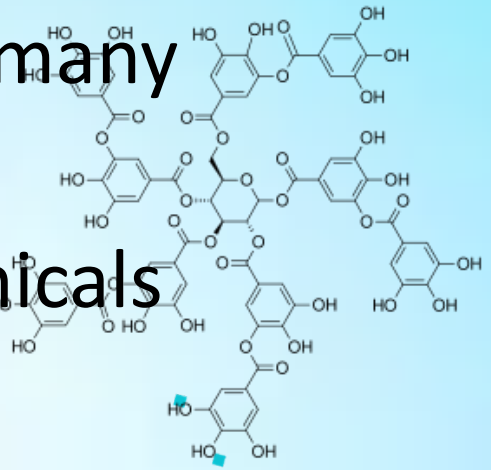
Minmin Su [1], Ying Jia [1], Yan Li [2], Dianyou Zhou [2], Jinsheng Jia [3]

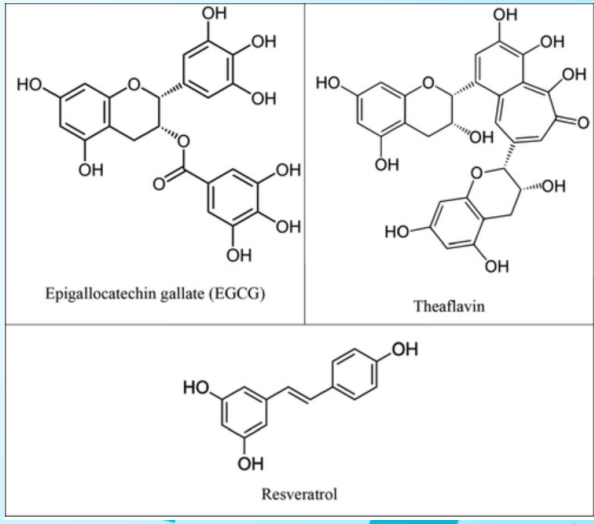
- 2020 Meta-analyses of RCTs
- 14 studies - n = 1,975
- RESULTS: probiotic strains reduce the incidence of ventilator-associated pneumonia

Su M, Jia Y, Li Y, Zhou D, Jia J. Probiotics for the prevention of ventilator-associated pneumonia: a meta-analysis of randomized controlled trials. Respir Care. (2020) 65:5:673-85.

Plant Polyphenols

- Antioxidant compounds found in many plants
- Comprise 4 families of phytochemicals
  - Phenolic acids
  - Flavonoids
  - Stilbenes
  - Lignans
- Antioxidant, Antiinflammatory, Immunomodulatory
- Antiviral
- Pleiotropic
- Bind to viral S protein



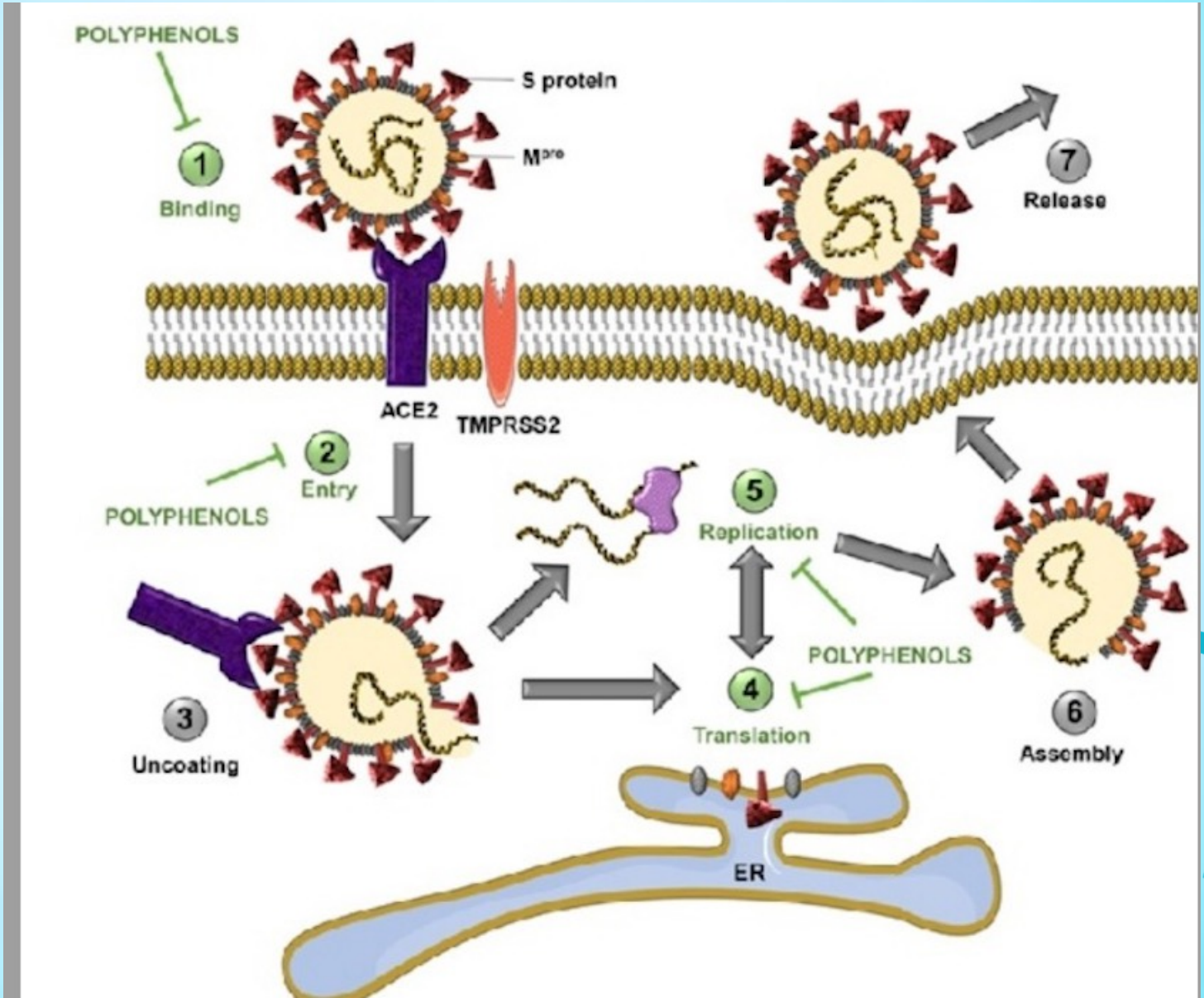


Tsao R. Chemistry and Biochemistry of Dietary Polyphenols. Nutrients. 2010;2(12):1231-46.

Polyphenol Activity vs. COVID

Target:

1. BINDING
2. ENTRY into cell
3. UNCOATING
4. TRANSLATION
5. REPLICATION
6. ASSEMBLY
7. RELEASE



Paraiso IL, et al. Potential use of polyphenols in the battle against COVID-19. Curr Opin food Sci. 2020;32:149-155,

Curcumin - Metaflammation

- From turmeric (*Curcuma longa)* root/rhizome

- Curcuminoids reported:
  - Anti-inflammatory
  - Decreases inflammasome signaling
  - Supports musculoskeletal system
  - Joints/connective tissue support
  - Helps improve flexibility and mobility

