Metagenics Institute

Personalizing Cognitive Health for Optimal Outcomes

University of Miami's 7th Annual Integrative Medicine Conference

Pre-Conference Session

April 26, 2018

Miami, FL

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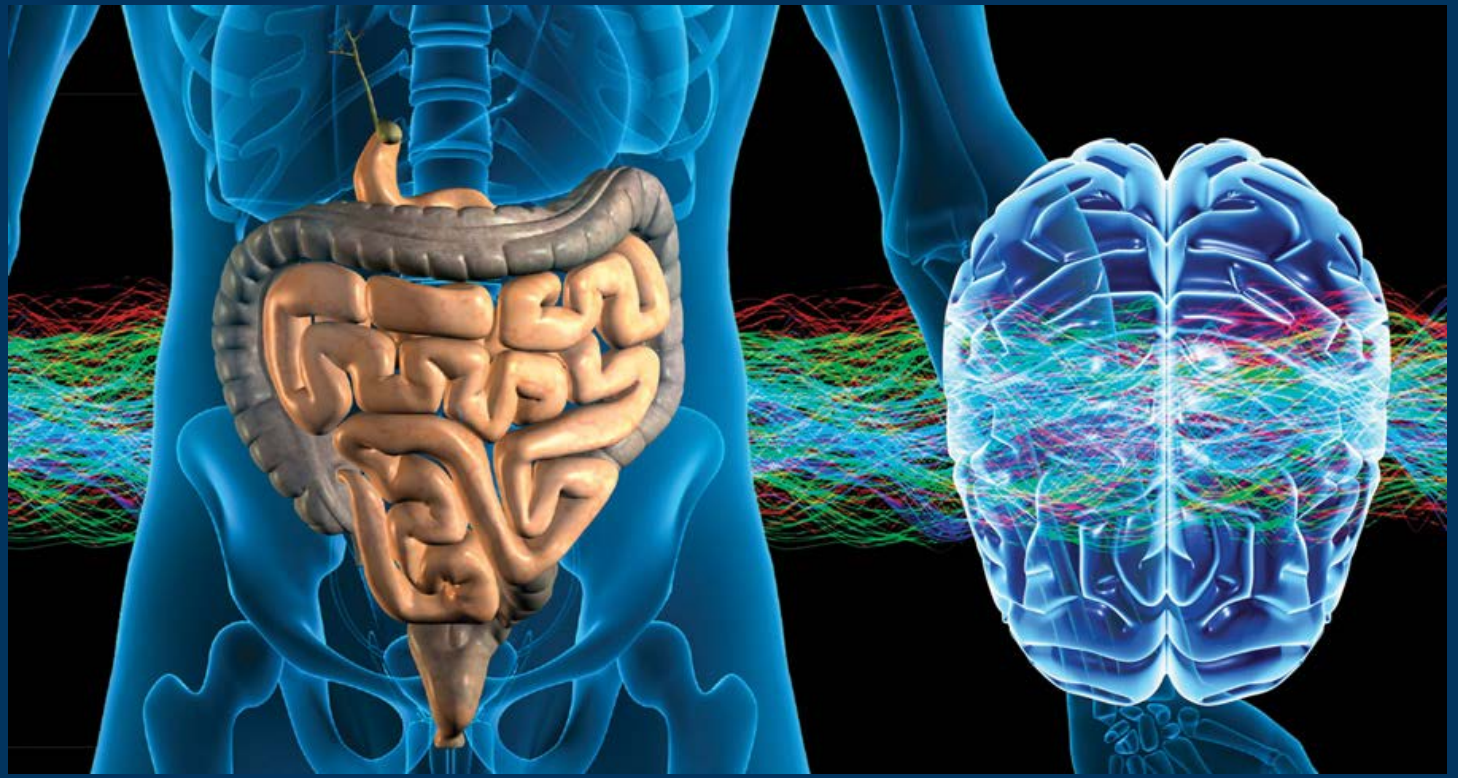
The Gut Brain Axis



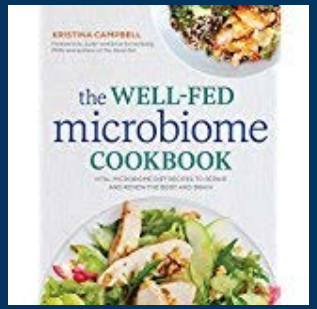
Robert Martindale MD, PhD

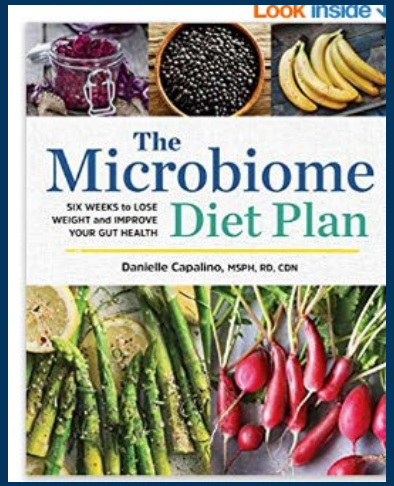
Chief, Division of Gastrointestinal and General Surgery

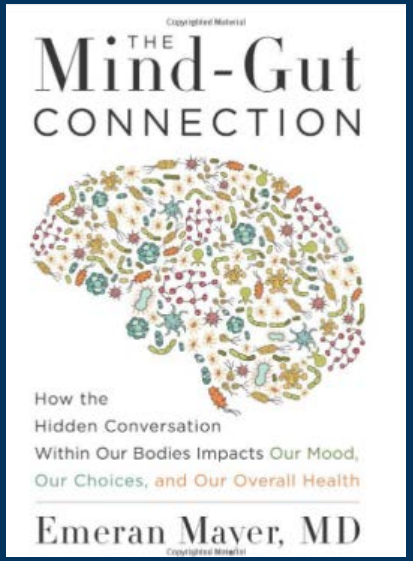
Oregon Health and Science University

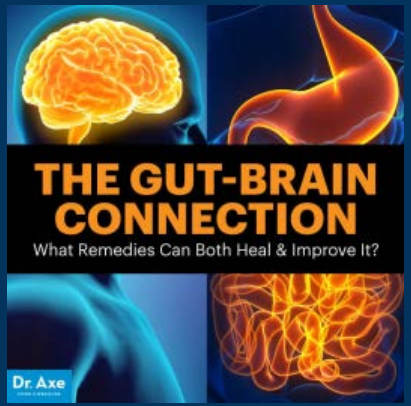
Portland Oregon

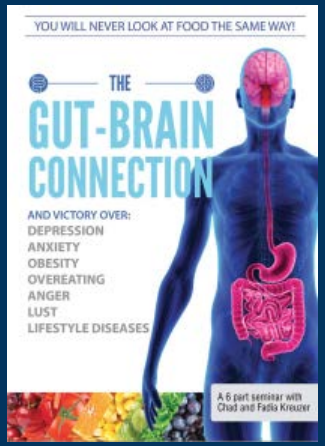
The Lay Press is Becoming Overwhelming

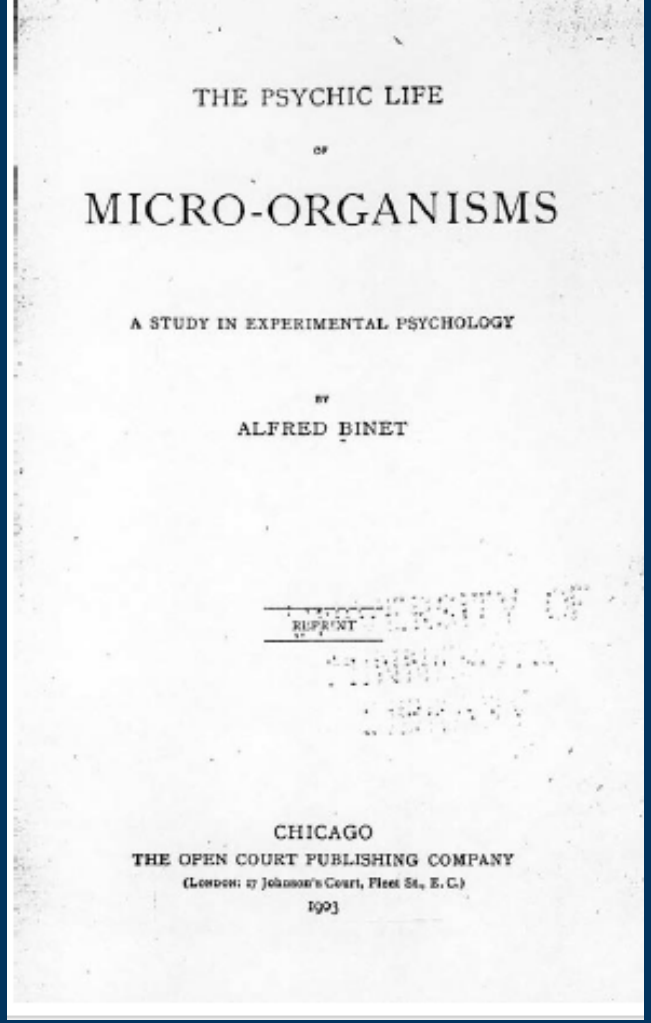












THE PSYCHIC LIFE

OF

MICRO-ORGANISMS

A STUDY IN EXPERIMENTAL PSYCHOLOGY

BY

ALFRED BINET

REPRINT

CHICAGO

THE OPEN COURT PUBLISHING COMPANY

(London: 17 Johnson's Court, Fleet St., E. C.)

1903

- **The Gut Brain connection is not new !**

- From 1914: *"The control of man's diet is readily accomplished, but mastery over his intestinal bacterial flora is not... They are the cases that present...malaise, total lack of ambition so that every effort in life is a burden, mental depression often bordering upon melancholia…A battle royal must be fought and when this first great struggle ends in victory for the Bacillus bulgaricus it must be kept on the field of battle forever at guard..."*
  - *Stow, Medical Record Journal of Medicine and Surgery, 1914*

Just as gut bacteria affect the brain, the brain can also exert profound influences on the gut microbiome—with feedback effects on behavior. Numerous studies, for example, have shown that psychological

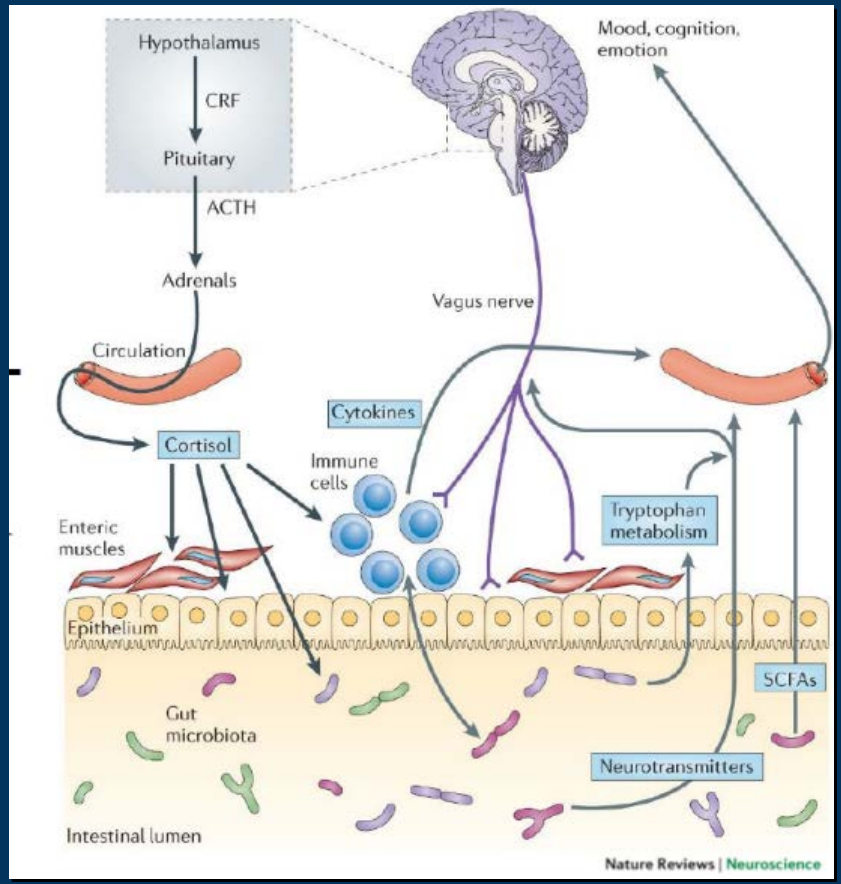
stress suppresses beneficial bacteria.

Statement from the American Psychological Association 2012

"that gut feeling"

Gut Brain Axis:
Which of these two is really in control?

Microbiome and Brain Function
"Gut-Microbiota-Brain Axis"



Recently shown to alter:

- Behavior
  - Anxiety, depression
  - Learning, memory
- Neurogenesis
- Neuroplasticity
- Microglial activity
- BBB integrity
- AD, Parkinson's

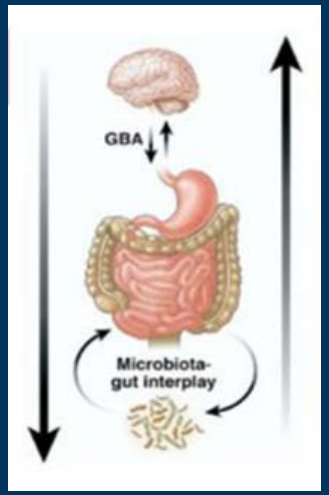
Human data for:

- Anxiety / stress
- Depression
- OCD / ADHD
- Others

Cryan FJ et al Nature Rev Neuroscience 2012
Minter MR et al Sci Rep 2016
Ho P 2017

Bidirectional involvement

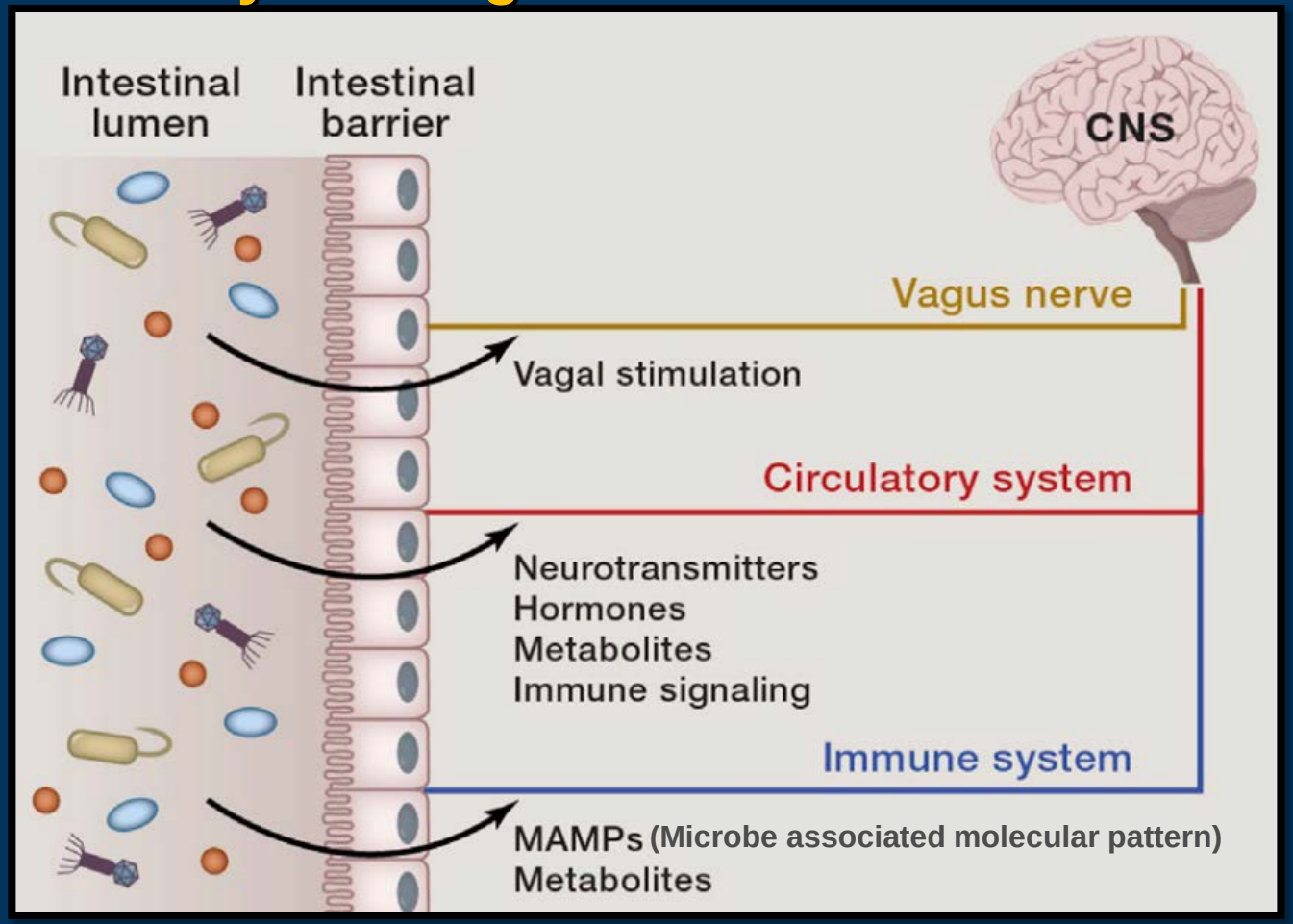
- From Gut to Brain
  - Neurotransmitters
    - GABA, Serotonin, BDNF
  - Protection of intestinal barrier
  - Modulation of sensory afferents
  - Bacterial metabolites
  - Mucosal immune regulation



- From Brain to Gut
  - Alteration of:
    - Mucus production
    - Motility
    - Permeability
    - Immune function

Carabotti M et al. *Ann Gastroenterology*. 2015

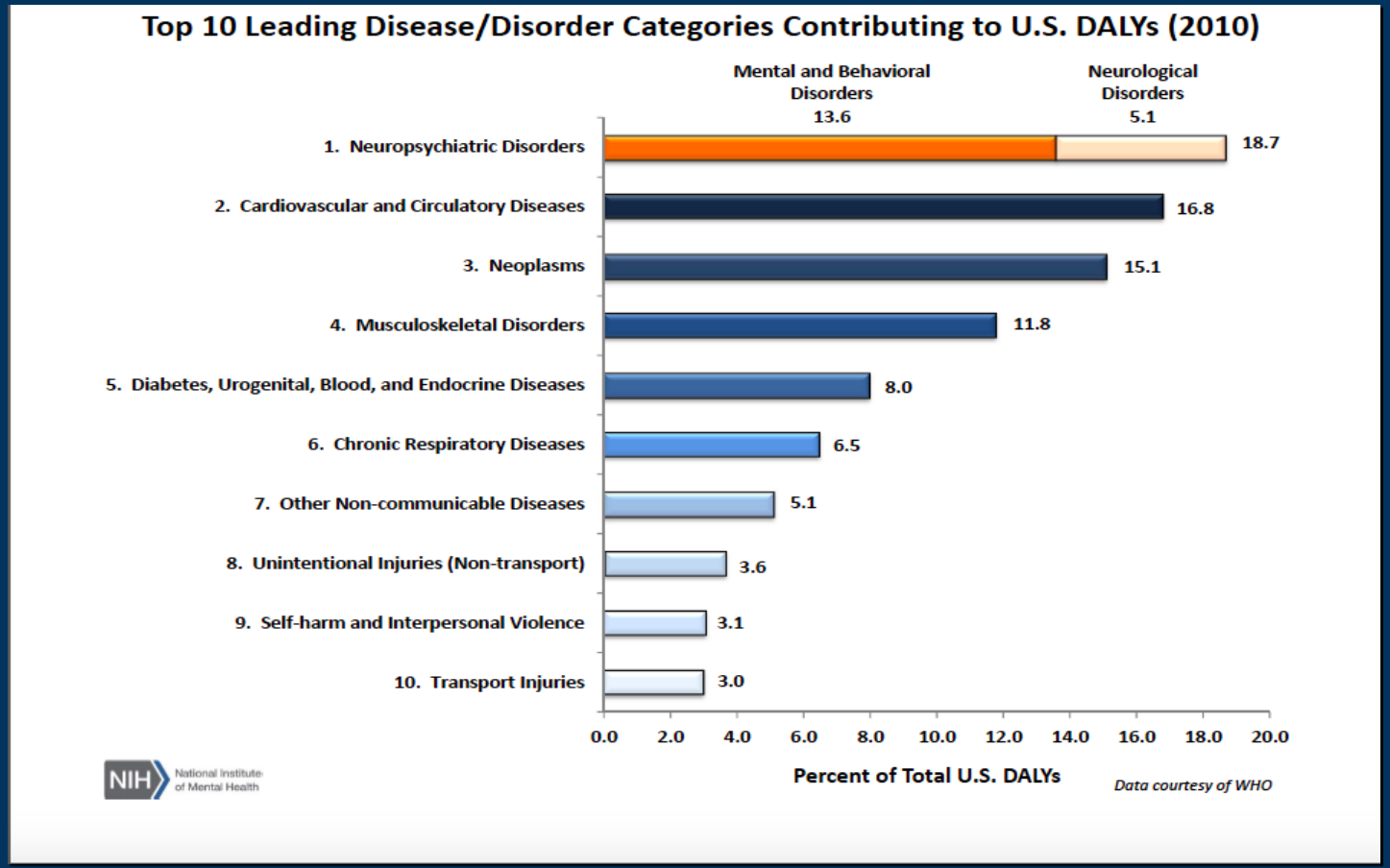
Pathways linking the microbiome and CNS



Sampson TR, Mazmanian SK. *Cell Host & Microbe*. 2015

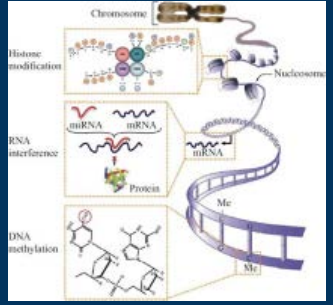
What are the connections ?

- Gut portion of Brain Gut Axis is controlled by the microbiome but mediated by:
-
  - Cytokines
  - Neurotransmitters
  - Endorphins
  - Endocannabinoids
  - Metabolomic changes



DALY- Disability-adjusted life year: measure of overall disease burden.
Expressed as # of yrs lost due to ill health disability or early death
DALY=YLL + YLD

Why has epigenetics and metabolomics exploded into modern medicine?



- Helps explain relationship between individual genotype and the environment during all stages of life
- Epigenetic mechanisms integrate environmental changes at the cellular level enabling the cellular plasticity
  - Methylation, acetylation, phosphorylation, biotinylation
- Epigenetic alteration can lead to serious acute and chronic health issues

Reprogramming of the Epigenome by Gut Microbiota Metabolome

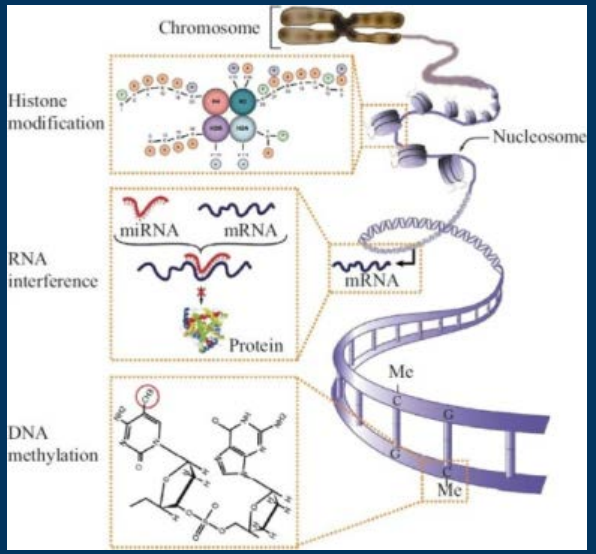
Chromatin undergoes sequence-independent epigenetic modifications like DNA methylation, histone acetylation, phosphorylation, biotinylation, and RNA interference:

- DNA methylation associated with the suppression of gene transcription
- Histone methylation mediate either transcription activation or transcription repression, depending on which amino acid residue of histone is methylated
- Acetylation and phosphorylation of histones typically enhance gene expression
- Biotinylation usually represses gene expression
- Micro RNA, via RNA interference, suppresses the expression of epigenetic-associated and other genes, either by binding directly with the respective messenger RNA (mRNA) sequences of the genes or indirectly by binding with different histone modifiers.

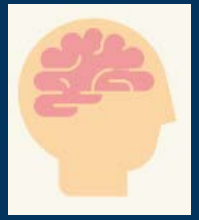
The Microbiome Produces Potent Epigenetic Modulators: Diet Dependent Low Molecular Weight Microbial Metabolites

- **Metabolites generated in gut:**
  - Vitamins B and K
  - SCFA
    - butyrate, proprionate, acetate, lactate, succinate
  - Polyamines
  - Polyphenols
  - Non-absorbed / digested CHO

- **Epigenetic modifications**
  - Acetylation
  - Methylation
  - Phosphorylation
  - Biotinylation



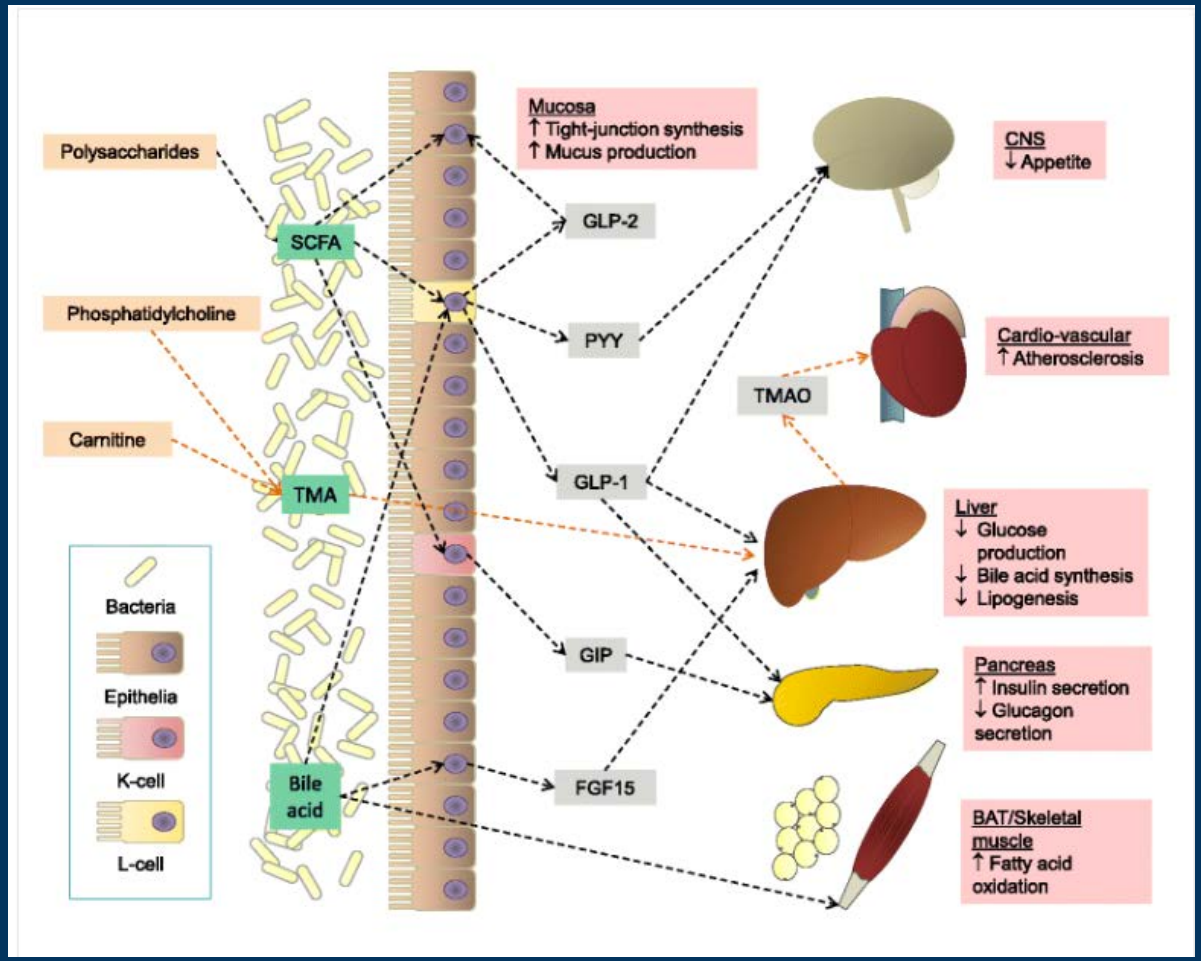
Gut Microbiota Communication with Other Organs



Organ	Process influences by gut microbiota	Disease associated with dysbiosis/microbial metabolites
Adipose tissue	Adipocyte volume Thermogenesis Browning Inflammation	Obesity/insulin resistance Insulin resistance
Liver	Bile acid metabolism Lipogenesis Energy expenditure	NAFLD/NASH
Pancreas	Insulin secretion	Type 2 diabetes
Whole body	Body growth	Undernourishment
Cardiovascular system		Stroke Atherosclerosis Thrombosis
Brain	Behavior Serotonin metabolism Intestinal gluconeogenesis Blood–brain barrier Appetite regulation	Autism spectrum disorder Stress response Metabolic disease
Lung	Gene expression	Allergic asthma

Schroeder BO, Backhed F. *Nature Medicine.* 2016

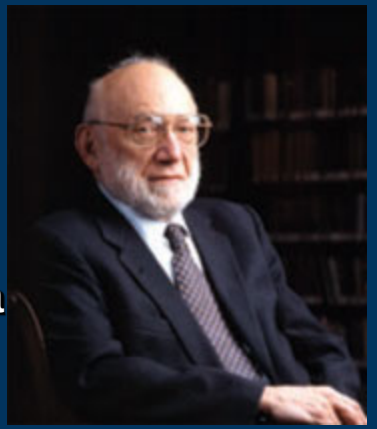
Gut Microbiota Metabolome – Host Interactions



Hansen. *Genome Medicine.* 2015;7:33

Human Microbiome

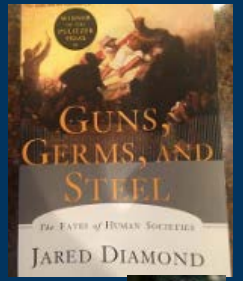
- Term suggested by Nobel Prize Winner Dr. Joshua Lederberg
- Describe the collective genome of our indigenous microbes (microflora), the idea that a comprehensive genetic view of *homo sapiens* as a life form should include the genes of our microbiome
- Microbiome = Microbiota
- Includes bacteria, fungi, archaea



Joshua Lederberg, PhD
1925-2008

99% of our total
genome is absent at birth

Why care about gut bacteria or the metabolic products ?



All life has evolved in presence of bacteria

As humans, they (bacteria) surround us and we surround them !

Our immune system reacts to bacterial presence.

3 to 6 pounds of bacteria is on or ”in” us

Bacteria produce numerous beneficial metabolites and peptides

Trophic

- Control of epithelial cell growth and differentiation
- Promote intestinal angiogenesis
- Development and homoeostasis of the immune system

Protective

- Protection against pathogens

Metabolic

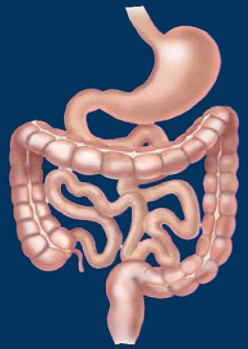
- Fermentation for SCFA
- Stimulates mucus
- Production of vitamin K
- Some AA, Neurotransmitters
- Xenobiotic metabolism
- Distant organ signaling

What have we learned from germ-free mice…

- When the microbiota is absent:
  - Altered sociability, decreased memory, and increase responses
- Bacteria produce neurotransmitters
  - norepinephrine, serotonin, dopamine,
- Germ free mice
    - More ACTH and corticosterone in response to stress
- Certain probiotic bacteria modulate the effects of neurotransmitters
  - Specific strains of *Lactobacillus rhamnosus* modulate stress mediated through the vagus nerve in mice
- Mucosa border maintenance

Cryan JF, Dinan T. *Nat Rev Neurosci.* 2012
Sharon G et al. *Cell.* 2016

Loss of Gut Mucosal Integrity
"Leaky Gut"



- Is "Leaky Gut Syndrome" real ?
  - Data supporting
  - Data refuting
- Gut permeability
- What are the proposed mechanisms ?

- It is time to start treatment ?
  - Who needs treatment ?
  - What is the treatment ?

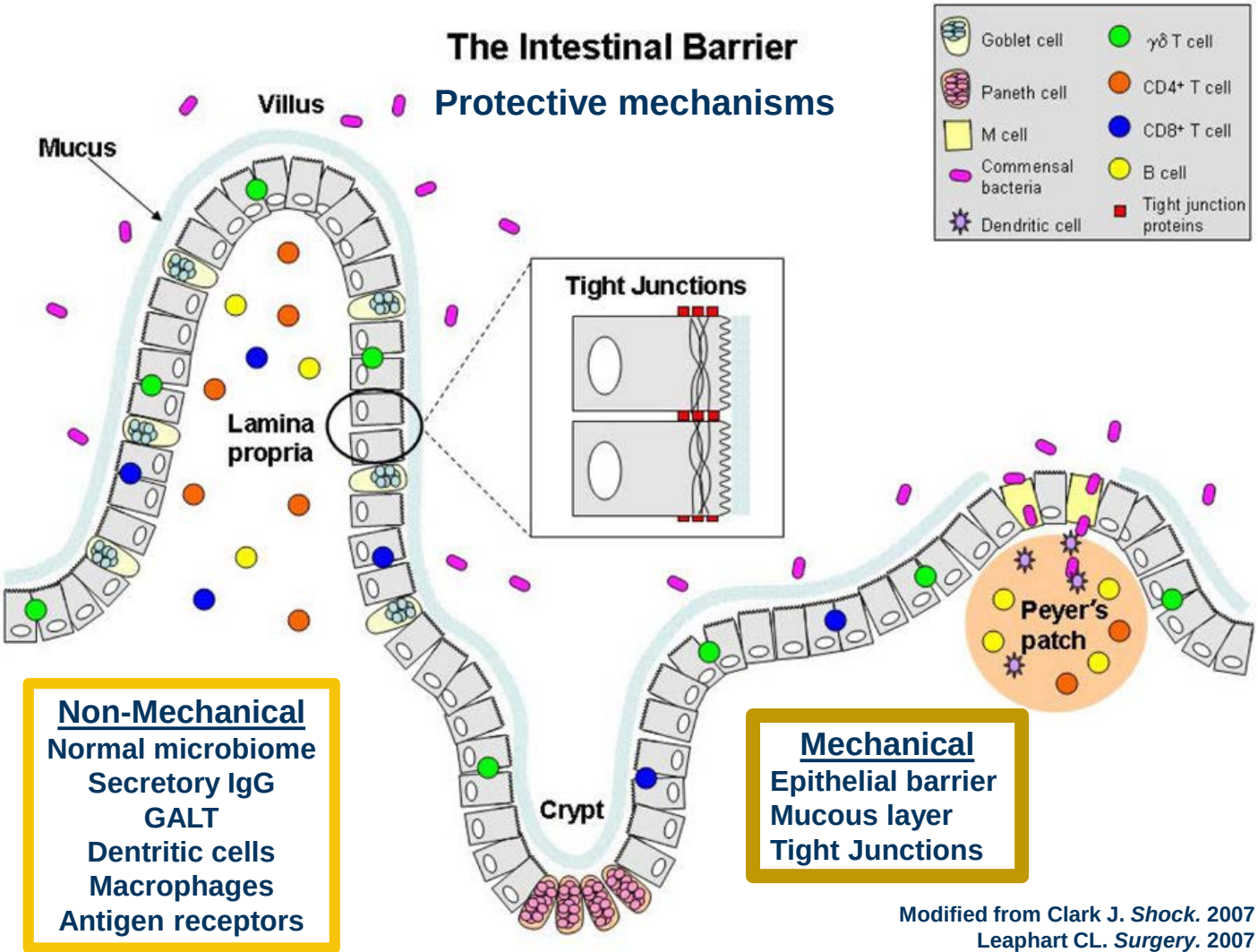
Odenwald MA et al. *Clinical GI and Hepatology*. 2013

Actions at the mucosal border: The Critical Balance !

Fishman JE et al. *Ann Surg.* 2014

The Intestinal Barrier

Protective mechanisms



Non-Mechanical

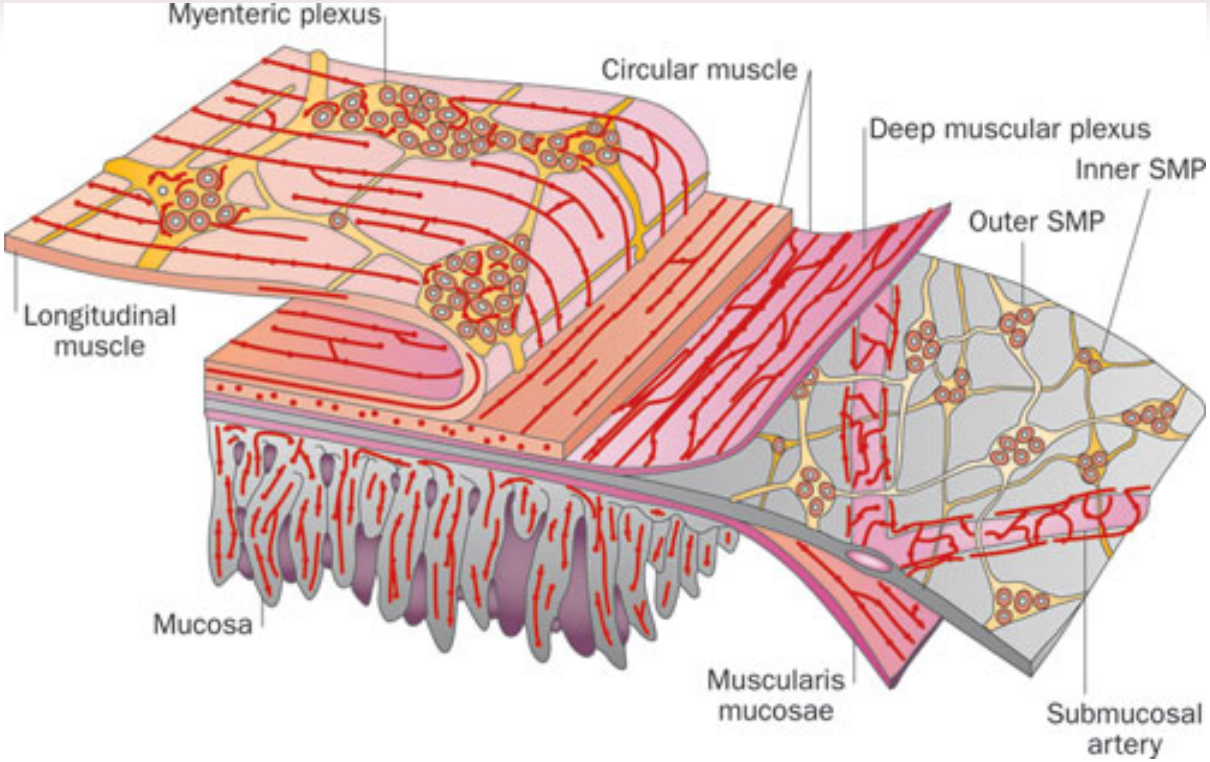
- Normal microbiome
- Secretory IgG
- GALT
- Dentritic cells
- Macrophages
- Antigen receptors

Mechanical

- Epithelial barrier
- Mucous layer
- Tight Junctions

Modified from Clark J. *Shock.* 2007
Leaphart CL. *Surgery.* 2007

The organization of the ENS of human



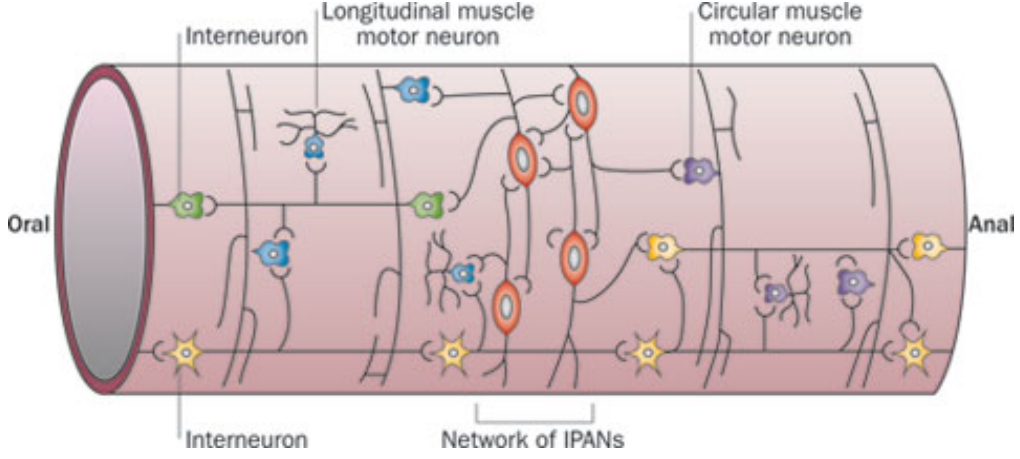
Innervation of the GALT and gut endocrine cells not illustrated here

Furness JB.The enteric nervous system and neurogastroenterology.
Nat. Rev. Gastroenterol. Hepatol. 2012.