Curcumin - Metaflammation

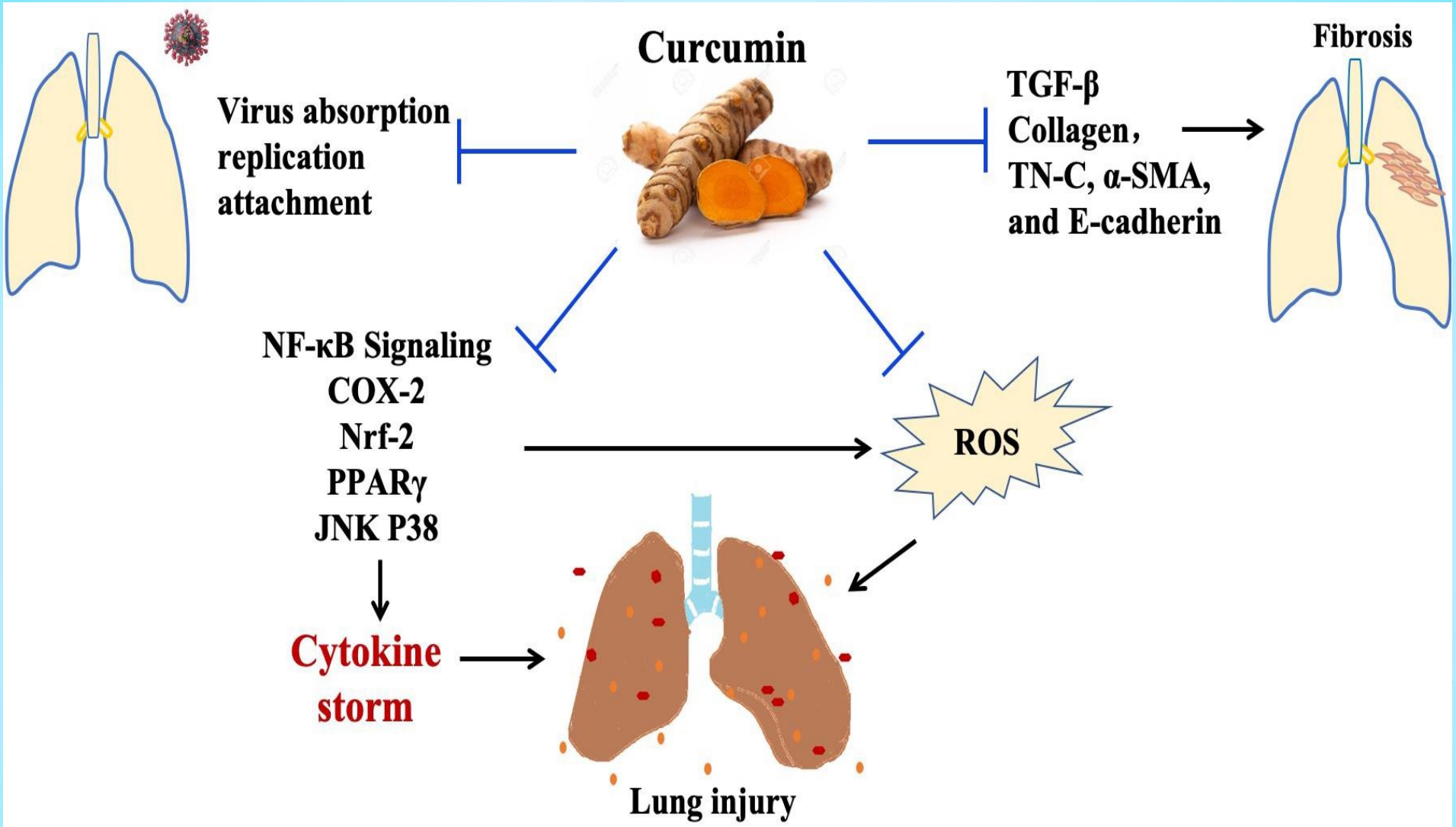
- Decreases oxidative stress via Nrf2-keap1 pathway
- Inhibits nuclear factor-kappaB
- Inhibits Toll-like receptor 4-dependent signaling pathways
- Inhibits activation of a peroxisome proliferator-activated receptor-gamma pathway.

Castro CN, et al. Curcumin ameliorates autoimmune diabetes,. Evidence in accelerated murine models of type 1 diabetes. Clin Exp Immunol. 2014;177(1):149-60.

Curcumin Metaflammation

- Modulates multiple cell signaling molecules
  - TNF-alpha
  - IL 1, IL-6
  - COX-2 and 5-lipoxygenase
  - NF-kappaB
  - CRP
  - PgE2
  - TGF-beta
  - AST/ALT
  - Malondialdehyde MDA
- Lab study reports curcumin ameliorates pancreatic beta cell destruction in autoimmune diabetes

Castro CN, et al. Curcumin ameliorates autoimmune diabetes. Evidence in accelerated murine models of type 1



Liu Z, et al. Front Cell Dev Biol. 2020;
https://doi.org/10.3389/fcell.2020.00479

Curcumin Indications

- Control metainflammatory responses
- Immune support
- Osteoarthritis
- MetS – insulin/blood glucose,
- Cardiovascular support – atherosclerosis;
- Exercise recovery
- Oncological indications - cancer
- IBDs - inflammatory bowel diseases
- Postoperative inflammation/pain
- Skin issues – psoriasis, eczema, dermatitis

Curcumin Dosage

General dosage

- 500mg turmeric root 2 times daily
- Std to 45-55% curcuminoids, 3-8% volatile oils and 2-6% turmerin

Zinc Immunity

- Essential micronutrient
- Involved in regulation of innate and adaptive immune responses
  - Modulates NF-kB – master regulator of proinflammatory responses
  - Controls oxidative stress
- Zn deficiency leads to cell-mediated immune dysfunctions
- Deficiency associated with chronic inflammation
  - Increased infections and poor outcomes

Gammoh N.Z., Rink L. Zinc in infection and inflammation. Nutrients. 2017;9:624

Zinc Adjunct in Pneumonia Mortality

- Meta analysis, 6 randomized double-blind placebo controlled
- n = 2216 patients w/ severe pneumonia
- Zinc tx as adjunct to antibiotic tx
- Significantly reduced mortality
- No change in treatment failure

Wang L, et al. Efficacy of zinc given as an adjunct to the treatment of severe pneumonia: a meta-analysis of

Zinc Autoimmunity

- 2018 Database review
- 62 studies included
- Relationship of Zinc status in autoimmune conditions
- For all models, Zn concentration in serum autoimmune patients significantly lower than controls

Sanna A, et al. Zinc status and autoimmunity: a systematic review and meta

Zinc

- 30-50mg elemental zinc daily
- Zinc glycinate more bioavailable
- Zinc can alter iron and copper absorption, so dose independently if possible
- Copper needed by lysyl oxidase in collagenesis and elastic tissue formation

Andrographis paniculata aerial parts

- "King of Bitters" – SE Asian botanical for immune/inflammatory conditions
- Andrographalide – phytochemical w/ Broad spectrum antibacterial, antiviral, antifungal, antiparasitic activity
- Hepatoprotective antioxidant
- Antiinflammatory
- Reported to help improve Th1/Th2 and Th17 modulation
- Dose = 300mg BID std 10-50% andrographolides

Jayakumar T, et al. Evid Based Complement Alternat Med.

Andrographis Upper Respiratory Tract Infections (uRTIs)

- 2010 randomized, double blind placebo controlled
- N=223 patients with uncomplicated URTI
- 200mg / day standardized andrographis or placebo
- RESULTS:
  - Significant reduction in symptoms scores for andrographis vs placebo
  - 2.1 x more effective than placebo in reducing symptoms of URTI

Saxena RC, et al. Phytomedicine. 2010;17(2-4):178-85

Andrographis Autoimmune Associated Fatigue

- 2016, 12 month double blind placebo-controlled pilot study
- N=25 Multiple Sclerosis patients (relapsing-remitting)
- 170mg BID standardized andrographis BID
- Andrographis significantly improved Fatigue Severity Scores (FSS) in patients receiving interferon

Bertoglio JC, et al. Andrographis paniculata decraeses fatigue in patients with relapsing-remitting multiple sclerosis: a 12 month double blind placebo controlled pilot study. BMC

Immune Support - Colostrum

- Produced mainly from birth to day 5 of lactation
- human breast milk essential for optimal growth and development of immune system
- Affects microbiome – milk oligosaccharides
- Human milk stem cells (hMSCs) discovered
- Help repair and regenerate infant
- As infants GI and immune system grow, mother's milk turns from immune factors to more calories and nutrients for growth
- Lack of breastfeeding linked to childhood diseases i.e. asthma, otitis media

Cacho NT, et al. Innate Immunity and breast milk. Front Immunol. 2017;8:584.
Stuebe A. The risks of not breastfeeding for mothers and infants. Rev Obstet Gynecol. 2009;2(4):222-31.

Bovine Colostrum Supplementation

- 12% Proline-Rich Polypeptides (PRPs)
- Increased levels of PRPs support optimal immune function
- Premium colostrum from U.S. bovine sources
- Natural source of IgA, IgD, IgE, IgG, IgM

Bovine Colostrum Supplementation

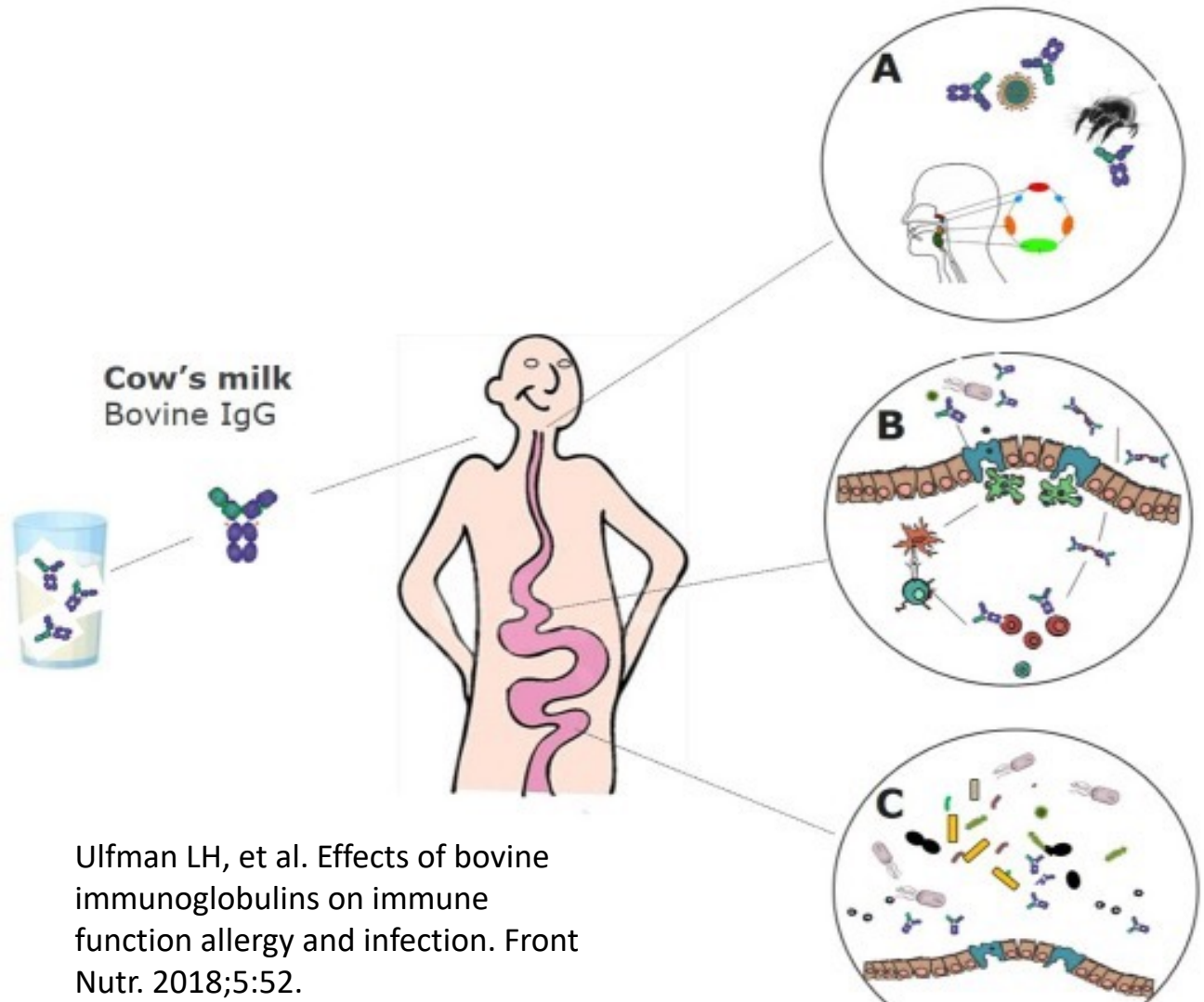
- Support immunity
- May positively affect bone and lean muscle mass
- Supports insulin resistance

Helps support healthy cognitive function

- Helps regulate anti-inflammatory cytokines
  - Th1/Th2 balance, NK cells, TNF-alpha, gamma interferon- (INF-γ), IL-2, IL-6, IL-10
- Support immunity against HSV, Epstein-Barr and Herpes virus 6 (HHV-6)

Ulfman LH, et al. Effects of bovine immunoglobulins on immune function allergy and infection. Front Nutr. 2018;5:52.

Bovine Colostrum Effects in Human GUT



Waldeyers ring
Respiratory virus exclusion
Allergen exclusion
Immune modulation (Treg, IgA)

PP
Immune exclusion of pathogens
Immune modulation (Treg, IgA)
Oral tolerance induction

GI lumen/epithelium
Prevent LPS leakage
Immune exclusion
Microbiota composition

Ulfman LH, et al. Effects of bovine immunoglobulins on immune function allergy and infection. Front Nutr. 2018;5:52.

Bovine Colostrum Study

- 2017 double blind placebo-controlled study n=16 athletes
- 20 days of colostrum, 500mg daily or placebo
- Gut permeability - determined by differential absorption of lactulose and mannitol + zonulin levels
- RESULTS:
  - Colostrum decreased intestinal permeability
  - Decreased zonulin production

Halasa M, et al. Oral supplementation with bovine colostrum decreases intestinal permeability and stool concentrations of zonulin in athletes. Nutrients. 2017. 9(4):pii:E370.

Bovine Colostrum Dosage

- Oral
  - 2.5 gm daily
  - Contains IgG 40%

Olive Leaf

- Olea europaea
- Widely used in traditional herbal medicine especially in Mediterranean countries
- Uses:
  - Immuno-modulating
  - Blood glucose and insulin regulation
  - Hyperlipidemia - CVDs
- Polyphenols – oleuropein

Magrone T, et al. Olive leaf extracts act as modulators of the human immune response. Endocr Metab Immune Disord Drug Targets. 2018;18(1):85-92.

Olive Leaf

- Antioxidant/anti-inflammatory
- Reported to increase IFN-γ production
- Improves #s of CD8+ and NK cells
- Increases NO
- T regulatory cell balance – Th17
- Good for intestinal inflammation

Magrone T, et al. Olive leaf extracts act as modulators of the human immune response. Endocr Metab Immune Disord Drug Targets. 2018;18(1):85-92.

Olive Leaf

- Dosage = 400mg 1-3 times a day std. to 20% oleuropein

- Can also add synergistic supplements such as
  - Arabinogalactan – Larch tree polysaccharides
  - Aloe vera leaf gel polysaccharides

How Can The Pharmacist Assess Nutritional Status in Patients?

- Unique/sophisticated health algorithms available with personalized reporting
- Uses health questions, biometrics, DIND info along with laboratory data to assess the health status of patients
- Targeted recommendations – dietary supplements, diet and exercise
- Systems biology approach to medicine – body systems communicate and interact with each other; when this is blocked or altered, metabolic disruptions can occur that lead to chronic health conditions

Initial Lab Testing

- Biometrics
  - BP, UpH, BMI, % body fat
- CBC and CMP
  - Includes MEB %
- Fasting glucose
- Hemoglobin A1c
- Insulin
- Thyroid panel – TPO/ThyAb, free T3, free T4, TSH, rT3
- Homocysteine
- Hs-CRP
- Estradiol, estrone, progesterone
- Stress/Cortisol urinary and serum
- DHEA
- Comprehensive Vitamin D Test
- Testosterone – free and total
- CRP
- Vitamin B12
- RBC Magnesium
- Iron/Ferritin/RDW/% sat/TIBC
- PSA and %free PSA
- NMR lipoprofile

Initial Lab Testing – Add-Ons

- 8-OHdG
- F2 isoprostanes
- Glutathione – total and reduced
- Adiponectin/Leptin
- TNF alpha / IL-6
- Lpa
- Lp-PLA2
- ApoB
- LDP-P
- oxLDL
- VEGF – vascular endothelial growth factor
- Vitamin B12
- MMA – methylmalonic acid
- MSH – melanocyte stimulating hormone
- MMP9 – matrix metalloproteinase-9
- MPO – Myeloperoxidase
- RBC Zinc
- Zonulin/histamine/LPS