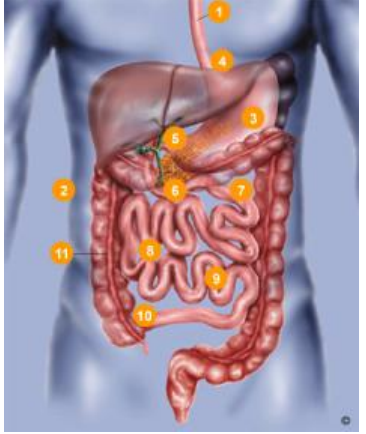
nature REVIEWS GASTROENTEROLOGY & HEPATOLOGY

Enteric Nervous System

- **Estimated 400-600 million enteric neurons**
  - **> total of all sympathetic and parasympathetic ganglia combined. ENS is almost equal to the number of neurons in the spinal cord**
- **Internal system which can function autonomously**
  - **Chemoreceptors**
  - **Baroreceptors**
  - **Subject to outside regulation under normal conditions (integration)**



Classification of Enteric Neuropathies



- **Congenital and developmental neuropathies**
  - **Ex: Hirschsprungs**
- **Sporadic and acquired neuropathies**
  - **Ex: IBS**
- **Neuropathies secondary to or associated with other diseases**
  - **Ex: Parkinson's, Alzheimers, Autism, depression, anxiety**
- **Iatrogenic or drug-induced neuropathies**
  - **Ex: ICU dysmotility**

Diseases “associated” with leaky gut

- **Strong data to support:**
  - IBD
  - IBS
  - Celiac
  - MOF
- **Organ system diseases with some supportive data**
  - DM1
  - GVHD
  - AIDS
  - Multiple sclerosis
  - Rheumatoid arthritis
  - Autism
  - Migraines
  - Food sensitivities
  - NASH (fatty liver)

- **Little objective data currently supports:**
  - Fibromyalgia
  - Depression
  - Allergies
  - Skin disorders

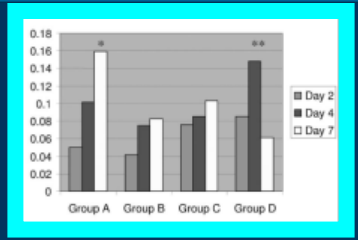
- **No objective data**
  - Weight gain
  - Chronic fatigue

Models evaluating barrier function

mostly rodent models

- Dextran sulfate
  - Epithelial injury
- IL-10 KO
- Immunodeficiency mouse models
- MLCK mouse model
  - Very promising – allows loss of barrier w/o damage
- Cytokine changes
  - TNF, IL-13
- Various infectious models
- Various inflammatory models
- Tight junction protein synthesis and redistribution
  - Claudin protein evaluations
- Expression of zona occuldens
- Electrical resistance (MAPK)
- Mucosal apoptosis

MLCK – Myosin light chain kinase



The beginnings of human data to show disease associated with loss of barrier dysfunction

MULTIPLE ORGAN FAILURE

B. Eiseman, M.D., F.A.C.S., R. Beart, M.D., *and* L. Norton, M.D., F.A.C.S.,
Denver, Colorado

Surg Gyn Obstet 1977

A New Syndrome

ICU Technology Allows Patients To Survive Single Organ Failure

Ben Eiseman

MULTIPLE ORGAN FAILURE

B. Eiseman, M.D., F.A.C.S., R. Beart, M.D., *and* L. Norton, M.D., F.A

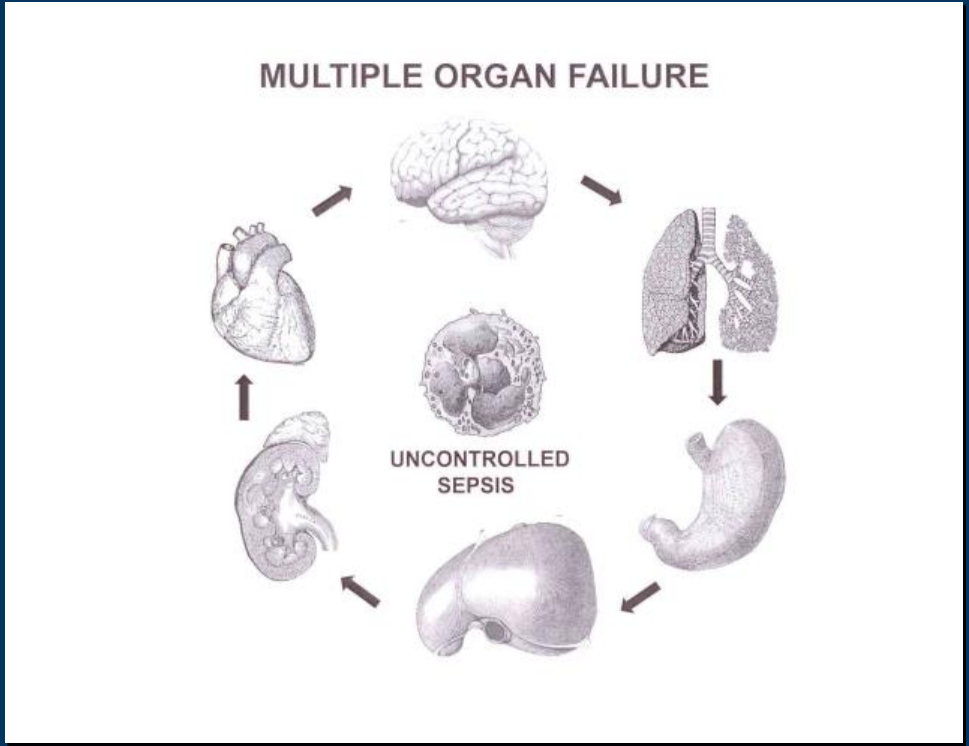
Denver, Colorado

Surg Gyn Obstet 1977

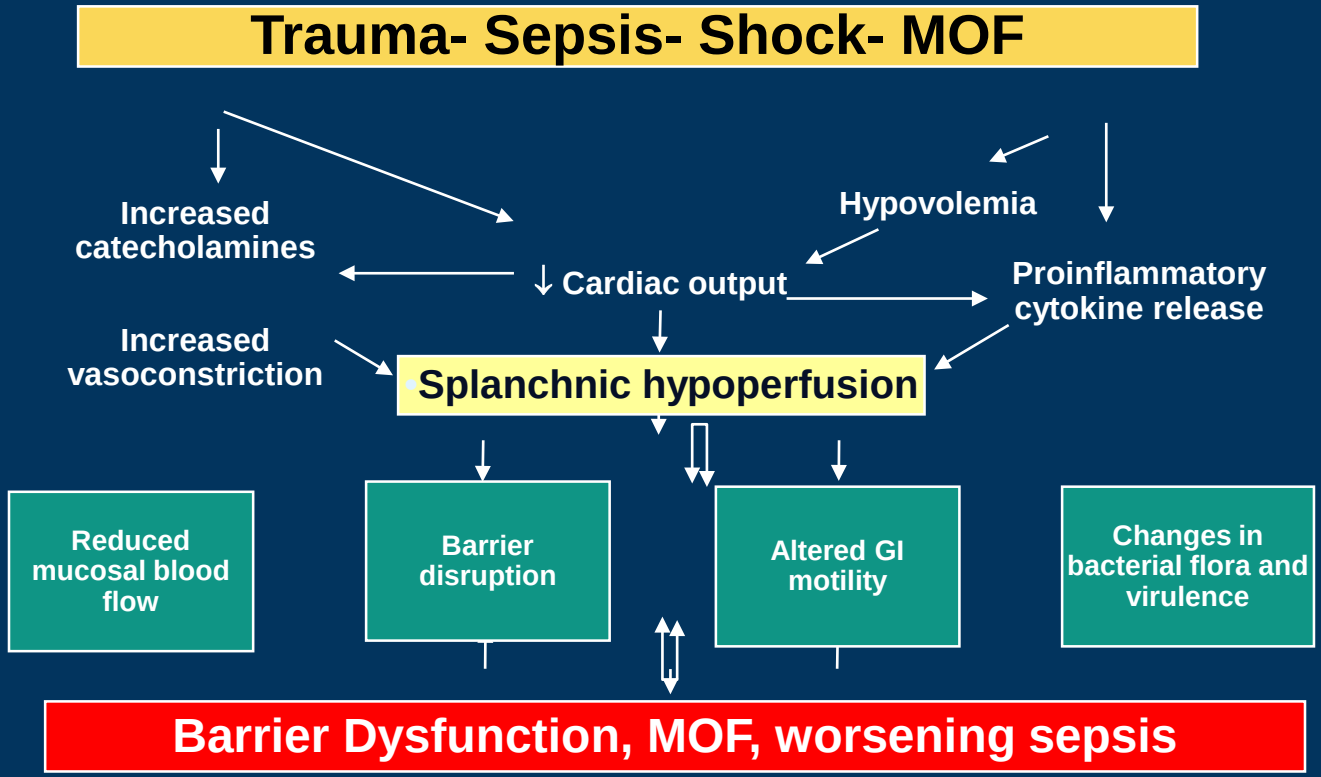
Infections felt to be the cause

Infectious etiology concept supported by key papers in 1970's Polk, Fry etc.

Research in the 70's focused on infectious etiology

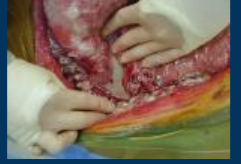


Pathophysiology of Splanchnic Hypo-perfusion



Martindale R et al CCM 2014, Schmidt H, Martindale R. *Curr Opin Nutr Metab Care*. 2003 Mutlu GM, et al. *Chest.* 2001

1970's > 50% of cases of MOF from intraabdominal infections



- By 1980's IAI showing better outcomes but MOF still occurring at the same rate as in the 70's ?
  - Better initial management of trauma and post op patients
  - More potent and appropriately dosed antibiotics
  - Earlier recognition of IAI with the use of CT
  - Interventional radiologic techniques allowing drainage of abscess without open surgery
- Series of papers from EU reporting MOF <u>without</u> infectious source

  - Faist- 1983 MOF in polytrauma
  - Nuytinck – 1987 "whole body inflammation in trauma…"
  - Waydhas – 1992 Inflammatory mediators infection, trauma, MOF
  - All supporting a convincing story that MOF in trauma often occurs without infectious etiology

Question 1980's: if not infection what was driving MOF ?

- Shock (septic, hemorrhagic, cardiogenic etc) seemed to be consistent with patients getting MOF
- Concept that low flow states and tissue ischemia / reperfusion is etiology becomes popular;
    - Giving rise to gut origin of sepsis (multiple authors)
        - » Gut as "Motor for Multiple Organ Failure"
    - "unrecognized flow-dependent oxygen consumption"
        - » Supranormal oxygen delivery (Shoemaker)
    - Supporting evidence at the time
        - – Animal models of bacterial translocation following trauma
        - – Selective gut decontamination in humans (+/-)
        - – Most patients dying with MOF with negative cultures
        - – Early enteral feeding showing benefit
            - » Primarily pneumonia outcome was decreased

Major research discoveries supporting hypothesis of gut as the "motor" for MOD

- **Moore et al**: shock and hypoperfusion allows gut release of proinflammatory cytokines increasing ARDS/Sepsis (1)
- **Fink et al**: epithelial tight junctions are compromised leading to increased permeability….inflammation (2)
- **Teixeira et al** : Germ free animal showing increased survival following I/R (4)
- **Clark et al** : epithelial apoptosis elevated in sepsis, prevented by over expression of anti-apoptotic protein Bcl-2 (6)
- **Deitch et al**: Toxin from gut damages lung via lymphatics (5)
- **Alverdy et al**: interaction between bacteria and host. Most patients dying of "MOF" have no + cultures (3)

1. Hassoun HT, Moore F et al. *Shock* 2001; 2. Fink MP et al. *Curr Opin CC* 2003; 3. Alverdy J. *CCM* 2003 and *J Leukocyte Biol* 2008; 4.Sousz DG et al. *CCM* 2003; 5. *Deitch Biosci* 2006; 6. Coopersmith CM et al. *JAMA* 2007

Temporal trends of postinjury multiple-organ failure: Still resource intensive, morbid, and lethal

Angela Sauaia, MD, PhD, Ernest E. Moore, MD, Jeffrey L. Johnson, MD, Theresa L. Chin, MD, Anirban Banerjee, PhD, Jason L. Sperry, MD, Ronald V. Maier, MD, and C. Cothren Burlew, MD

J Trauma Acute Care Surg 2014

- Data collection from 20 institutions 2003 to 2010
  - 1643 patients with MOF
- Strict criteria for sepsis / injury / MOF
- Results
  - MOF incidence decreased over time 17% 2003 to 9.8% in 2010
  - MOF death 33% 2003 to 36% 2010
    - » No change in ventilator days or length of stay in ICU
  - Most MOF death occurred within 2 days of MOF diagnosis
  - Lung dysfunction decrease 58 to 51%
  - Cardiac dysfunction decrease from 21 to 13 %
  - Renal and hepatic failure rates did not change
    - » Now shown to be most likely from gut failure leading to hepatic and renal failure

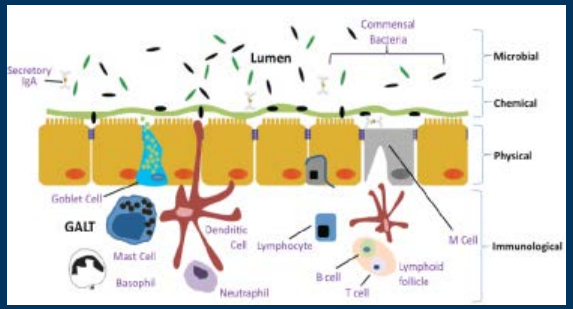
Sauaia A et al. *J Trauma and ACS.* 2014

Most of discussion on “leaky gut” or loss of mucosal integrity is not in the ICU.
What are symptoms for the “routine” leaky gut syndrome ?

- **Commonly reported:**
  - Bloating
  - Gas
  - Cramping
  - Food sensitivities
- **Less commonly reported:**
  - Asthma
  - Chronic joint and muscle pain
  - Recurrent vaginal infections
  - Constipation
  - Behavior changes
  - Anxiety
  - Depression
  - Autism

All seem vague and difficult to collect objective data

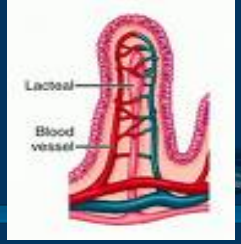
Treatment: Protecting mucosal barrier function



- Maintaining visceral blood flow
- Glycemic control
- Lower inflammatory stimuli
  - Dietary changes
  - Anti-inflammatory or agents to enhance resolution of inflammation
- Enteral feeding
- Minimize pharmaceutical agents which alter flora and motility
- Pro and prebiotic supplements

What are the mechanisms ?

Splanchnic Hemodynamics



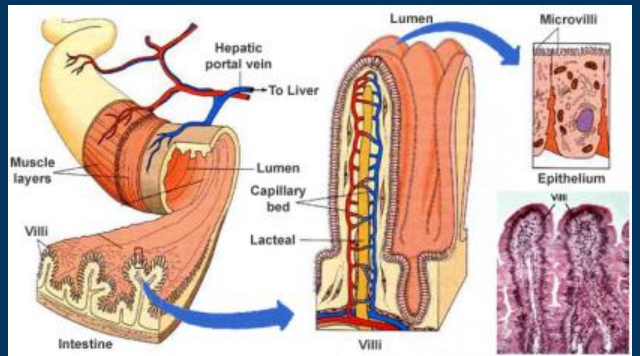
GI tract receives 25% of cardiac output (varies widely)

- 1.25 L/min at rest, 3.0 L/min with meal, 0.5 L/min with exercise
- Dilates to nutrient bolus in segmental fashion

Uses 20 to 30% of total body 0_2 consumption at rest

Small intestine receives nearly 50% of arterial blood flow to splanchnic bed (uneven distribution)

Villous tips are at highest risk

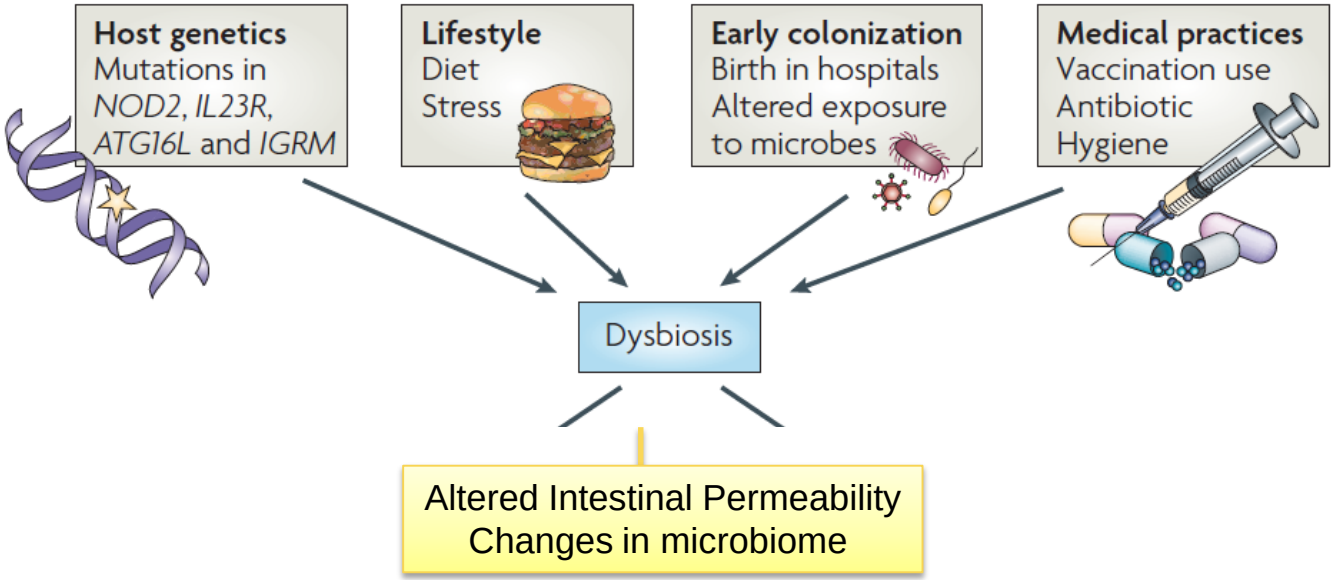


Blood flow (ml/min*100g)	
Splanchnic	50
Kidneys	400
Brain	55
Skeletal Muscle	3
Heart	80

Etiology of Induced Changes in Commensal Microflora

- Broad spectrum antibiotics
- PPI / H_2RI
- Vasoactive pressor agents
  - Changes in pH,
  - Decrease pO_2
  - Increase pCO_2
- Opioids
  - Decrease motility and bacterial clearance mechanisms
- Anticholinergic agents
- Decrease in luminal nutrient delivery
- “Stress”

What contributes to dysbiosis

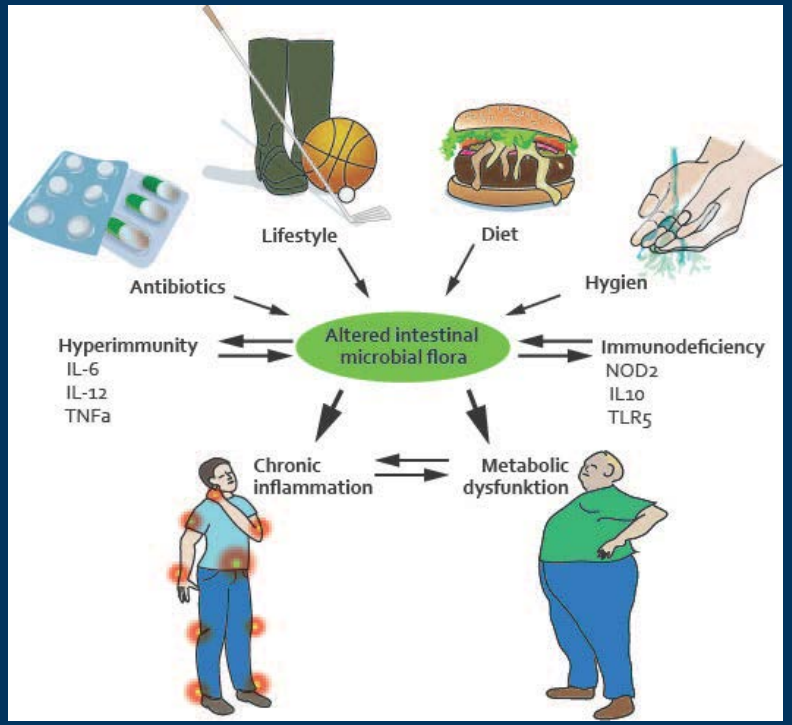


Modified from:

Nat Rev Immunol. 2009 May;9(5):313-23. Round, Mazmanian. The gut microbiota shapes intestinal immune responses during health and disease.

Why care about gut bacteria?

- All eukaryotes have evolved in presence of bacteria.
- They surround us and we surround them !
  - Our immune system reacts to bacterial presence.
  - Bacteria produce metabolites and peptides.