Urinary and Saliva pH

- pH critical in determining biochemical balance
- Optimal pH salivary = 7 - 7.2

(trending low 6.1-6.9, trending hi 7.3-7.8)

- Optimal pH urinary = 6.5-7 (trending low 6-6.49, trending hi 7.1-7.2)
- The more acidic (lower pH) = more inflammation
- More lactic acid produced at lower pH
- Mitochondria less efficient

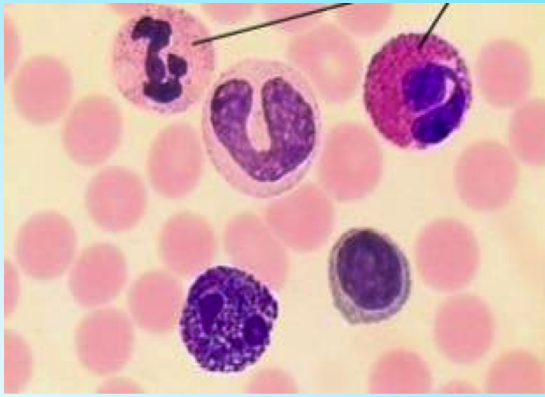
pH

- A trending high or high pH means body too alkaline
  - Digestive issues (hypochlorhydria)
  - Detoxification and drainage problems (liver , lymph, kidney)
- Use digestive enzymes (with HCL if no problems with gastric pain), 2 tabs with each meal
- Probiotics, anti-candida (cat's claw + berberine)
- Kidney, lymph drainage support

MEBs - %

- Monocytes, basophils, eosinophils = MEB
- Part of white blood cells
- Immunity, GUT, food reactivity, inflammatory markers

Note - Intense Exercise Effects on CBC -WBC



- Impaired neutrophils
- Altered Lymphocytes
- MEB's tend to increase – eosinophils, basophils, monocytes
- Impaired CD3 CD4 CD8 and NK cells
- Increased ROS = oxidative stress
- Increased time to healing
- Increased rate of illness

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
CBC With Differential/Platelet					
WBC	5.7		x10E3/uL	4.0-10.5	01
RBC	5.27		x10E6/uL	4.10-5.60	01
Hemoglobin	15.4		g/dL	12.5-17.0	01
Hematocrit	44.1		%	36.0-50.0	01
MCV	84		fL	80-98	01
MCH	29.2		pg	27.0-34.0	01
MCHC	34.9		g/dL	32.0-36.0	01
RDW	13.7		%	11.7-15.0	01
Platelets	268		x10E3/uL	140-415	01
Neutrophils	47		%	40-74	01
Lymphs	46		%	14-46	01
Monocytes	6		%	4-13	01
Eos	1		%	0-7	01
Basos	0		%	0-3	01
Neutrophils (Absolute)	2.6		x10E3/uL	1.8-7.8	01
Lymphs (Absolute)	2.6		x10E3/uL	0.7-4.5	01
Monocytes(Absolute)	0.4		x10E3/uL	0.1-1.0	01
Eos (Absolute)	0.1		x10E3/uL	0.0-0.4	01
Baso (Absolute)	0.0		x10E3/uL	0.0-0.2	01
Immature Granulocytes	0		%	0-1	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	01

MEB ranges

Monocytes %

- Range = 3-12
- Alert low = < 3
- Trending low = 3-4.5
- OPTIMAL = 4.6-8
- Trending high = 8.1-12
- Alert high = >12

Basophils %

- < 1 optimal
- Trending high = 0.9-1

Eosinophils %

- <5 optimal
- Trending high = 3.5-5

Comprehensive Vitamin D Test

- Vitamin D range = 30-100
- Optimal vitamin D level = 50-90 ng/ml
- Trending low = 30-49.9
  - 5,000 IU daily
  - Recheck in 90 days
- Alert Low = <30
  - 5-10,000 IU daily
  - Recheck in 90 days
- Alert high = >100
  - Can lead to toxicity including calcium deposits in soft tissue

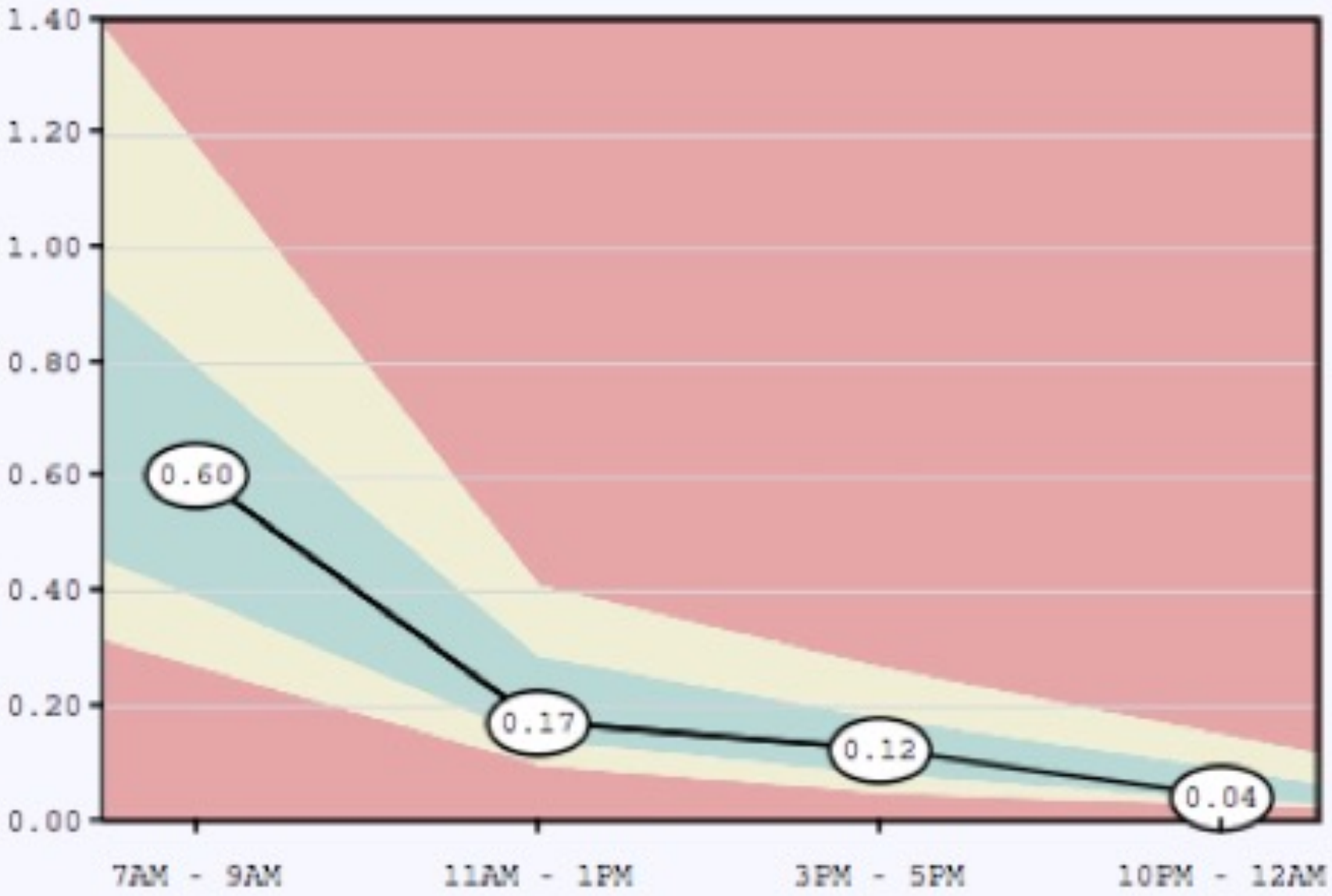
Cortisol Level: Triggers

- Wired and Tired
- Tired and flat
- Poor sleep
- Poor performance
- Weight gain around the abdomen
- Mind racing
- Immune problems
  - Allergies and Asthma
  - Inflamed Joints
  - Poor exercise recovery

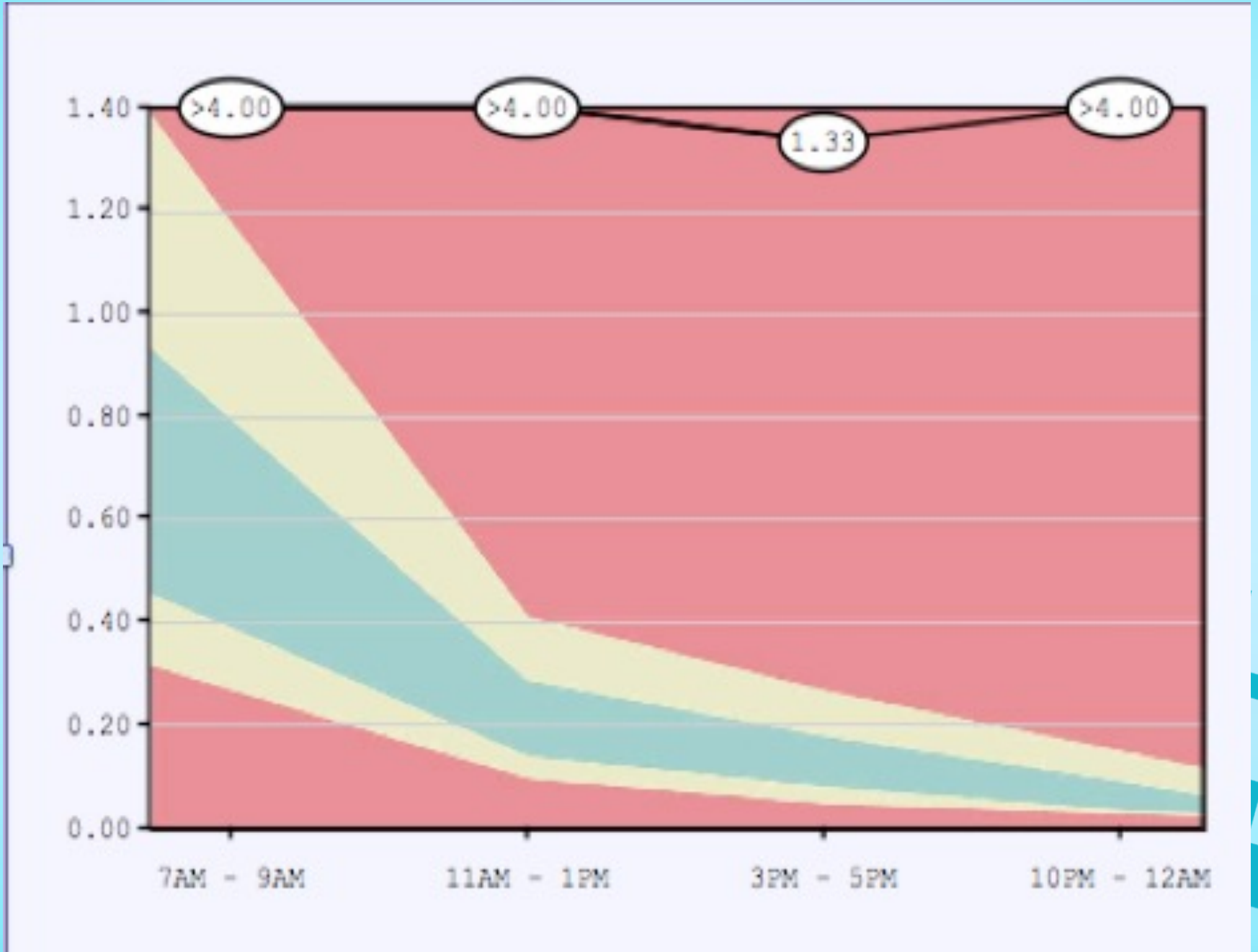
Serum Cortisol Ranges

- 8 am serum cortisol range 4 – 22 mcg/dL
  - Alert LOW = <4
  - Trending Low = 4-11.9
  - OPTIMAL = 12-17
  - Trending high = 17.1-22
  - Alert High = >22
- Trending or alert Low
    - Adrenal concentrate is no anxiety and fatigue
    - or Adrenal cortex if anxiety present
    - or Licorice if BP stable
- Trending or alert High
    - Adaptogens
    - Adaptogens + Magnolia/Phellodendron if cravings and feeling stressed
    - Adaptogens + Theanine combination if perseverating and no anxious
    - Adaptogens + theanine/kava if significant anxiousness bordering on panic

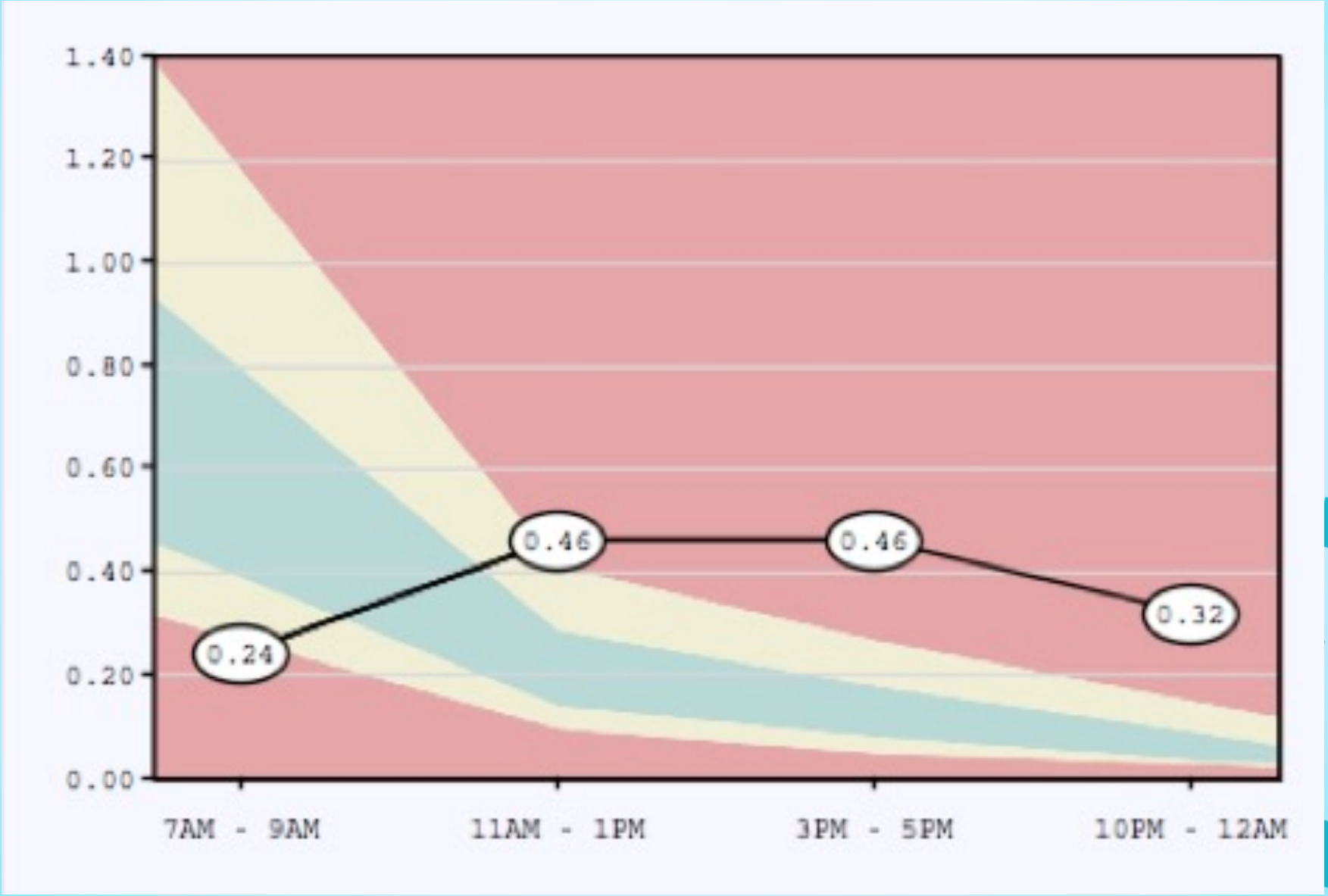
Cortisol – Normal Pattern



Cortisol - STRESS



Cortisol - STRESS



Cortisol - STRESS

GLUCOSE

- Fasting blood glucose and 2 hour post prandial (glucose tolerance test or GTT)
- Chronic stress, sleep disorders, environmental toxicity and microbiome disruption, nutrient deficiencies = lead to higher risk for developing insulin resistance and type 2 diabetes
- HbA1c, insulin, cystatin C, fructosamine
- Important in Immunity