Trophic

- Control of epithelial cell proliferation and differentiation
- Promote intestinal angiogenesis
- Development and homoeostasis of the immune system

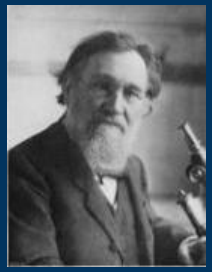
Protective

- Protection against pathogens

Metabolic

- Fermentation for SCFA
- Endogenous mucus
- Production of vitamin K
- Some AA, Neurotransmitters
- Xenobiotic metabolism

Where "man meets microbe"
Dynamic Interplay of Mutualism

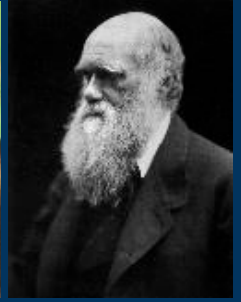


Metchnikoff 1906

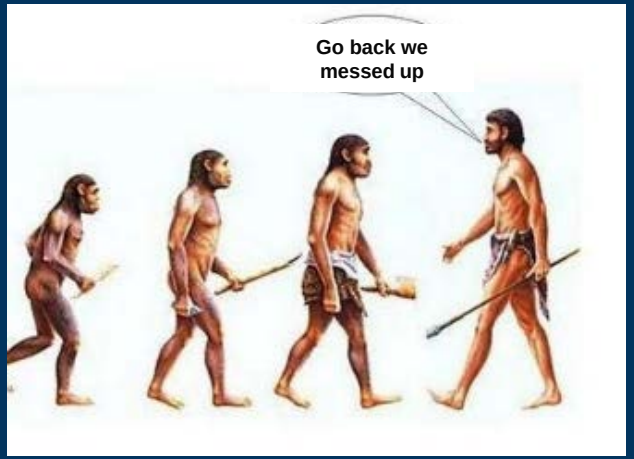
- Concepts are not new
  - Reference in Bible, Koran and in Hindu text
  - Metchnikoff "father" of modern probiotic concepts

- Surface area of GI 300 to 400 sq meters
- > 8 million genes in the bacterial genome vs >20,000 in the human
  - 100 trillion living bacteria in the human intestine
    - » Only about 10 trillion cells in human body
  - Several thousand species in human colon, many non-culturable
  - Extensive # of microenvironments (skin, R v L hand etc)
- Exposed to "pro and prebiotics" from day one of life
  - 13 to 15% of CHO in breast milk not absorbed by infant
- New areas of medicine specifically targeting the metabolic issues
  - Psychobiome or psychobiotics, etc

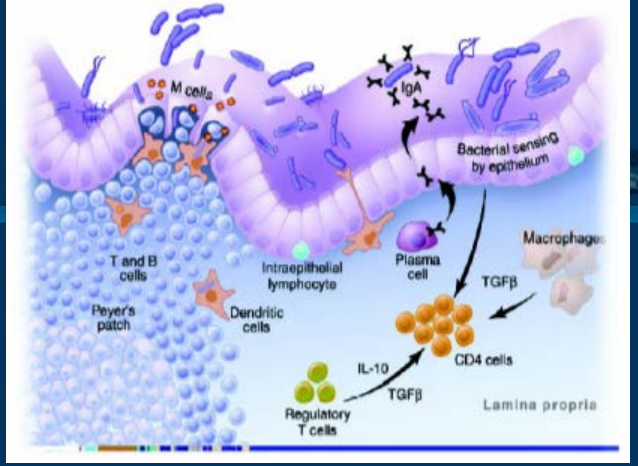
Man and Our Microbiome Continue to Evolve in "Darwinian" Fashion



- Changes in activity
  - Sedentary lifestyles
- Newborns in USA
  - 1/3 C section, majority bottle fed
- Immunizations
- Domestic pets
- Decrease in parasitic infection
- Refrigeration
- Sanitation and hygiene standards
- Urban life in cities and concrete
- Increased use of antibiotics
  - Indicated or not !
  - Now beginning to understand "collateral damage" of antibiotics
- Major dietary changes
  - Fats, protein, fiber, additives, emulsifiers, sweeteners, anti-oxidants, preservatives, insecticides, refining grains, de-germination of grains
- Dramatic changes in the way we feed our sick patients



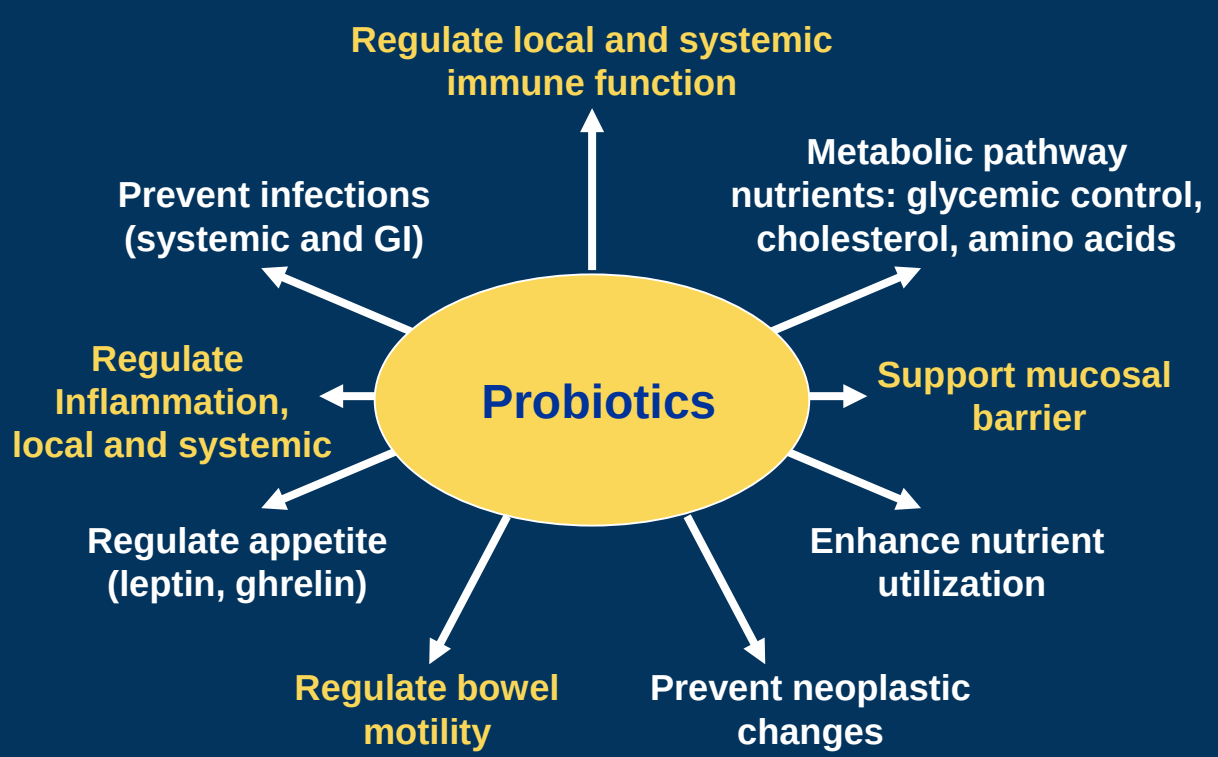
Does the microbiome change ?

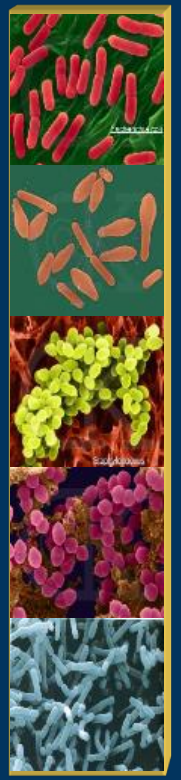


- Diet, inflammation, pH, drugs
- Bacterial changes with host stress situations
  - Bacterial use environmental clues
    - pH, temperature, redox potential, osmolality
  - When energy supply is limited genes “switch on” virulence factors
    - Ex: E.coli and Pseudomonas can rapidly become virulent with host stress (epinephrine, cortisol, morphine etc)
- New data showing microbiome even changes between meals

Babrowski T et al. *Ann Surg.* 2012
Alverdy J et al. *CCM.* 2003;31:598-607
Alverdy J et al. *Molecular Biol.* 2008
Cani PD et al. *Curr Opin Biotech.* 2015

Probiotics: Exploring the Mutually Beneficial Effects of Bacteria and Their Substrates in the Human Host





Starting From Day 1 We Are Exposed To Pro and Prebiotics

What are they?

- **Probiotics**: live microorganisms that confer a health benefit on the host when administered in adequate amounts
- **Prebiotics**: substrate for probiotics

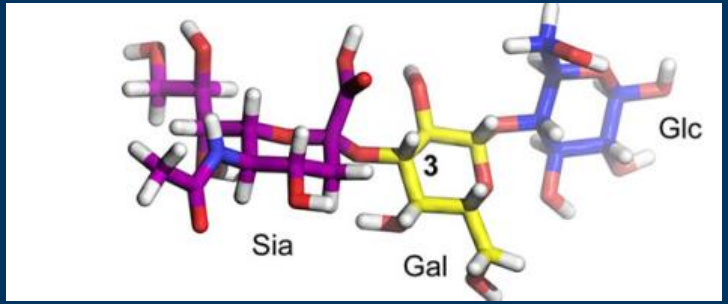
Where found ?

- **Probiotics**: found in fermented foods
- **Prebiotics**: found in many unprocessed foods

Probiotics + Prebiotics = Synbiotics

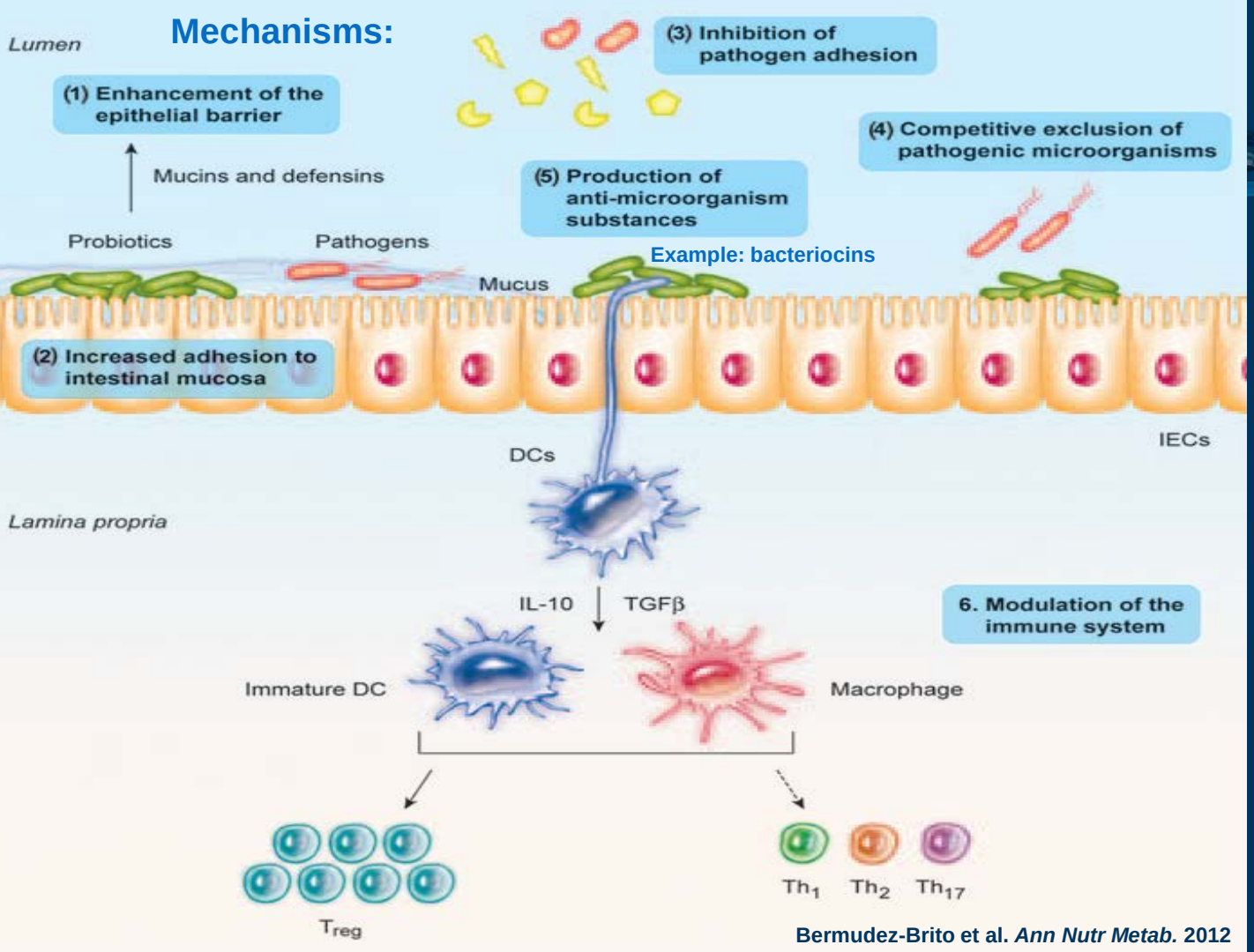
Human Milk Oligo-saccaride (HMO)

- **HMOs have prebiotic effects:**
  - **selectively enhancing desired colonic bacteria**
  - **Anti-adhesive for pathogens**
  - **Blocking pathogen colonization and invasion**
  - **Changing glycosylation of epithelial cells altering expression to limit infections**

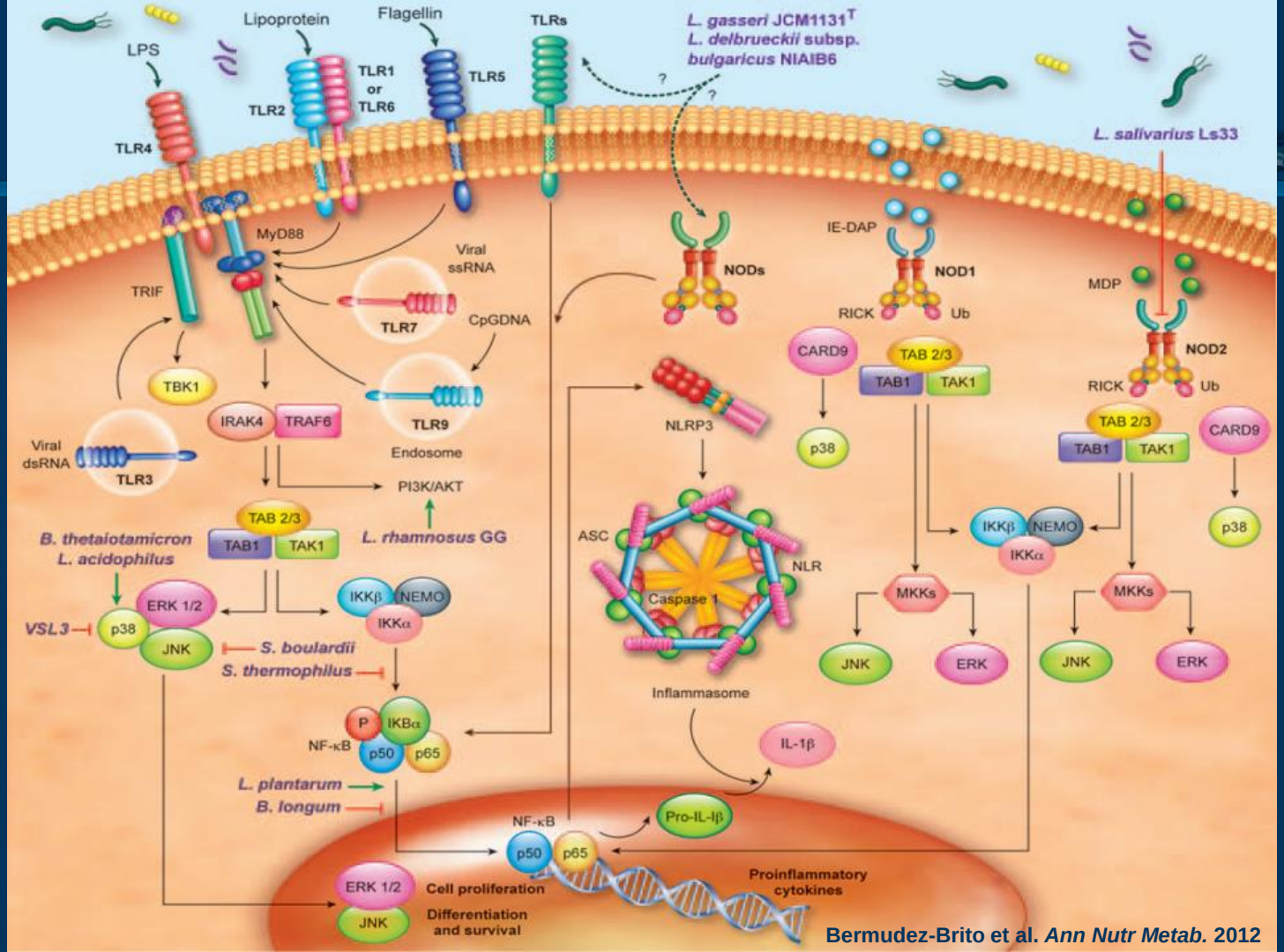


Human breast milk: 15 % of the carbohydrates are not able to be absorbed in the proximal gut and are clearly there as substrate for optimizing colonic bacteria

Mechanisms:



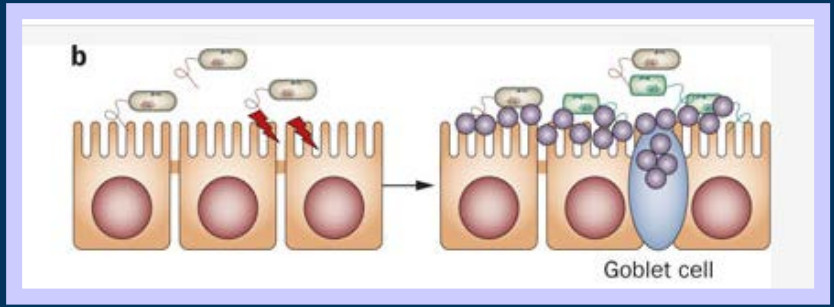
Bermudez-Brito et al. *Ann Nutr Metab.* 2012



Bermudez-Brito et al. *Ann Nutr Metab.* 2012

Mechanisms:

Colonization Resistance
Antimicrobial Factors



L. reuteri* inhibits *H. pylori

L. reuteri* inhibits *Staph aureus

Mechanisms

- Competitive inhibition
- Physical barrier (mucous)
- ↓ Adherence, attachment
- Produce bacteriocins
  - Defensins, Trefoil
  - Bind pathogens
- ↓ pH reduces growth
- Interferes quorum sensing
  - ↓ Virulence expression
- Breaks up biofilms

Bacteria

- *Escherichia coli (pathogenic)*
- *Salmonella typhimurium*
- *Shigella spp.*
- *Campylobacter jejuni*
- *Streptococcus mutans*
- *Bacillus subtilis*
- *Clostridium perfringens*
- *Helicobacter pylori*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
- *Pseudomonas fluorescens*

Fungi

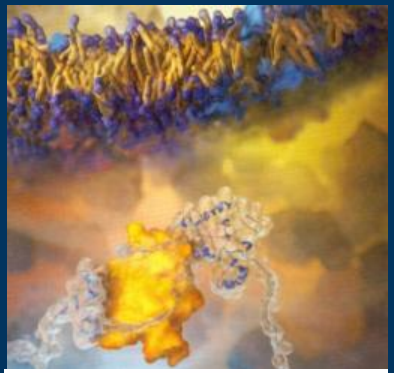
Candida albicans
Aspergillus flavus

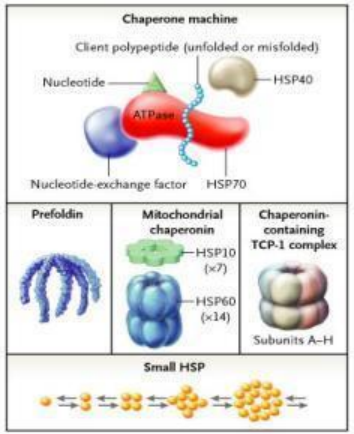
Sherman PM. *NCP.* 2009
Morowitz MJ. *SCNA.* 2011

Protecting the mucosal lining:

"Soluble factors for *Lactobacillus rhamnosus GG* activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells"

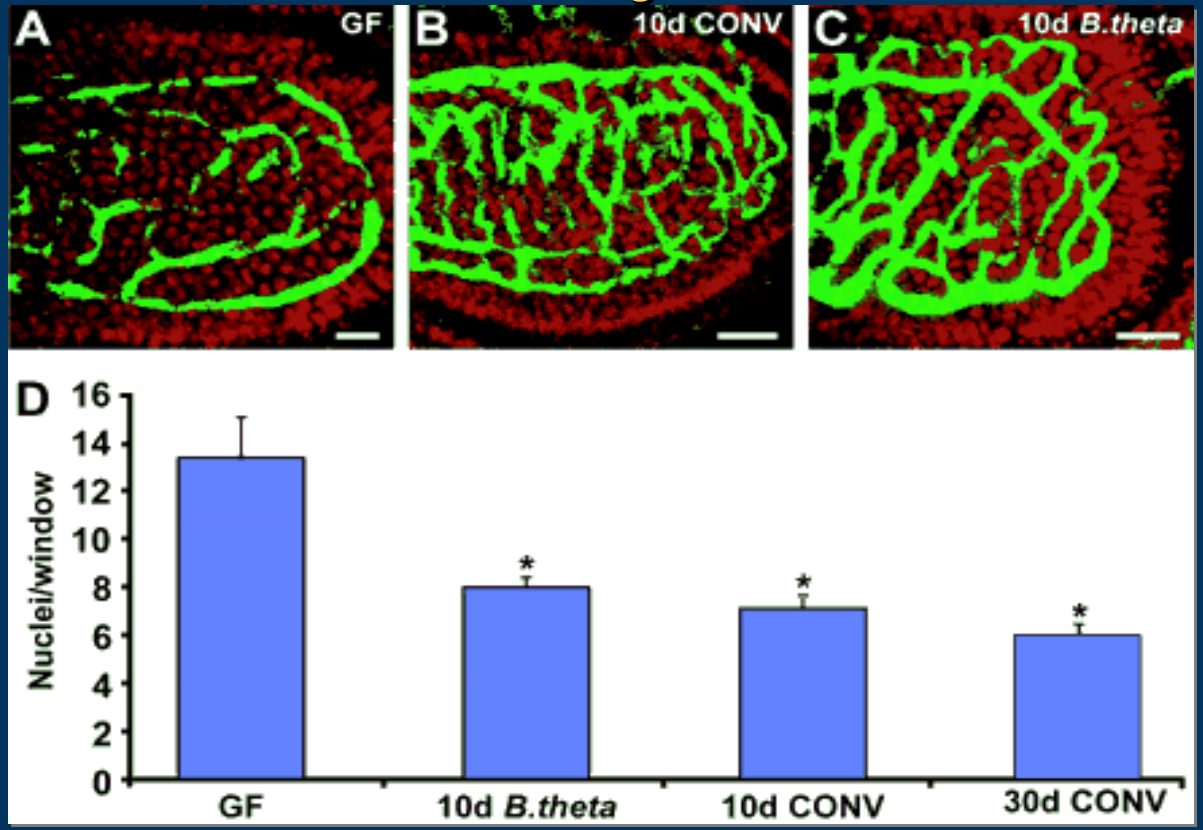
- 70% of energy for colonocyte derived from luminal butyrate
- Cell culture model
- DNA microarray methods, real-time PCR and electrophoretic mobility shifts studied
- Studies confirm:
  - L. GG modulates signaling pathways
  - Activates via MAP kinase
  - L.GG protects mucosa from oxidant stress via expressing HSP