GLUCOSE TARGET RANGES

- Fasting blood glucose
  - Alert Low = <65
  - Trending Low = 65-72
  - OPTIMAL = 73-89
  - Trending high = 90-99
  - Alert High = >99

- Postprandial
  - 1 hr Target = < 125
  - 2 hr target = < 95

Magnesium

- Magnesium is necessary for over 300 biochemical reactions
- Over 75% of Americans are Magnesium deficient
- Deficiency report in overweight and obese individuals
- Important in:
  - Muscle and bone health
  - Immune health
  - Sleep
  - Nervous system/Brain
  - Blood pressure and vasculature
  - Energy production
  - Blood glucose and insulin regulation

Huerta MG, Roemmich JN, Kington ML, et al. Magnesium deficiency is associated with insulin resistance in obese children. Diabetes Care. 2005;28(5):1175-81.

Magnesium

- Demands in serum go up 10 fold during training
- Less than adequate levels result in reduced performance
- Improves exercise via enhancing glucose availability in blood, brain and muscle

Char V, Nizamliogly M, Mogulkoc R, et al. Effects of magnesium supplementation on blood parameters at rest and after exercise. Biol Trace Elem Res. 2007;115(3):205-12.

Chen HY, Cheng FC, Pan HC, et al. Magnesium enhances exercise performance via increasing glucose availability in the blood, muscle and brain during exercise. PLoS One. 2014;9(1):e85486.

Magnesium

- Manage with 200-300 mg BID (elemental) magnesium glycinate, citrate, AA chelate, taurate
  - 7.5-10mg/kg elemental – mainly for building storage or athletes
- If sleep a problem, dose Mag 200-300 mg at bedtime
- Mag. Threonate – 2,000 mg 1-2 times daily , contains 144mg magnesium

RBC Magnesium

- Magnesium serum levels do not give accurate cellular levels
- Need to perform RBC magnesium level - amount of intracellular Mag
- Range RBC Mag: (4-6.4 mg/dL)
  - Alert Low = <4
  - Trending Low = 4.1-5.6
  - OPTIMAL = 5.7-6.2
  - Trending high = 6.3-6.4
  - Alert High = >6.4

Serum Magnesium

- Serum 1.5-2.5 mg/dL

- < 1.5 low
- 1.5-2.1 trending low
- 2.2-2.4 optimal
- 2.41-2.5 trending hi
- > 2.5

Zonulin Testing

- Zonulin family protein discovered in 2000 Univ. of Maryland
- Only known physiological modulators of the intercellular tight junctions
- Only human protein known to reversibly regulate intestinal permeability
- Generally, tightly controlled
- Innate defense mechanism against bacterial colonization of the small intestine
- Dysregulated by changes in microbiome composition and function
  - Antigen trafficking control is lost
  - Leads to loss of mucosal tolerance
  - Leaky GUT

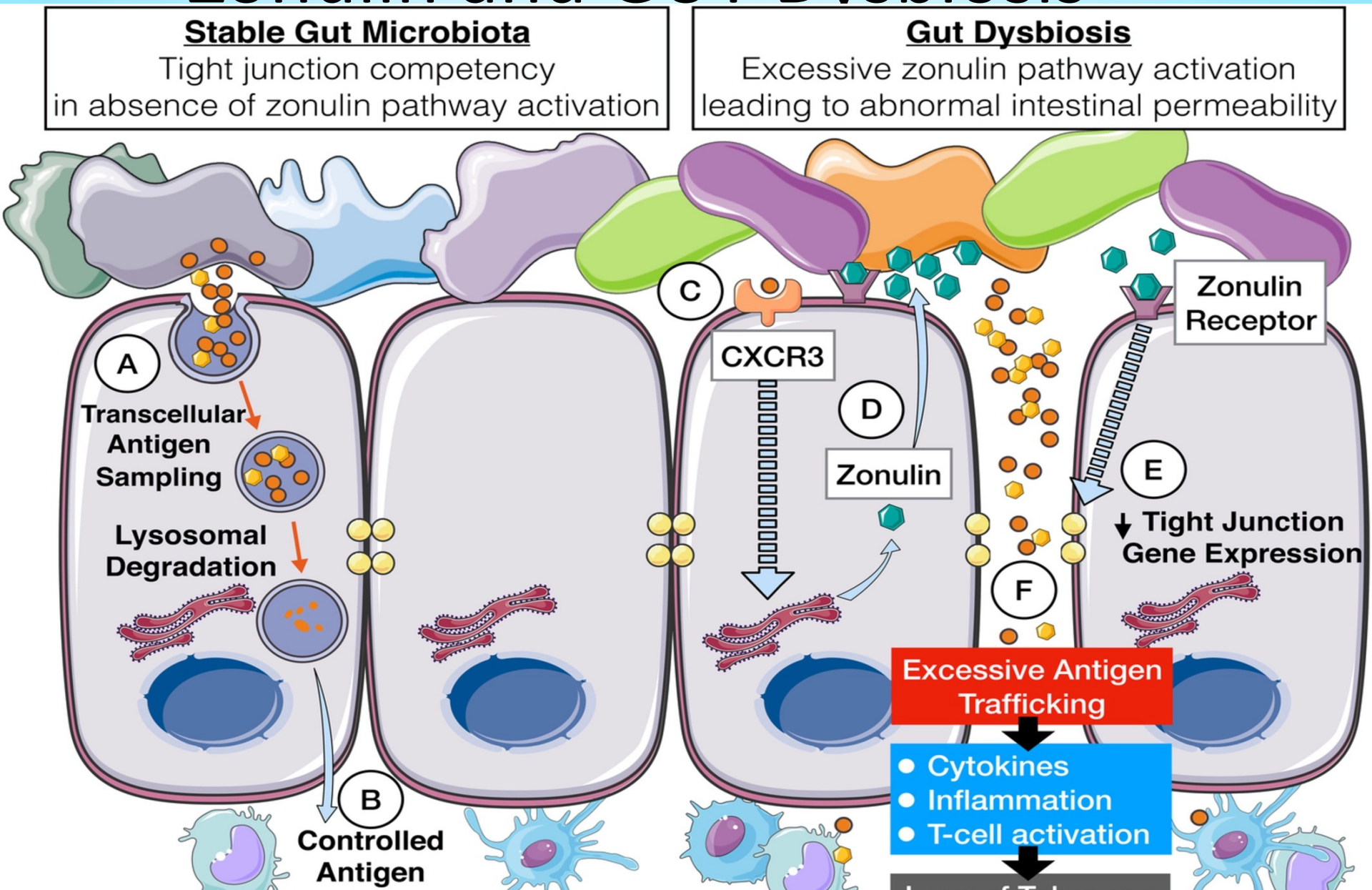
Heickman LKW, et al. Zonulin as a potential putative biomarker of risk for shared type 1 diabetes and celiac disease autoimmunity.

Zonulin

- Gliadin - glycoprotein from wheat
- Activates zonulin signaling via zonulin receptor-positive IEC6 and Caco2 cells
- Zonulin released in cell medium with subsequent zonulin binding to the cell surface
  - Engagement of the chemokine receptor CXCR3
  - Rearrangement of the cell cytoskeleton
  - Loss of occludin-ZO1 protein-protein interaction
  - Increased monolayer permeability
  - Increases immune/autoimmune consequences

Drago S, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 2006;41(4):408-19.

Zonulin and GUT Dysbiosis



Metainflammatory Conditions Associated w/ Increased Zonulin Levels – The Evidence

Chronic inflammatory diseases in which zonulin has been linked as a biomarker of gut permeability.

Disease	Model	References
Aging	Human	37, 38
Ankylosis spondylitis	Human	39
Attention deficit hyperactivity disorder	Human	40
Autism	Human	41, 42
Celiac disease	Human	15– 20, 23– 27, 43– 48
Chronic fatigue syndrome/myalgic encephalomyelitis	Human	49
Colitis – inflammatory bowel diseases	Human	50, 51
Colitis	Mouse	52
Environmental enteric dysfunction	Human	53
Gestational diabetes	Human	54, 55
Glioma	Human	56
Glioma	Cell	57
Insulin resistance	Human	58
Irritable bowel syndrome	Human	59, 60
Hyperlipidemia	Human	61
HIV	Human	62– 66

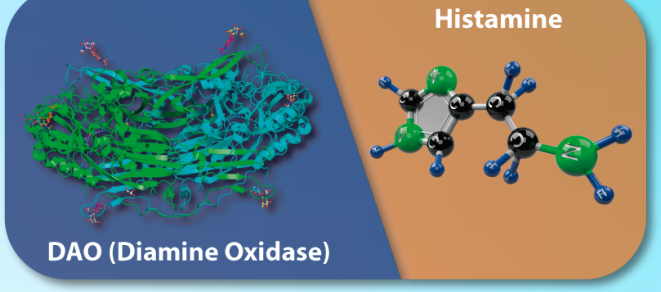
Fasano A. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F10000 Res. 2020;9:10.12688/f1000r

Zonulin

- High levels of zonulin indicative of leaky gut
- Recent study directly links increased zonulin w/ obesity, hypertension, Impaired fasting glucose and metabolic syndrome
  - Ohlsson B, et al. Higher Levels of Serum Zonulin May Rather Be Associated with Increased Risk of Obesity and Hyperlipidemia, Than with Gastrointestinal Symptoms or Disease Manifestations. Int J Mol Sci. 2017;18(3):582.
- < 34ng/ml optimal
- 30-34ng/ml Trending hi
- > 34ng/ml high

Drago S, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 2006;41(4):408-19.

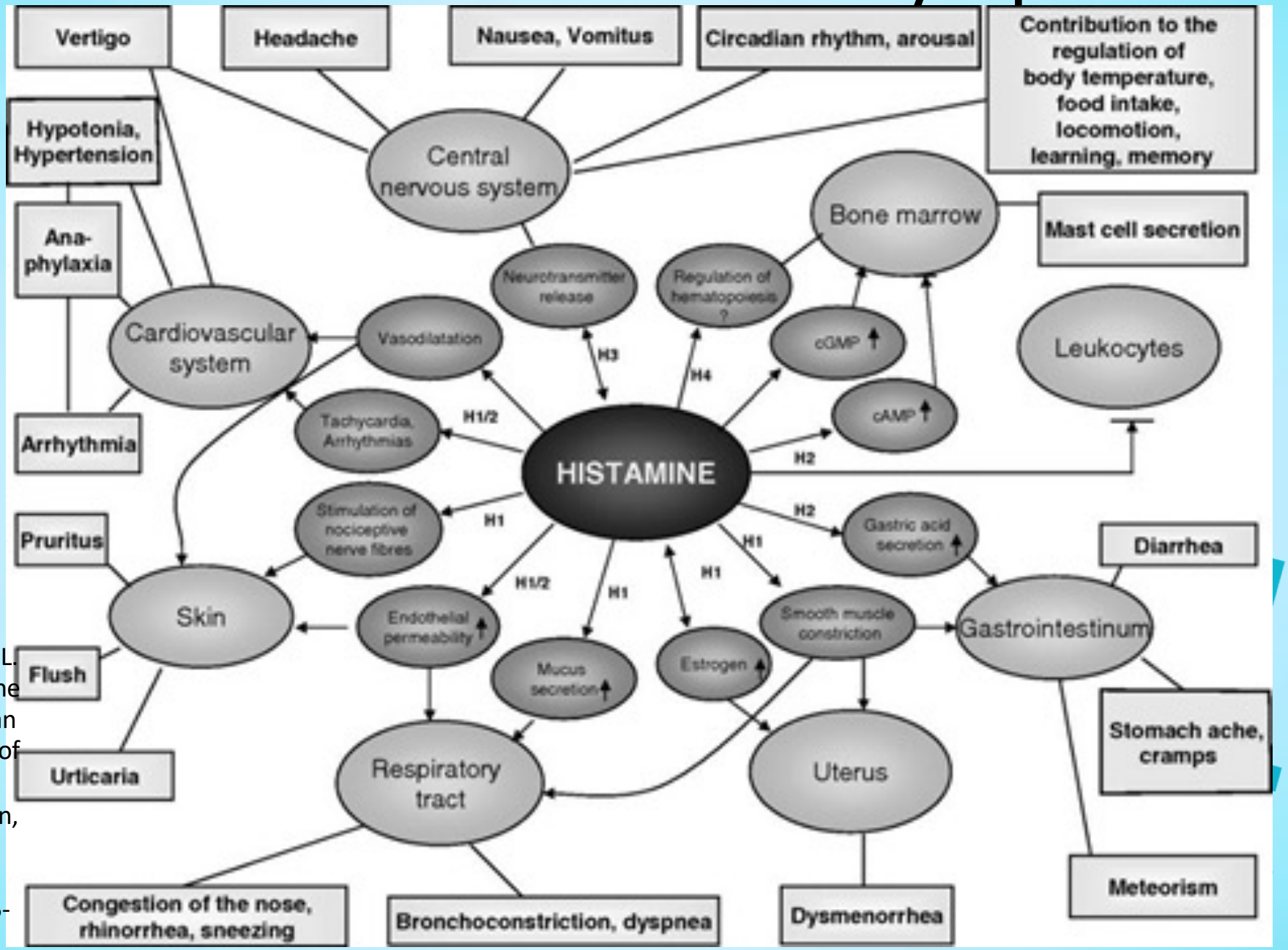
Histamine Testing



- Histamine is a biogenic amine that occurs in various degrees in many foods
  - Neurotransmitter
- Histamine Intolerance
  - Results from disequilibrium of accumulated histamine and the capacity for histamine degradation
- Causes:
  - Dysregulation of GUT microflora
  - Impaired degradation of orally supplied histamine due to diamine oxidase (DAO) deficiency – genetic or acquired
- Can lead to histamine toxicity