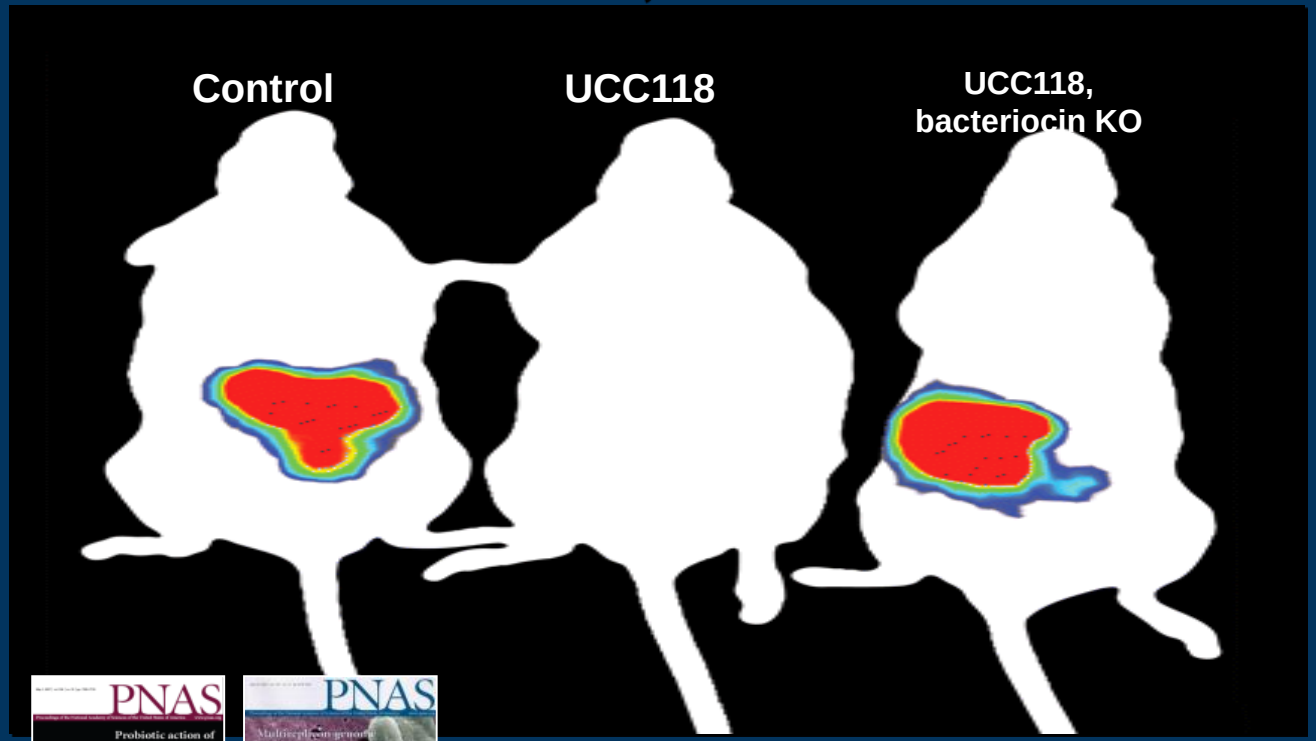
Tao K et al. *Am J Physiol Cell Physiol.* 2006;290;1018-1030

Mechanisms:Enhancing mucosal blood flow



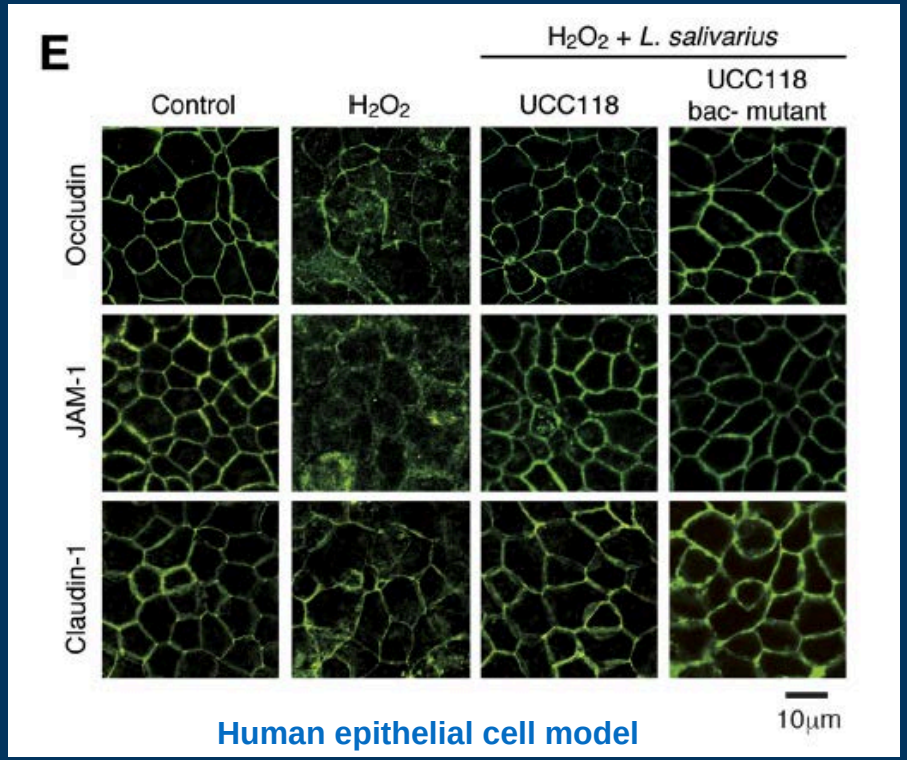
Stappenbeck TS, Hooper LV, Gordon JI. *Proc Natl Acad Sci USA.* 2002;99:15451-15455

L. salivarius (UCC118) prevents *Listeria* infection, in mice

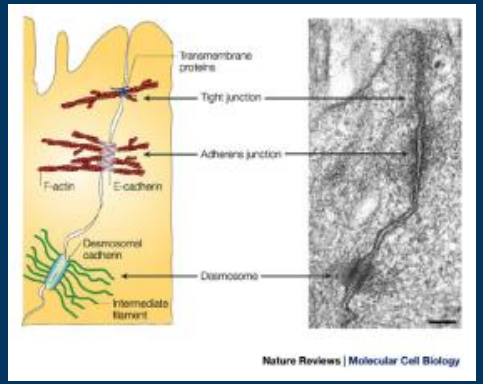


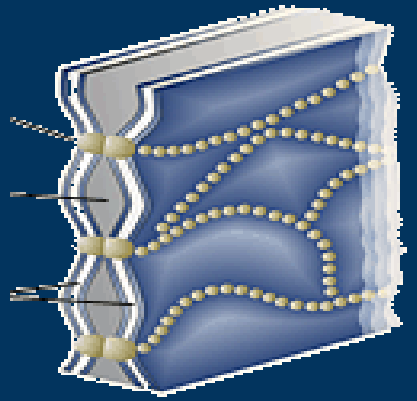
Corr SC. *PNAS* 2010

Lactobacillus salivarius (UCC118) prevents disruption of epithelial cell tight junctions



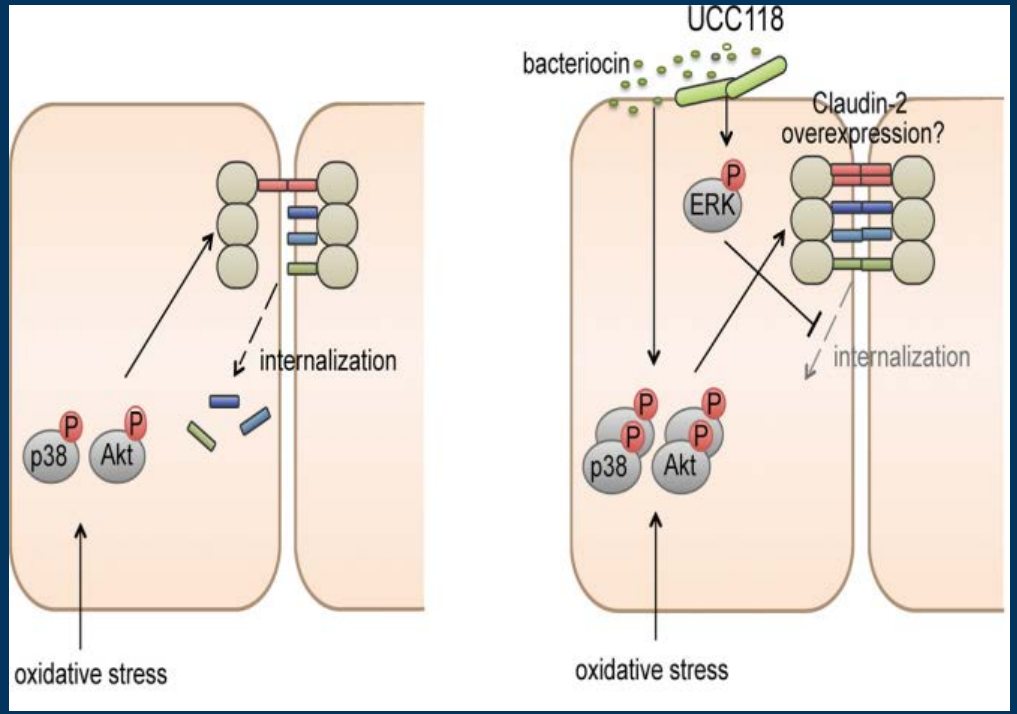
Human epithelial cell model





Miyauchi et al. *Am J Physiol Gastrointest Liver Physiol.* 2012

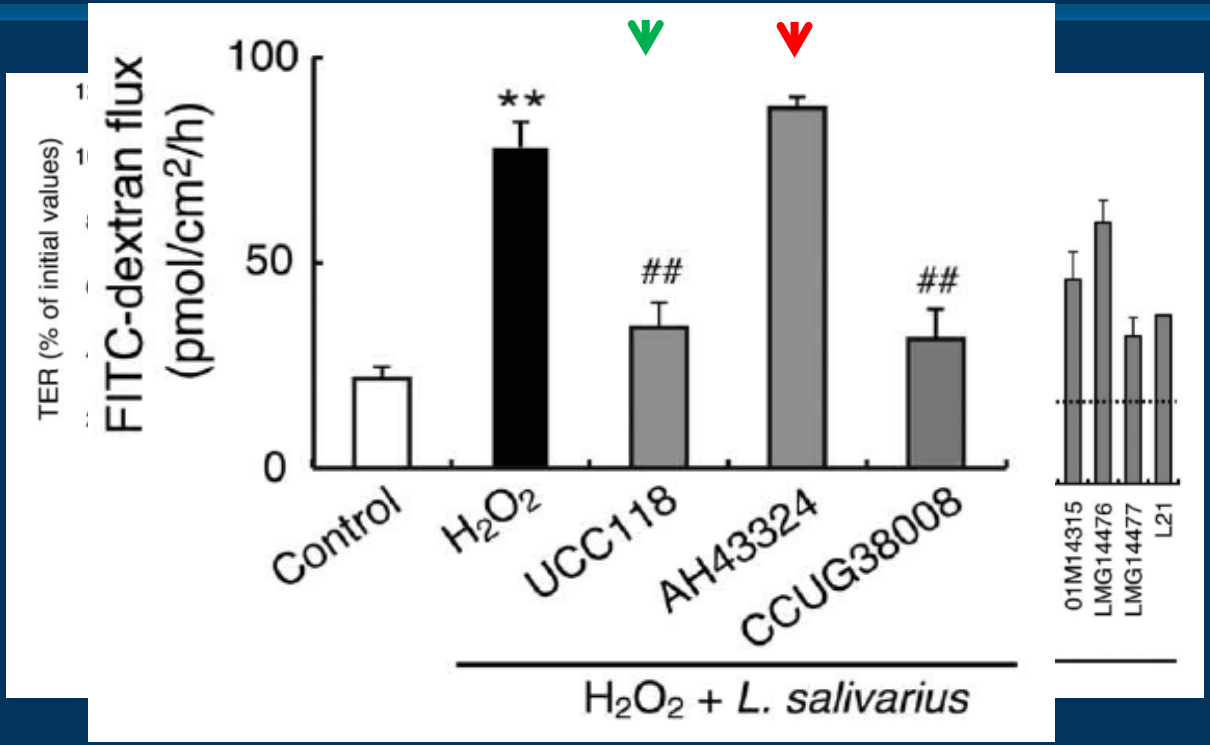
UCC118 alters tight junction protein localization.



•**Tight junction proteins**

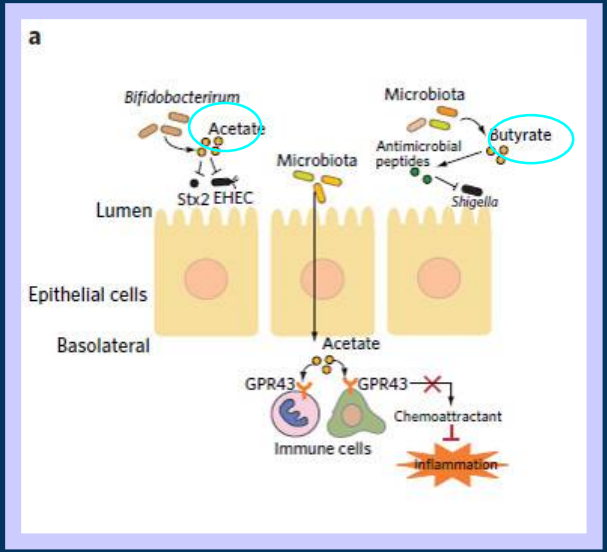
Miyauchi et al. *Am J Physiol Gastrointest Liver Physiol.* 2012

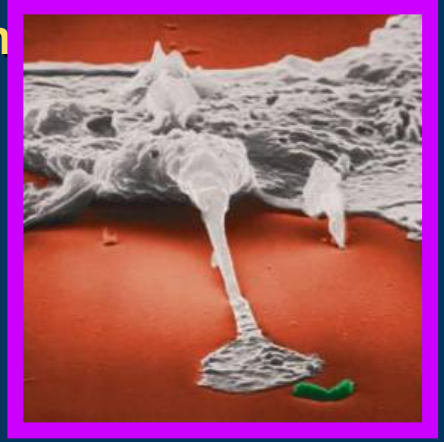
L. salivarius UCC118 and *L. salivarius* AH43324 have very different effects upon barrier function

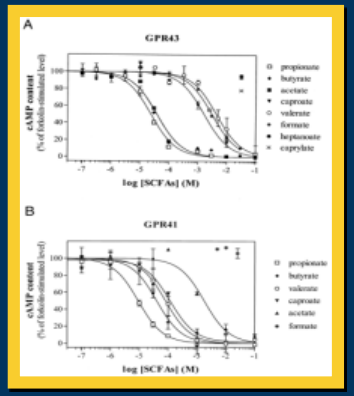


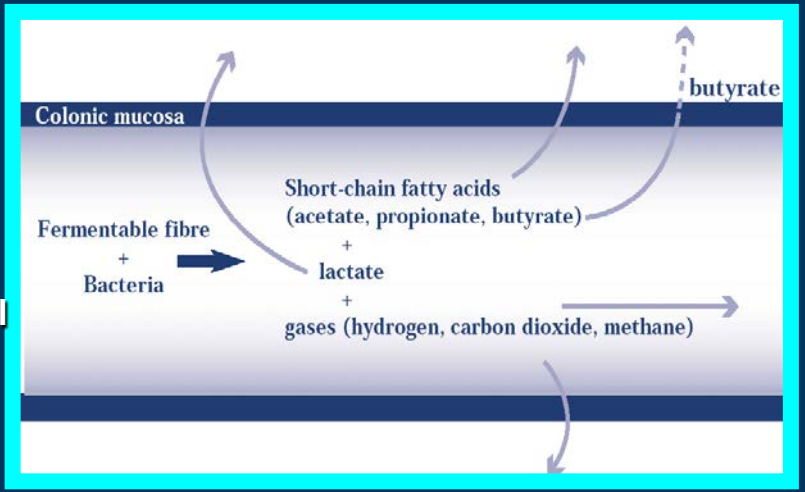
Miyauchi *et al*. *Am J Physiol Gastrointest Liver Physiol*. 2012;303(9):G1029-41.

SCFAs, Fiber Fermentation and Butyrate Receptors





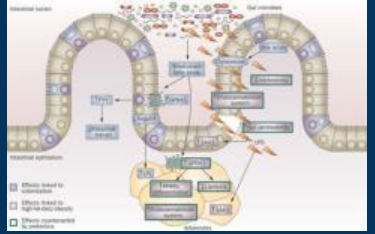




- Trophic effect, colonocyte fuel
- Anti-inflammatory
- Enhance WBCs, macrophage
- ↓Adhesion molecules
- (↓microvascular thrombosis)

Thangaraju M et al. *J GI Surg.* 2008
Ganapathy V 2011

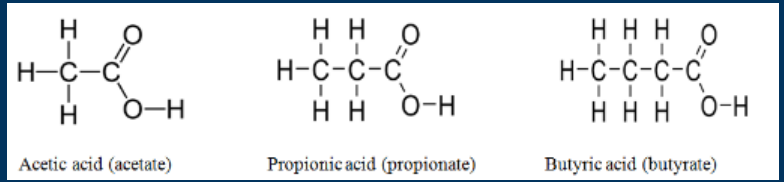
SCFA = Fermentation end product of some probiotics (from prebiotics):
Multiple Mechanisms Described



- Energy source;
  - Colonic mucosa;
    - Stimulates cell proliferation, Promotes sodium and water absorption
  - Cardiac, skeletal muscle, brain
    - Acetate, butyrate, proprionate
- Regulation of gene expression for ICAM-1 and E-Selectin on endothelial cells
- Decrease COX-2 expression
    - (butyrate and proprionate)
- Prevention of neoplastic transformation
    - Inhibits histone deactylase by DNA hypermethylation to promote differentiation in cancer cell lines
- Enhances Leptin secretion
- pH control; Inhibition of pathogen overgrowth in gut lumen,
- ROS scavenger
    - Pyruvate is anti-inflammatory and decreases NFKB expression
- Activation of polymorphonuclear cells
    - Both local and systemic immune benefit
    - G-protein receptors on circulating PMN's

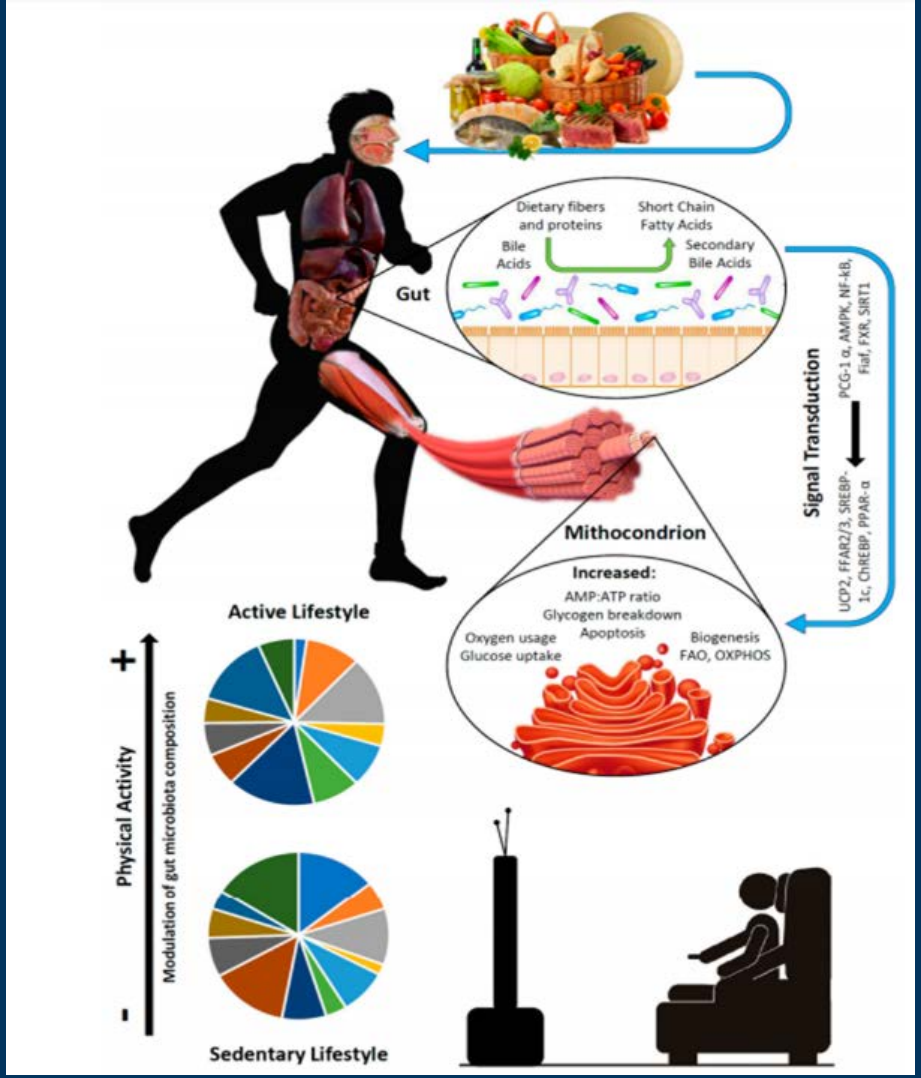
Thangaraju M et al. *J GI Surg.* 2008
Morowitz MJ. *Surg Clin N Am.* 2011

SCFA's:



- Known transporters described in colonic mucosa
- Activates G-protein-coupled receptors
  - GPR41, GPR43
- Regulates transepithelial fluid transport
- Reduces mucosal inflammation
- Strengthens epithelial defense barrier
- Lowers cholesterol
- Decreases insulin resistance
- Improves recovery in I/R injury
- Regulator of HDAC and the anti-mitogenic activity

Bhat MI et al. *Nutrition Rev*. 2017



Evidence supports SCFA enhancing muscle function and mitochondrial biogenesis in the myocyte.

Essentially potentiating the benefit of exercise in muscle maintenance

Ticinesi A et al. *Nutrients.* 2017

G Protein couple receptor ligands encoded in bacteria

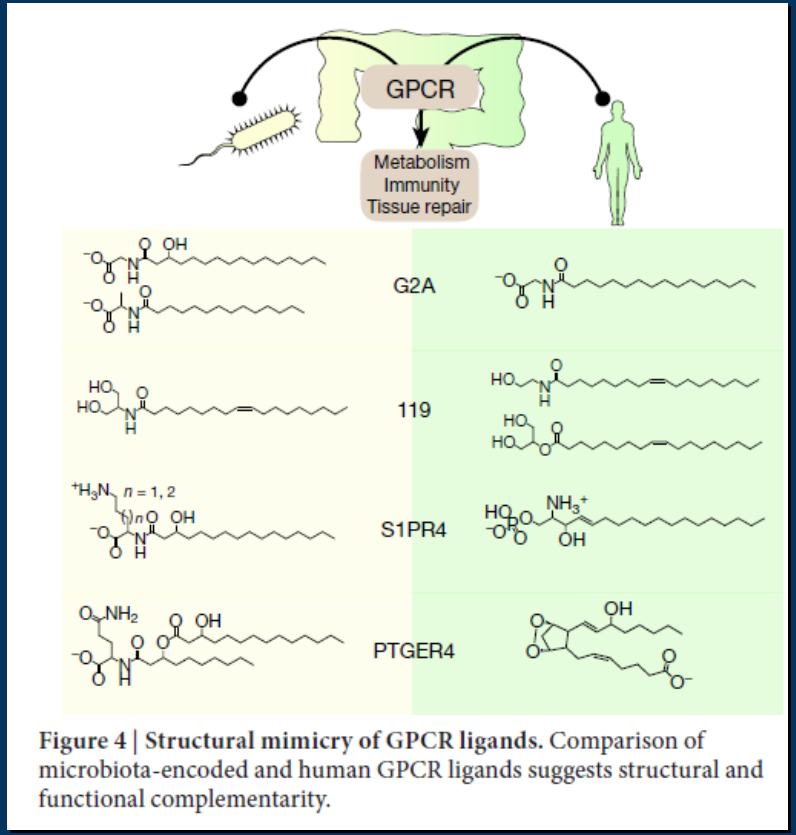


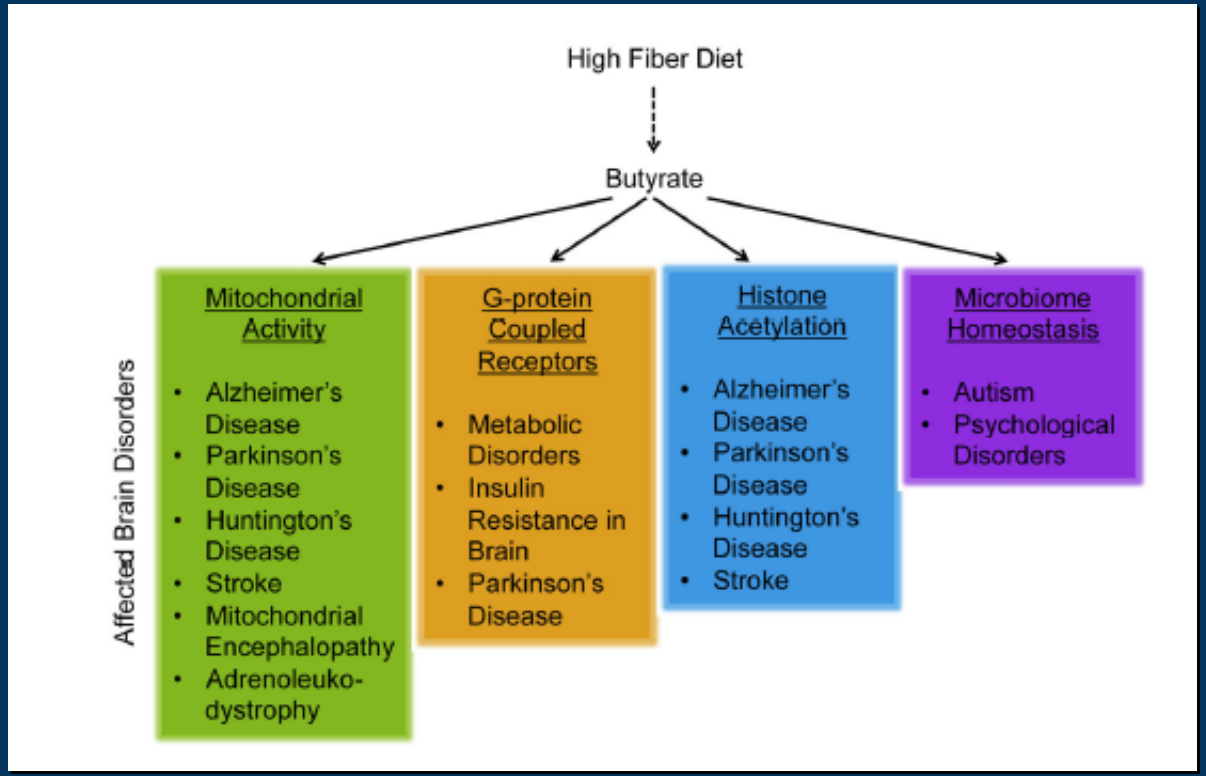
Figure 4 | Structural mimicry of GPCR ligands. Comparison of microbiota-encoded and human GPCR ligands suggests structural and functional complementarity.

Chemical mimicry

Cohen LJ et al. *Nature.* 2017

Butyrate, Neuroepigenetics and the Gut Microbiome: Can a High Fiber Diet Improve Brain Health?

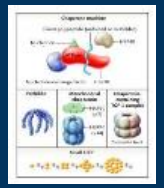
Megan W. Bourassa[a,b], **Ishraq Alim**[a,b], **Scott J. Bultman**[c], and **Rajiv R. Ratan**[a,b,*]

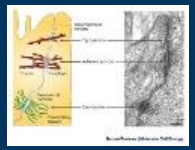


Bourassa MW et al. *Neurosci Lett.* 2016

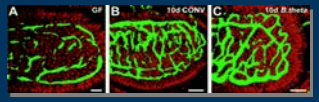
Multiple clinical mechanisms of probiotics well described

- Competitive inhibition of pathogens





- Enhance HSP in gut mucosa
- Tight junction protein synthesis
- Enhance mucosal blood flow



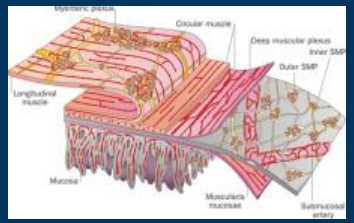
- Stimulate gut immunity
- Butyrate (fermentive end product) enhances neutrophil killing, chemotaxis, resolution of inflammation

- Butyrate: anti-neoplastic activity
- Increases return of GI motility
- Helps maintain microbiome diversity in colon

- Activation of G-protein receptors

Additional mechanisms

- Alterations in metabolism/energy utilization
  - Vitamin production in infant greatest effect (folate, B12)
  - Production and absorption of AA
- Stimulation of intestinal motility
- Butyrate anti-neoplastic activity



- Interacts with ENS bidirectional communication
  - Nerve Growth Factor stimulated by *Lactobacillus sp*
  - Increases IL-10 which attenuates inflammation
  - Alters GABA in brain and shown to be anxiolytic with 28 day continuous feeding (blocked by vagotomy)
  - Microbiome required for normal gut brain signaling
  - Microbiome required for gut Ca++ binding protein expression

Bienenstock J et al. *Gut Microbes.* 2013
McVey-Neufeld KA et al. *Neurogastro and Motility.* 2015

Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis

Susan Westfall[1] · Nikita Lomis[1,2] · Imen Kahouli[1,2] · Si Yuan Dia[1] · Surya Pratap Singh[3] · Satya Prakash[1,2]

Cell Molecular Life Science 2017

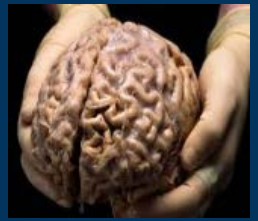
- Central Nervous System
- Autonomic nervous system
  - Sympathetic and parasympathetic
- Enteric nervous system
- Hypothalamic pituitary adrenal axis

- Microbiome manipulation is target for biotherapies
  - Mechanisms
    - Direct neural communication (vagus nerve)
    - Via endocrine mechanism
    - Via immune response

Early data showing bi-directionality

- Preclinical/animal studies demonstrate that probiotic effects on brain are dependent on vagal afferent signals
  - *Lactobacillus rhamnosus* directly activates vagal neurons
  - Induces region-dependent alterations in GABA receptor expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-like symptoms via vagus nerve signaling in mice
- Vagotomized mice do not exhibit this effect

- **Human:**
  - **DBPCRCT Messaoudi M et al Br J Nutr 2011**
    - **Decrease psychological stress, urinary cortisol**
  - **Rao AV et al Gut Pathogens 2009: Chronic fatigue**
    - **L casei Shirota v placebo x 2 months**
    - **Improved fatigue, less anxiety**

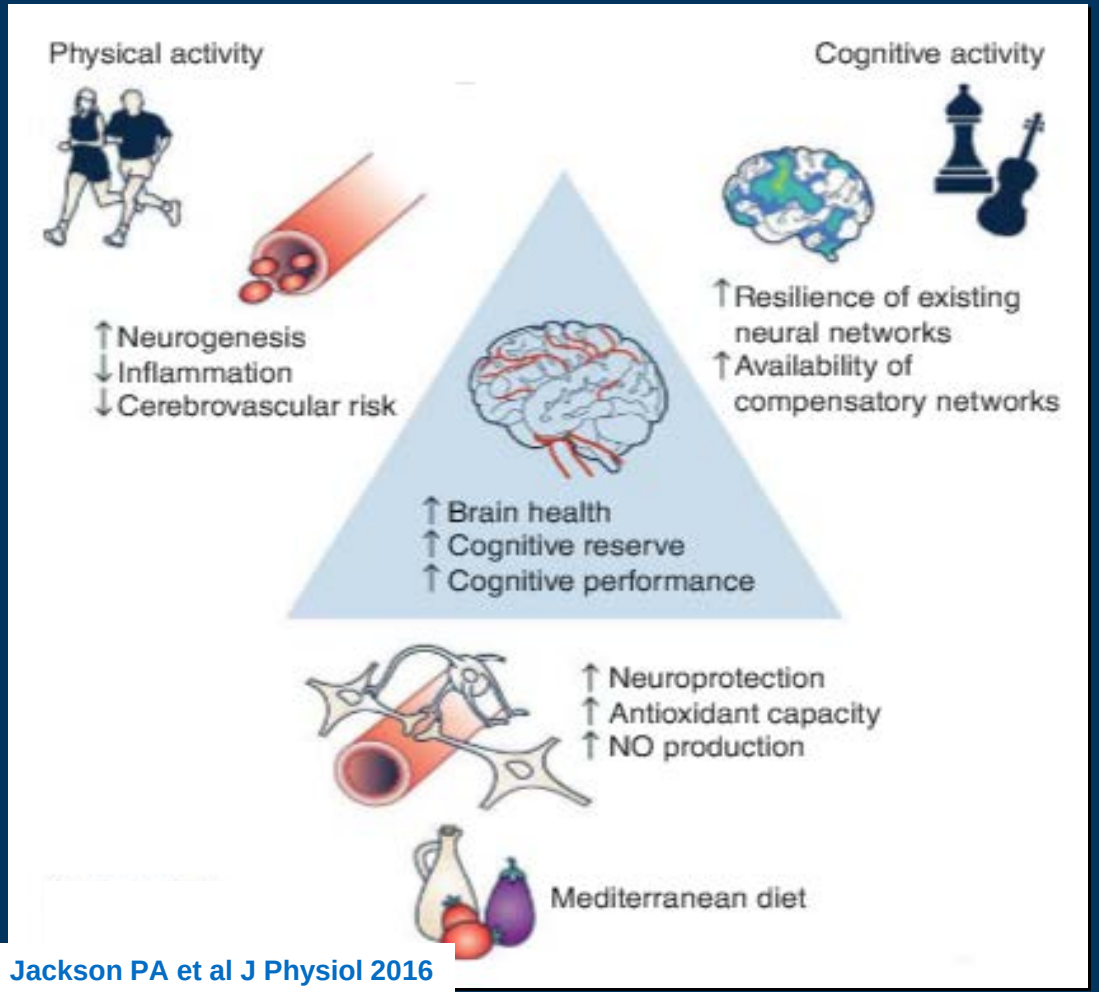


Bravo JA et al. *PNAS.* 2011
Bienenstock K et al. *Nutrition Rev.* 2015
Messaoudi M et al. *Br J Nutrition.* 2011

Diet and Brain Function

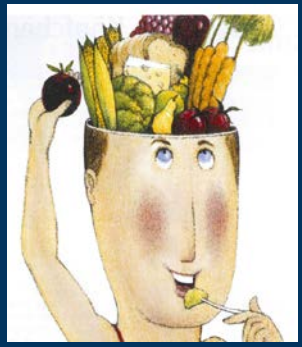
- Global observations regarding brain function;
  - Increase in chronic and acute neurologic disorders
    - Alzheimer's, depression, autism, cognitive decline
  - Longevity is increasing making life long cognitive function a greater challenge
  - Dramatic increase in poor dietary habits
- Dietary adjustments and supplements suggested as powerful modulators of brain function
  - Omega-3 fatty acids
  - Specific amino acids
  - Probiotic / prebiotics
  - Antioxidants
  - Vitamins
  - Mediterranean diet

It is Not Just the Food We Eat !



Jackson PA et al J Physiol 2016

Can Nutrition Prevent Dementia and Cognitive Decline ?



Traditional hypothesis:

- Brain function is not influenced by individual nutrients in foods
- Blood-brain barrier is virtually impermeable !

Current understanding:

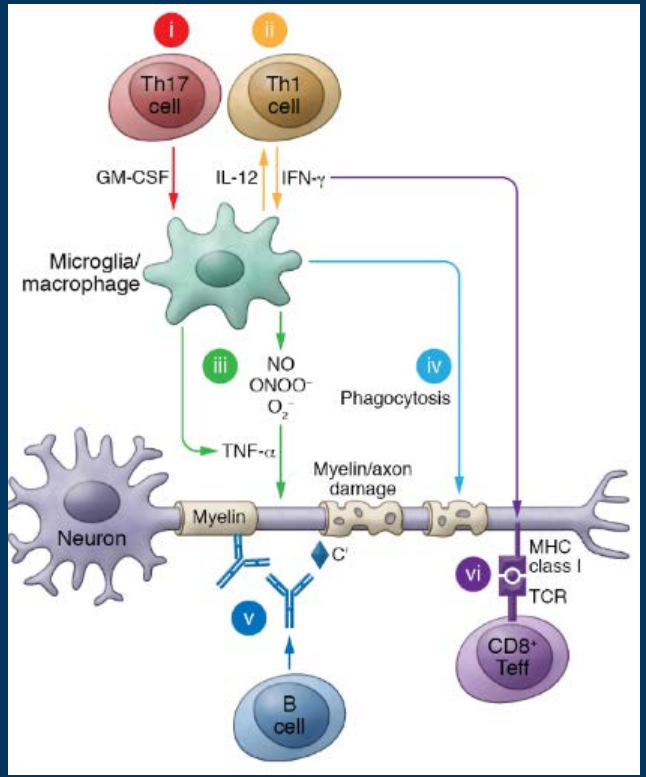
- Brain function can be dramatically influenced by diet and nutrient intake

CNS inflammation and neurodegeneration

Tanuja Chitnis and Howard L. Weiner

Ann Romney Center for Neurological Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

Chitnis T et al J Clin Investigation 2017



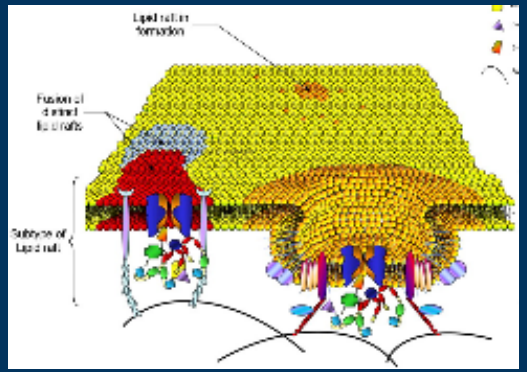
Mechanisms of Degeneration

- Apoptosis
- Necroptosis
- Neuronal autophagy
- Retrograde degeneration
- Wallerian degeneration
- Demyelination
- Astrogliopathy

J Clin Investigation 2017

From Energy Metabolism to Cognition

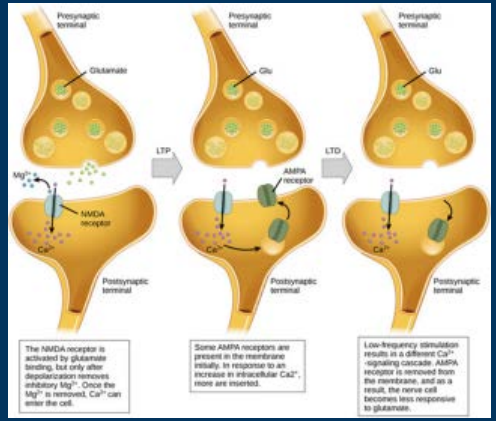
- Brain consumes 20% of total oxygen utilization at rest while only making up 2% of body weight
    - High energy requirement to maintain electrochemical gradients for nerve transmission
- DHA most abundant phospholipid in brain
- DHA critical for normal “lipid rafts” formation



Gomez-Pinella F et al. *Nature Rev Neurosci.* 2013

Diet and Cognition: interplay between cell metabolism and neuronal plasticity

- Dietary factors exert their effects via:
  - Energy metabolism
  - Synaptic plasticity
    - Epigenetic regulation

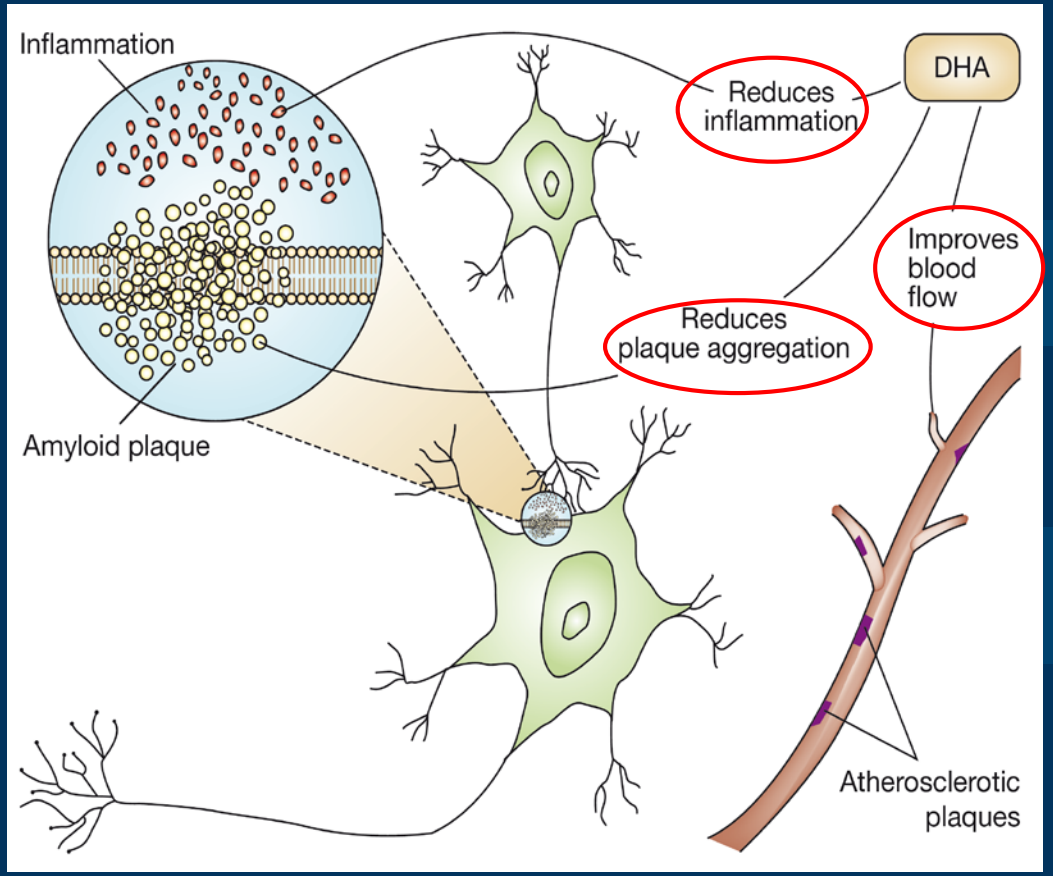


- Oxidative stress

- DHA
  - Key for inter-neuronal signaling and cognition
- Brain-derived neurotrophic factor
  - Crucial for activating signaling cascades which are diet dependent

Gomez-Pinilla F et al. *Curr Opin Clin Nutr Metab Care.* 2013

PUFA: Mechanisms of action to reduce dementia



n-3 Fatty acids ↑
(e.g. DHA)