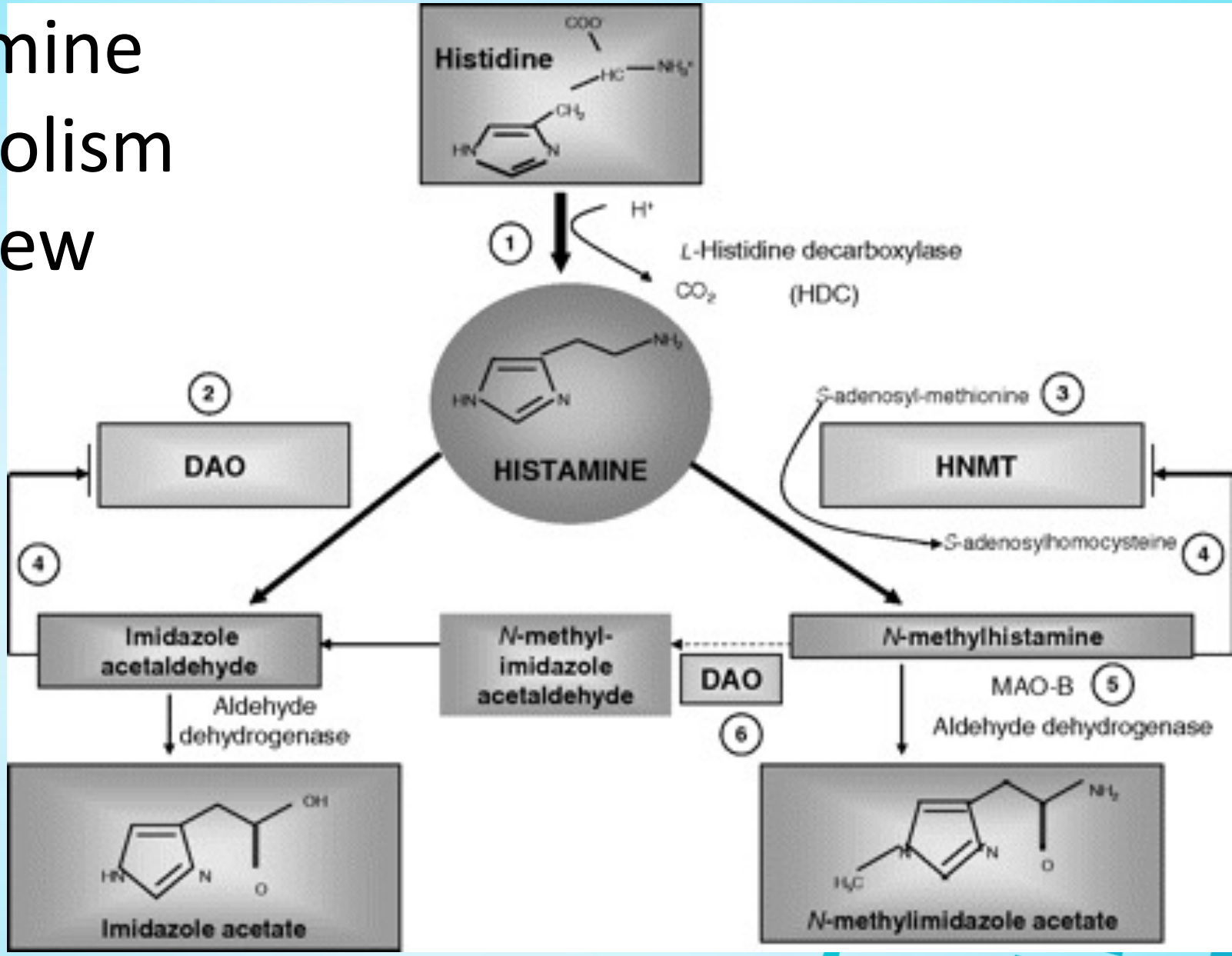
Maintz L, etla. Histamine and histamine intolerance. Am J Clin Nutr. 2007;85(5):1185-96

Histamine Intolerance Symptoms



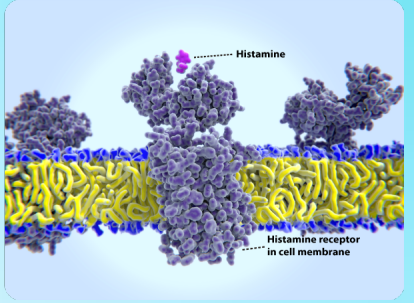
Maintz, L. 2007. The American Journal of Clinical Nutrition, [online] 85(5), pp.1185-1196.

Histamine Metabolism Review

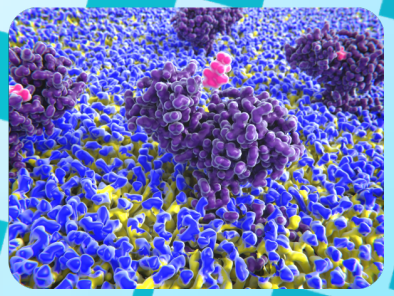


Maintz, L. and Novak, N., 2007. Histamine and histamine intolerance. The American Journal of Clinical Nutrition, [online] 85(5), pp.1185-1196.

Histamine Testing



- Histamine intolerance symptoms include:
  - Diarrhea, **headache (migraine)**, rhinoconjunctival symptoms, asthma, hypotension, arrhythmia, urticaria, pruritus, flushing, digestive issues, fatigue, SNS dominance
- Symptoms reduced by histamine-free diet
- TEST DAO and histamine
- Plasma histamine range - $\leq$ 1.8 ng/ml



Maintz L, etla. Histamine and histamine intolerance. Am J Clin Nutr. 2007;85(5):1185-96.

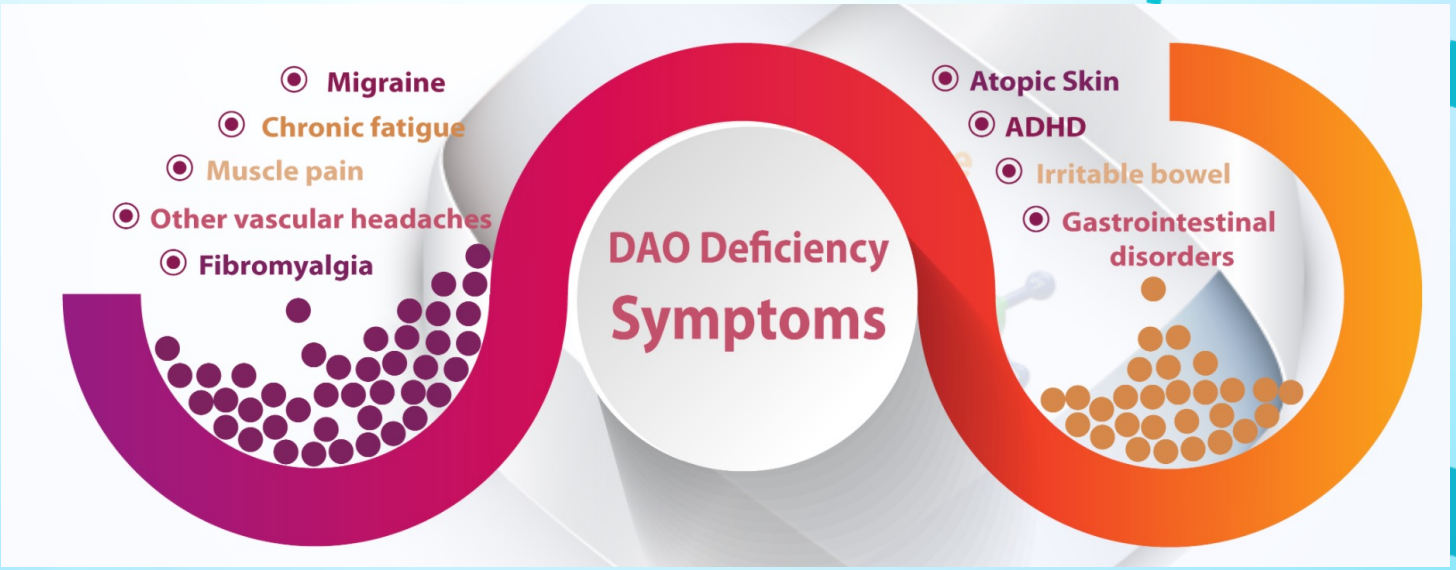
Histamine Testing

- Urinary histamine range – varies w/ sex, age

Sex	Age	
Both	0-99 years	16-53 mcg/24Hrs
Both	13-16 years	14-44 mcg/g creatinine
Female	0-3 years	18-60 mcg/g creatinine
Female	4-12 years	14-51 mcg/g creatinine
Female	13-16 years	14-44 mcg/g creatinine
Female	17-99 years	14-44 mcg/g creatinine
Male	0-3 years	18-60 mcg/g creatinine
Male	4-12 years	14-51 mcg/g creatinine
Male	13-16 years	14-44 mcg/g creatinine
Male	17-99 years	12-30 mcg/g creatinine

Foods Reported to Block DAO Enzyme

- Alcohol – aggressively attacks DAO
- Black tea
- Energy drinks
- Mate tea



Support for Histamine Symptoms

- Consider these supplements that have been reported to assist in histamine breakdown and decrease degranulation of mast cells:
  - Vit C – ascorbic acid 500mg – 2gm daily
  - B6 – pyridoxine-5-phosphate 25-50mg daily
  - Zn chelate – 30-50mg daily
  - Cu chelate – 1-2mg daily
  - Magnesium bisglycinate chelate – 400-800mg daily
  - Mangosteen – SE Asian antioxidant fruit; 250mg BID std. 95% flavonoids and 40% mangostins
  - Quercetin – 500mg BID
  - Stinging nettle leaf (Urtica dioica) – antihistaminic; 500mg 3-4 times daily; freeze dried preferred
  - Tinospora (*Tinospora cordifolia*) – mast cell protection; 20% polysaccharide 450mg BID

Lab Values

- Histamine Lab test
  - < 2.0ng/ml optimal
  - 1-2ng/ml trending high
  - > 2ng/ml HIgh