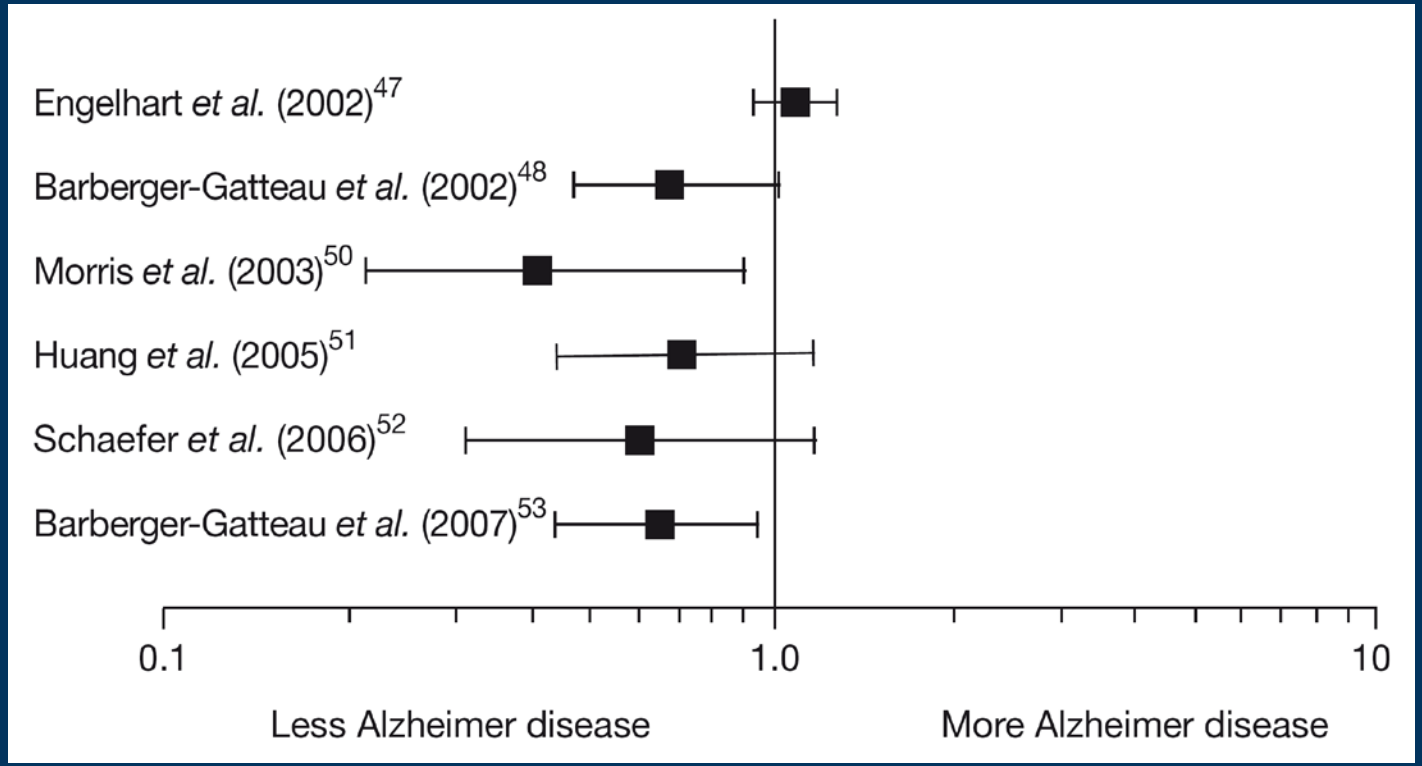
↓

Low risk of Dementia and Alzheimer's

Fotuhi et al. *Nat Clin Pract Neurol.* 2009;5:140-52
Schaefer et al. *Arch Neurol.* 2006;63:1545-50
Franga VG et al. *J Neuroimmunology*. 2017

Fish Consumption & Omega-3-Supplements and Dementia

Risk of dementia identical, cognitive impairment slightly ↓



Fotuhi et al. *Nat Clin Pract Neurol.* 2009;5:140-52

Omega-3 fatty acids to decrease cognitive loss with aging

- Prospective study plasma fatty acids and cognition
- N=2251 patients
- Multiple neuropsychological testing modules
- Hypothesis: oxidative stress related to neurodegenerative disease
- Conclusions:
    - Omega-3 FA have substantial benefit in reducing cognitive decline

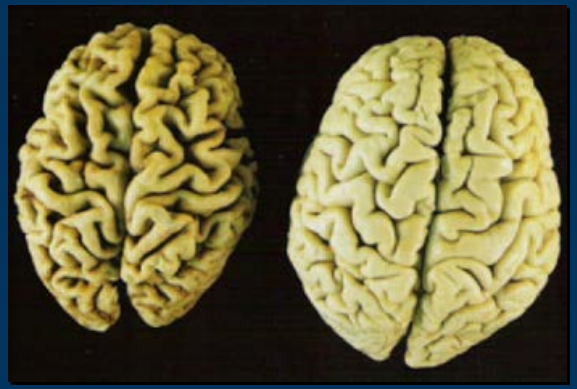
Beydoun MA et al. *Am J Clin Nutr.* 2007;85:1103-11

- Prospective RCT EPA/DHA supplements in cognitive function
- N= 867 > 70 yo
- 200 mg EPA/500 mg DHA vs olive oil for 24 months.
- Conclusion:
    - No difference between groups in loss of cognitive function

Dangour AD et al. *Am J Clin Nutr.* 2010,91:1725

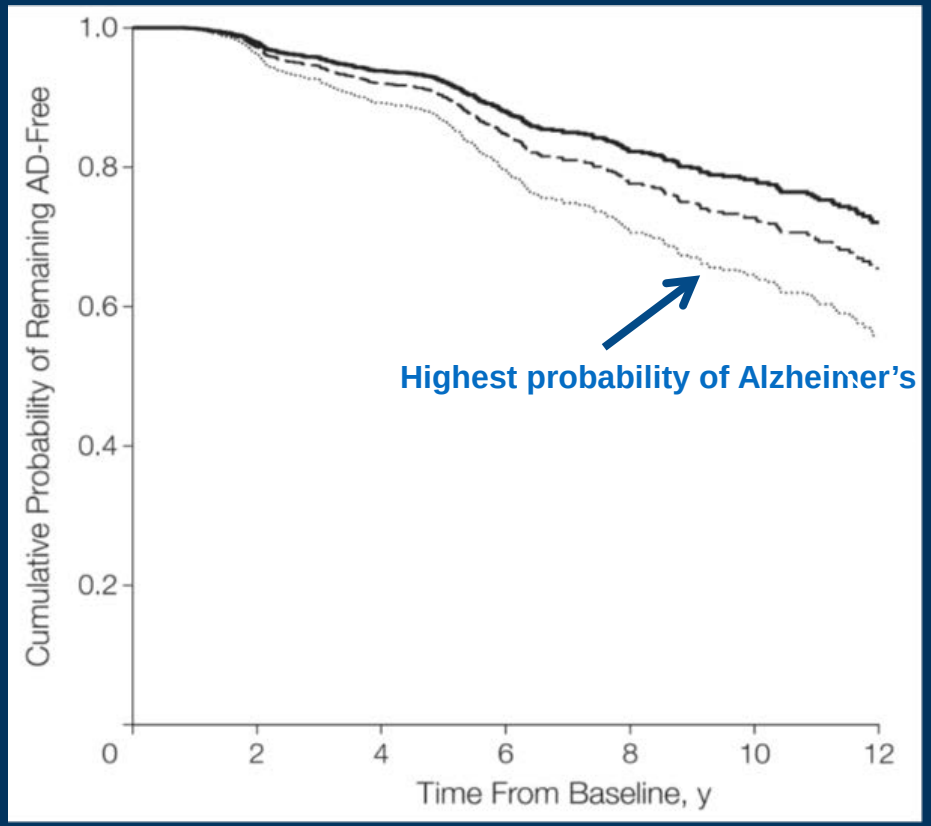
“Plasma fish oil and atrophy of medial temporal lobe”

- Prospective observational study
- N=281 (MRI evaluation)
- Objective: associate fish oil with depression, dementia, Alzheimer Disease
- Results:
  - Higher plasma EPA/DHA less gray matter loss
  - Atrophy associated with lower decline in memory and depression



Samieri C et al. *Neurology*. 2012

Relationship of Med Diet and Exercise: Onset of Alzheimer's Disease



Highest physical activity
++ Mediterranean Diet

Low physical activity
++ Mediterranean Diet

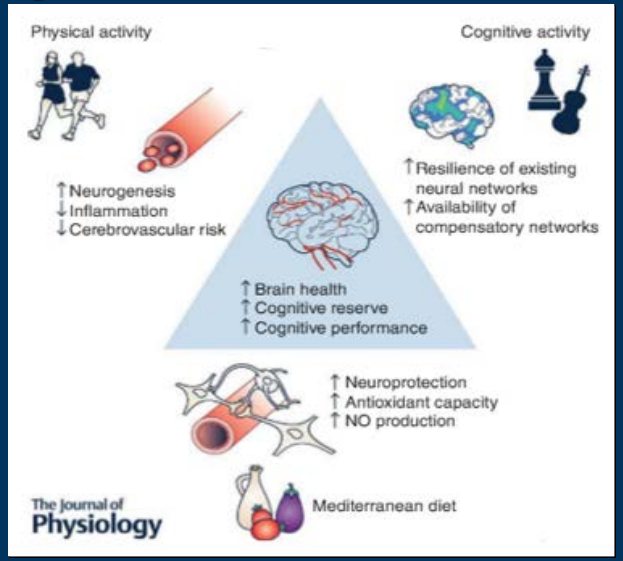
Low physical activity
+ Mediterranean Diet

N=1880 subjects

Scarmeas. *JAMA*. 2009

Cognitive health and prevention of neurodegeneration: Summary

- Combinations of:
  - Dietary Modifications
    - Anti-inflammatory diet
  - Exercise
  - Cognitive Activity



- Fish oils with consistent high quality data to support
- Maintaining microbiome diversity (preventing dysbiosis)
- Anti-oxidants agents
  - Resveratrol, curcumin, vitamin E, flavanols etc

Jackson PA et al. *J Physiology*. 2016
Clare L et al. *PLOS Med*. 2017

Microbiome – CNS Maladies

- Autism
- Alzheimers
- Cognitive decline
- Depression
- Anxiety

Producing a Healthy Gut-Brain Connection:
Practical Applications for Everyday Life !

- **Eat a wide variety of foods**
  - Try to add fermented foods and prebiotics when possible
  - Minimize food additives (sweeteners, emulsifiers, etc)
- **Daily intake with a good prebiotic is beneficial**
- **Be cautious of overstatement of claims of benefit**
  - Association does NOT Equal causation

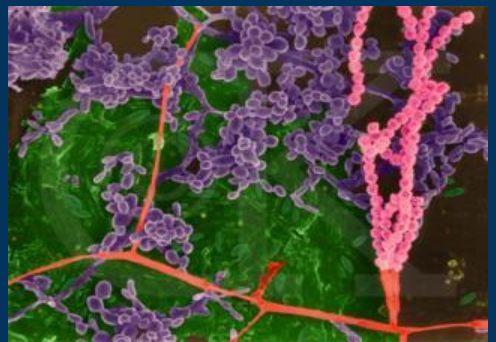
It time for a paradigm shift in clinical medicine and surgery !

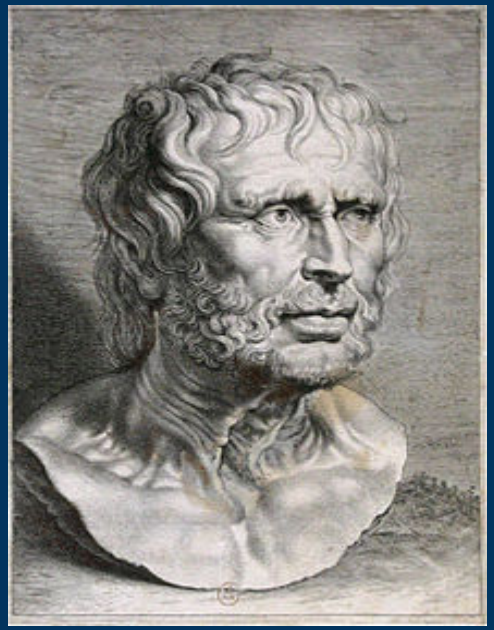
Maintain a diverse non-pathogenic microbiome.

Bioecological control:

we have come along way from

“Germ theory to germ therapy”

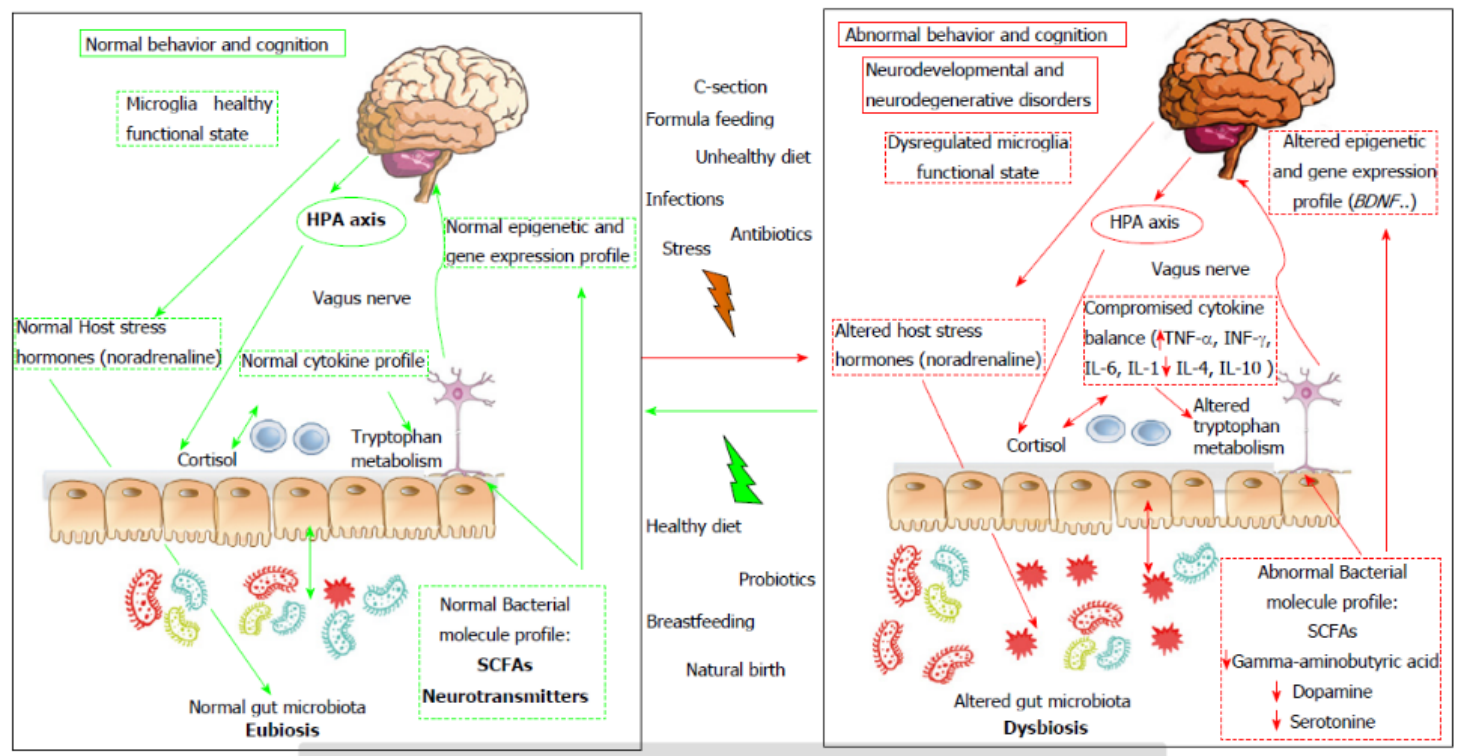




Man does not die he kills himself.

- Seneca

Cognitive decline and loss of muscle mass (sarcopenia) can be attenuated by dietary changes. These are *modifiable behavioral* issues that along with resistance exercise will PREVENT and TREAT many of the brain maladies today


Normal behavior and cognition
Microglia healthy functional state
HPA axis
Normal epigenetic and gene expression profile
Vagus nerve
Normal Host stress hormones (noradrenaline)
Normal cytokine profile
Cortisol
Tryptophan metabolism
Normal Bacterial molecule profile:
SCFAs
Neurotransmitters
Normal gut microbiota
Eubiosis
C-section
Formula feeding
Unhealthy diet
Infections
Antibiotics
Stress
Healthy diet
Probiotics
Breastfeeding
Natural birth
Abnormal behavior and cognition
Neurodevelopmental and neurodegenerative disorders
Dysregulated microglia functional state
Altered epigenetic and gene expression profile (BDNF..)
HPA axis
Vagus nerve
Compromised cytokine balance (↑TNF-α, INF-γ, IL-6, IL-1↓ IL-4, IL-10)
Altered host stress hormones (noradrenaline)
Cortisol
Altered tryptophan metabolism
Abnormal Bacterial molecule profile:
SCFAs
↓Gamma-aminobutyric acid
↓ Dopamine
↓ Serotonine
Altered gut microbiota
Dysbiosis


Metagenics Institute

Heather Zwickey, PhD

Professor of Immunology

Psychoneuroimmunology:

Stress, Chronic Inflammation, and Immune Cell Activation

Learning Objectives

- Describe how the nervous system, endocrine system, and immune system interact
- Report relationships between cytokine, neurotransmitter, and hormones
- List diagnoses that are known to have microbiota relationships
- Suggest treatment possibilities based on system relationships

Blame Descartes

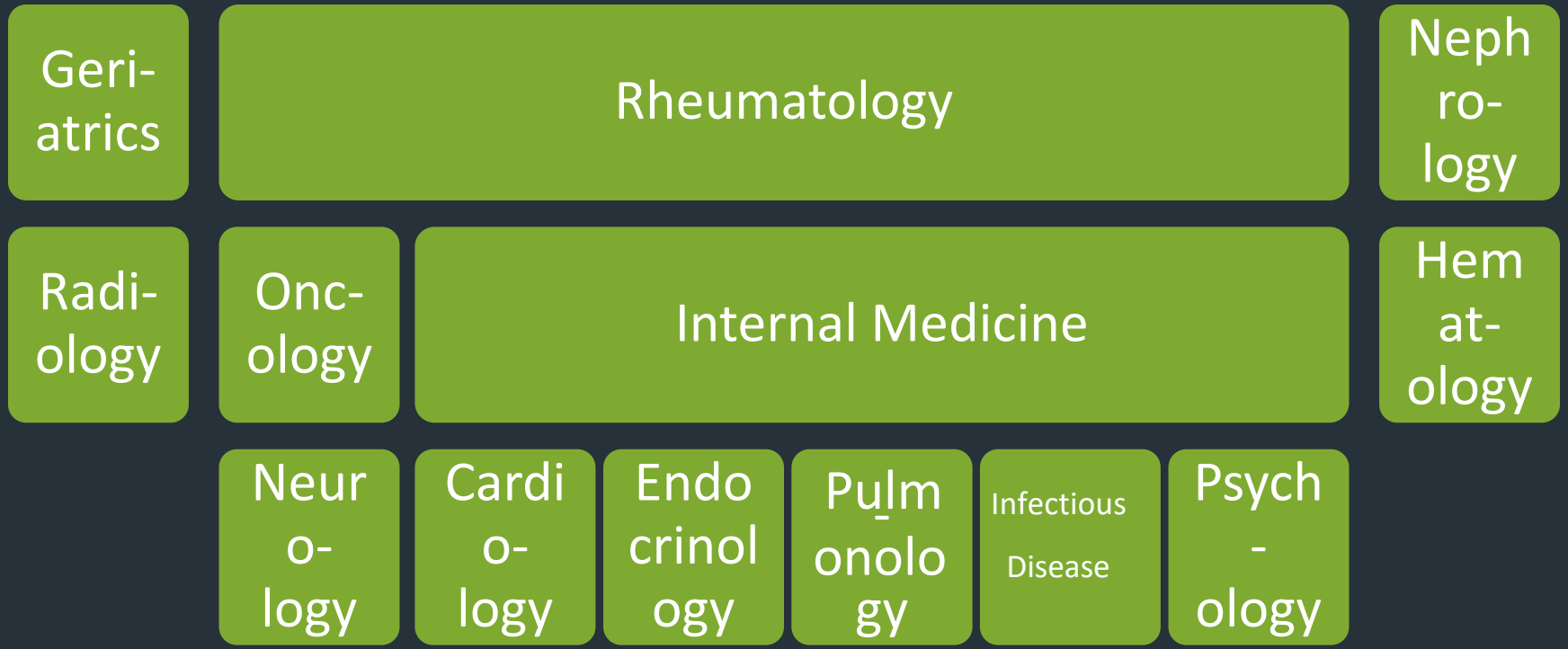
Hippocrates

Intertwined body, mind, and spirit in the 4th century

Descartes

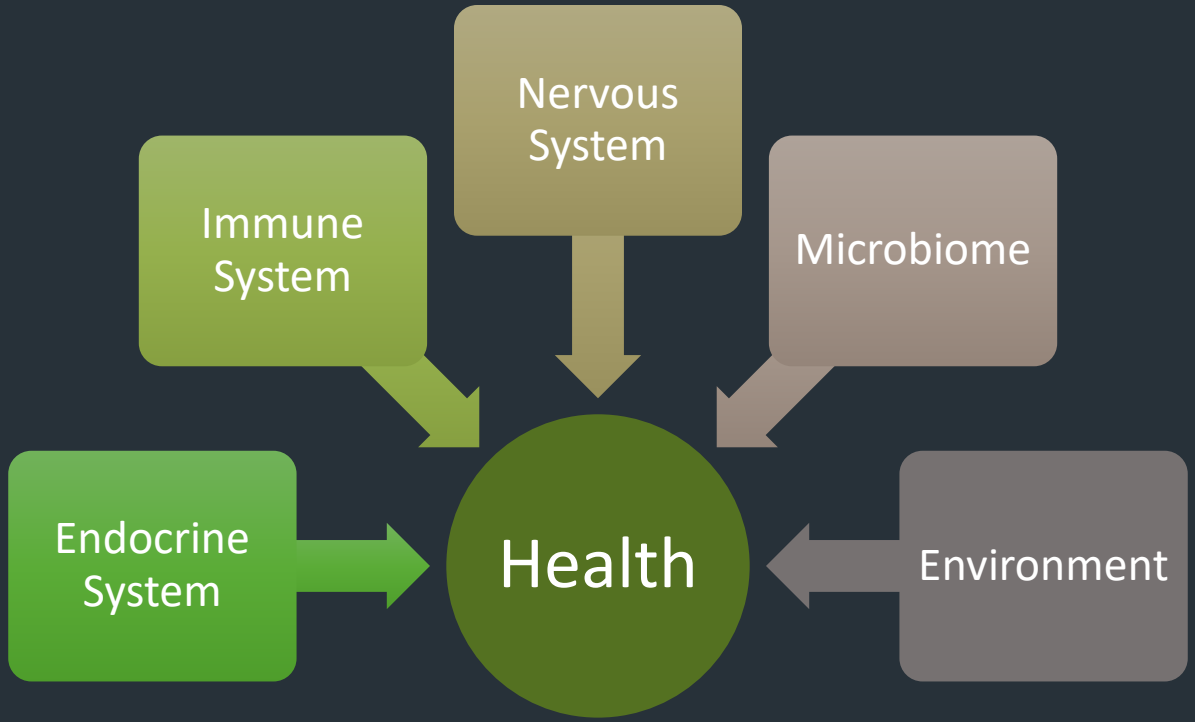
Reductionist of the 17th century

Specialties, such as…

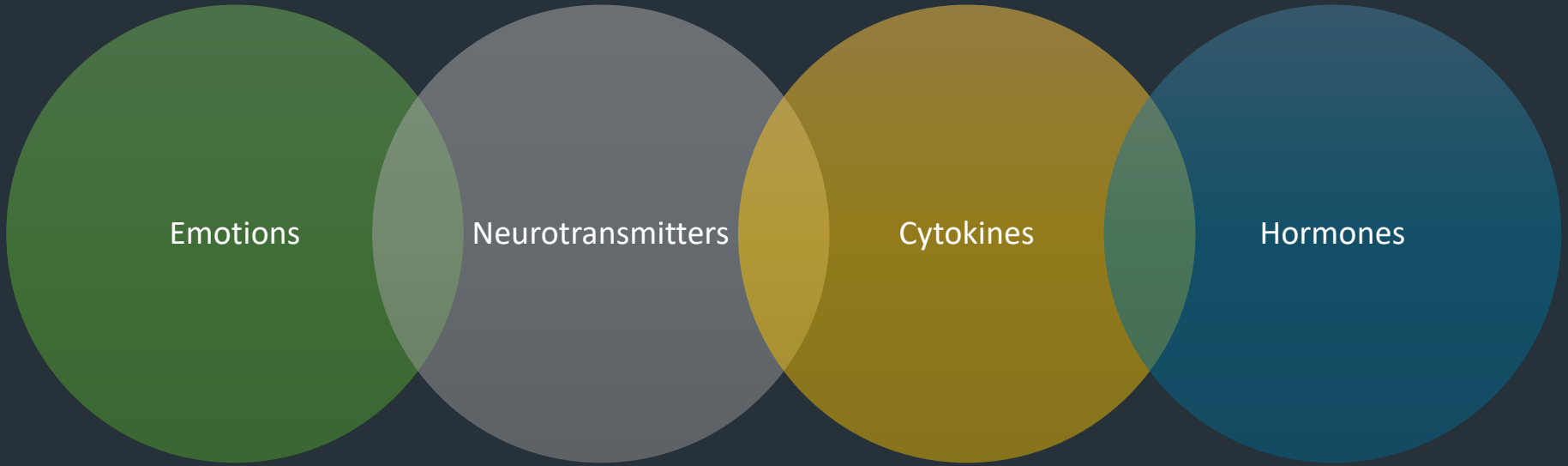


Put the pieces back together

Systems


Nervous System
Immune System
Microbiome
Endocrine System
Health
Environment

Psycho-neuro-endocrin-immunology

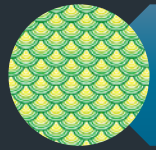


Present

Cytokines

Neurotransmitters

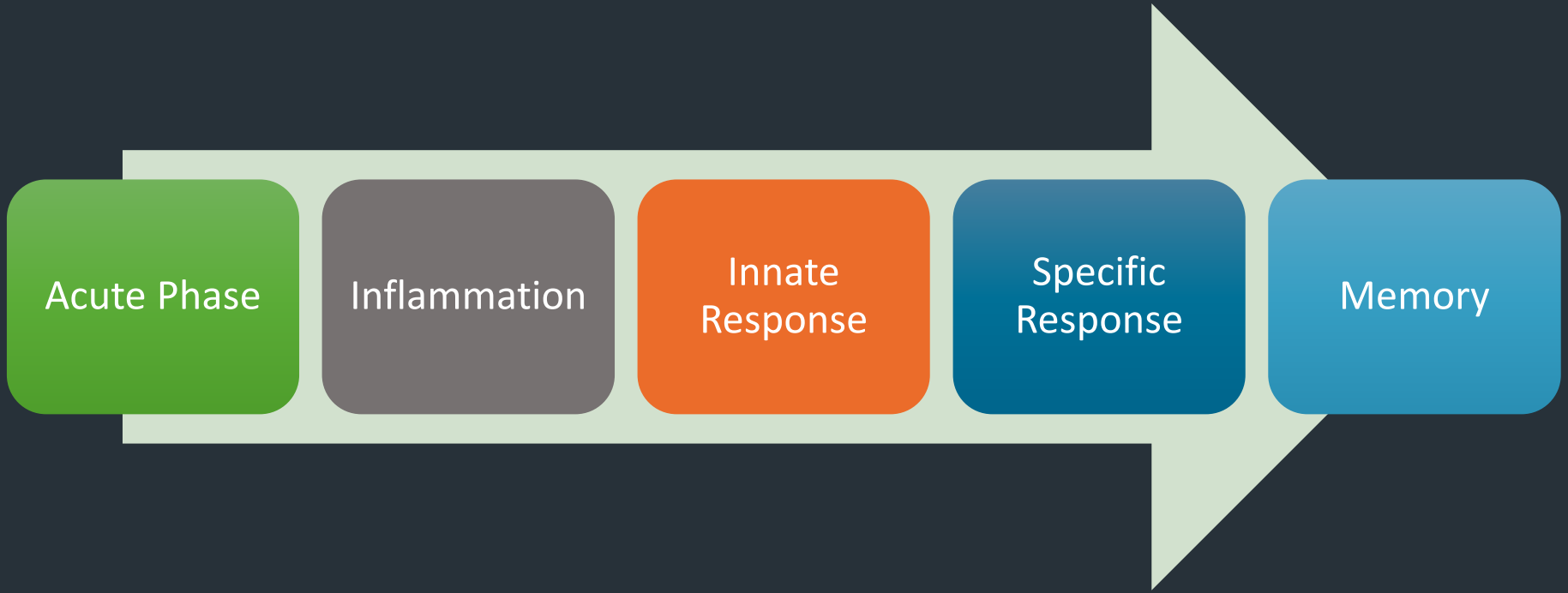
Endorphins



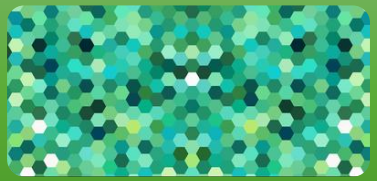
Endocannabinoids

Quick Immune Review

Immune Response



Acute Phase



Defensins

- Anti-microbial peptides that control microflora growth

Kinins

- Peptides that cause vasodilation and smooth muscle contraction

Complement

- Proteins involved in microbial cell death and inflammation

Neurological Effects

Defensins

- Bind to sensory neurons
- May reduce excitability
- May be involved in neuroinflammation

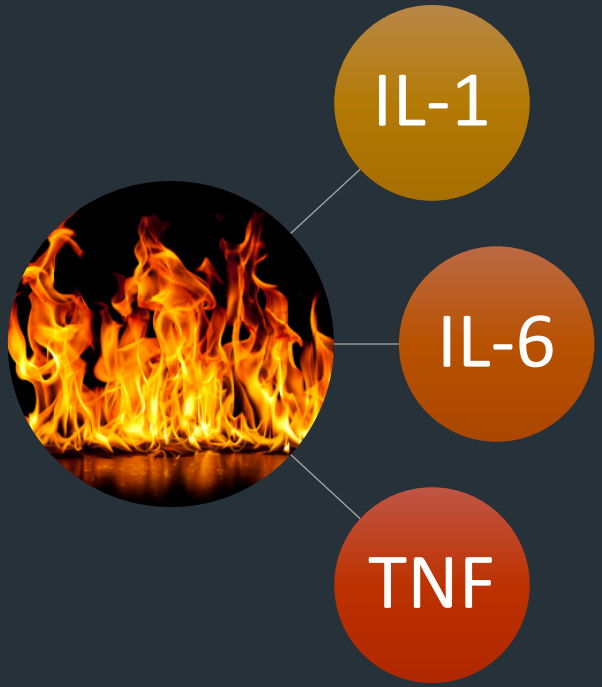
Kinins

- Neurogenesis and neuroprotection
  - Bradykinin following brain injury
- Control blood flow

Complement

- Glial cells express C’ receptors
- Produced in the brain in response to injury
- Mis-regulated in AD

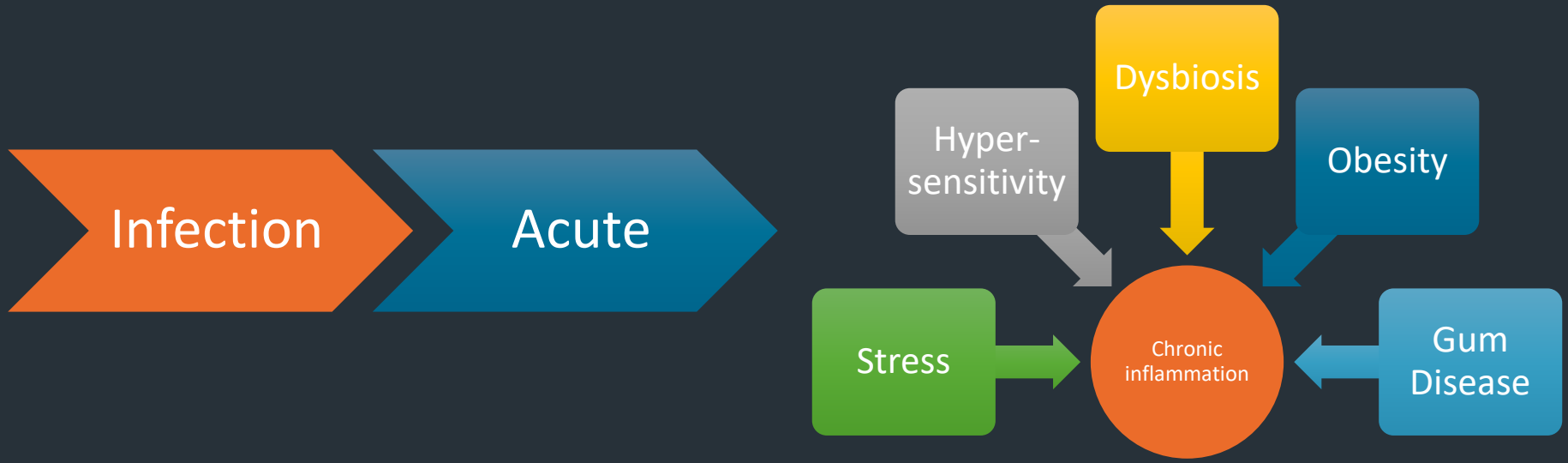
Inflammation – Acute and Chronic



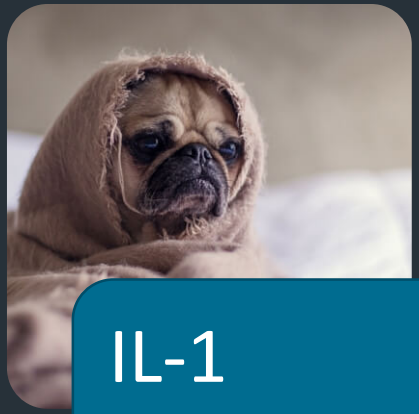
- Degree of pathology associated with amounts of these cytokines

- Immune system
- Brain

Acute vs Chronic

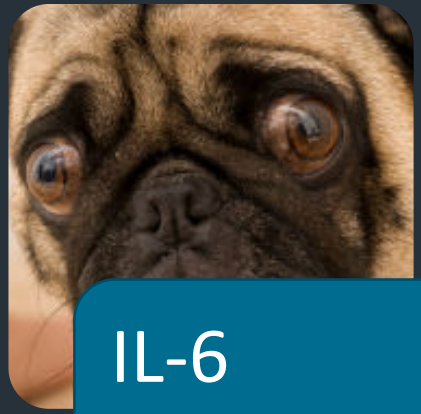


Inflammation – Neurological relationship



IL-1

- Depression



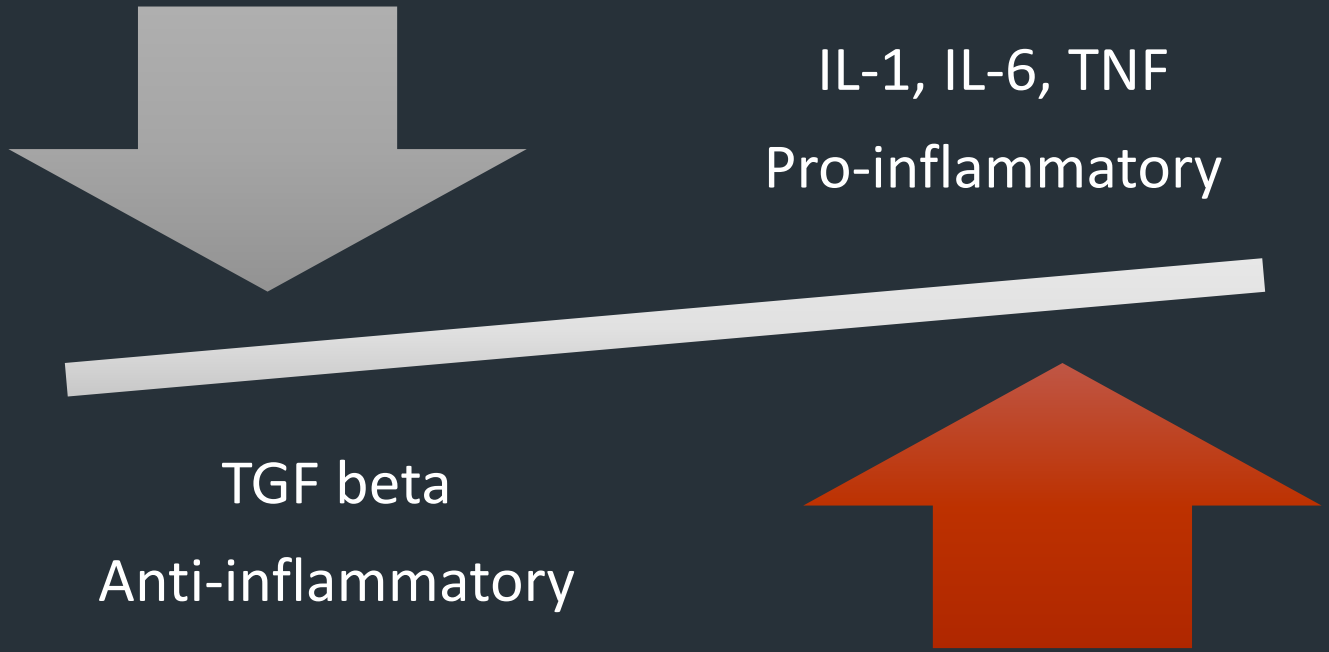
IL-6

- Anxiety

TNF

- Hostility

Balance



TGF beta

Nutrition

Microflora (*Bifido*)

Vitamin A

Innate Immunity

Macrophages & Dendritic Cells

- Phagocytose pathogens, activate specific immunity
- Oxidative stress → Kill pathogens, damage tissue

Neutrophils

- Phagocytose and kill pathogens

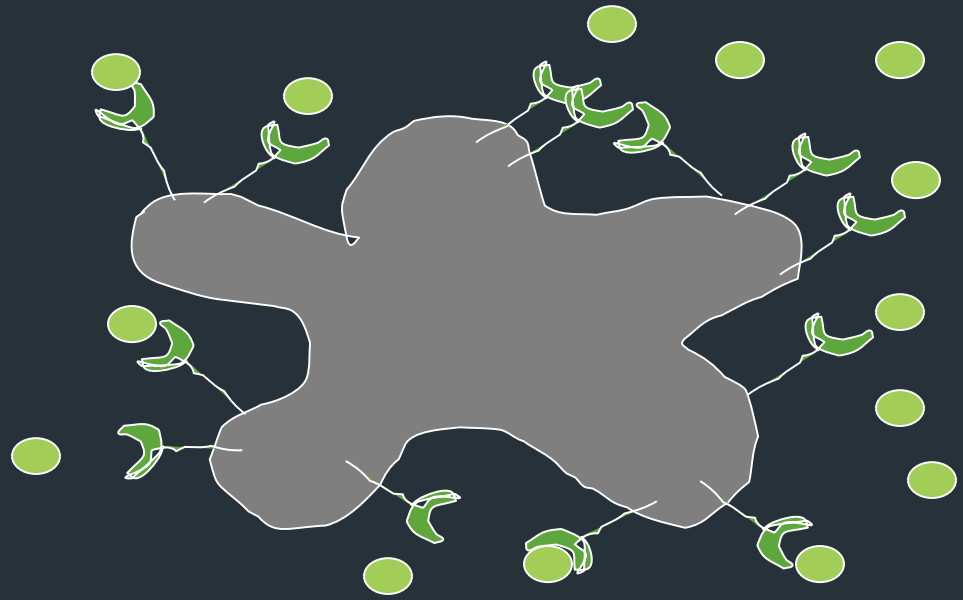
Relationship with neuro-endocrine system

Macrophages express both α and β adrenergic receptors

Szelenyi J, et al. Differential involvement of sympathetic nervous system and immune system in the modulation of TNF-alpha production by alpha2- and beta-adrenoceptors in mice. *J Neuroimmunol*. 2000;103(1):34–40.

α receptors are high affinity-

Bind low concentrations of epi



β receptors are low affinity-

Bind high concentrations of epi

α receptors are high affinity-

Bind low concentrations of epi

Low Stress– bind α-receptor

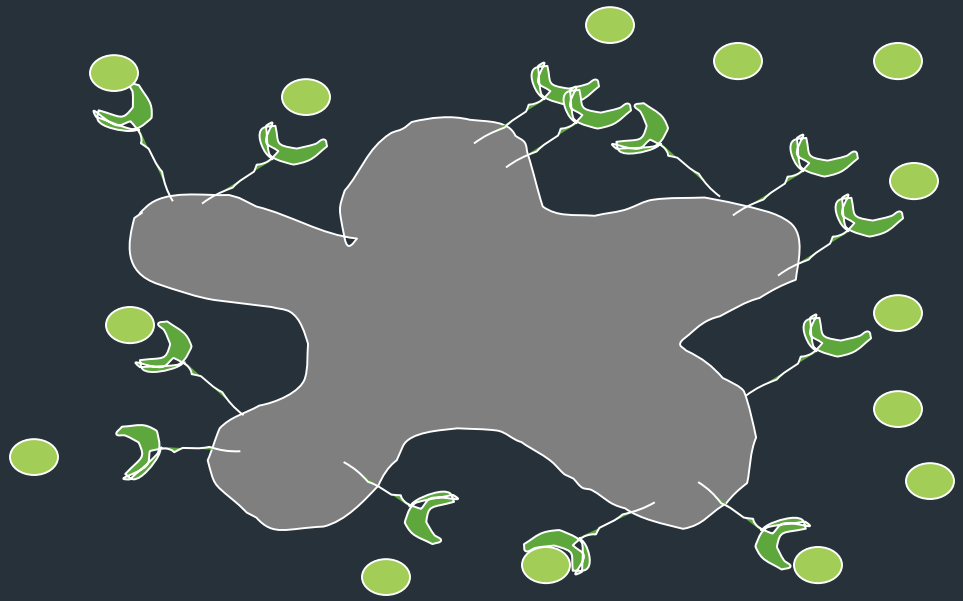
Results upon infection:

- increases phagocytosis upon infection
- increases TNF-α upon infection
- increases IL-6 upon infection

High Stress– bind β–receptor

Results upon infection:

- decreases phagocytosis,
- decreases antigen processing and presentation,
- decreases production of IL-12.



β receptors are low affinity-

Bind high concentrations of epi

Implications

Mind-body therapies stimulate alpha

- Result in increased macrophage activity

Stress (acute and chronic) stimulate beta

- Result in prolonged infections

Specific Immunity

B cells

- Produce antibodies

CD4 T cells

- Produce cytokines

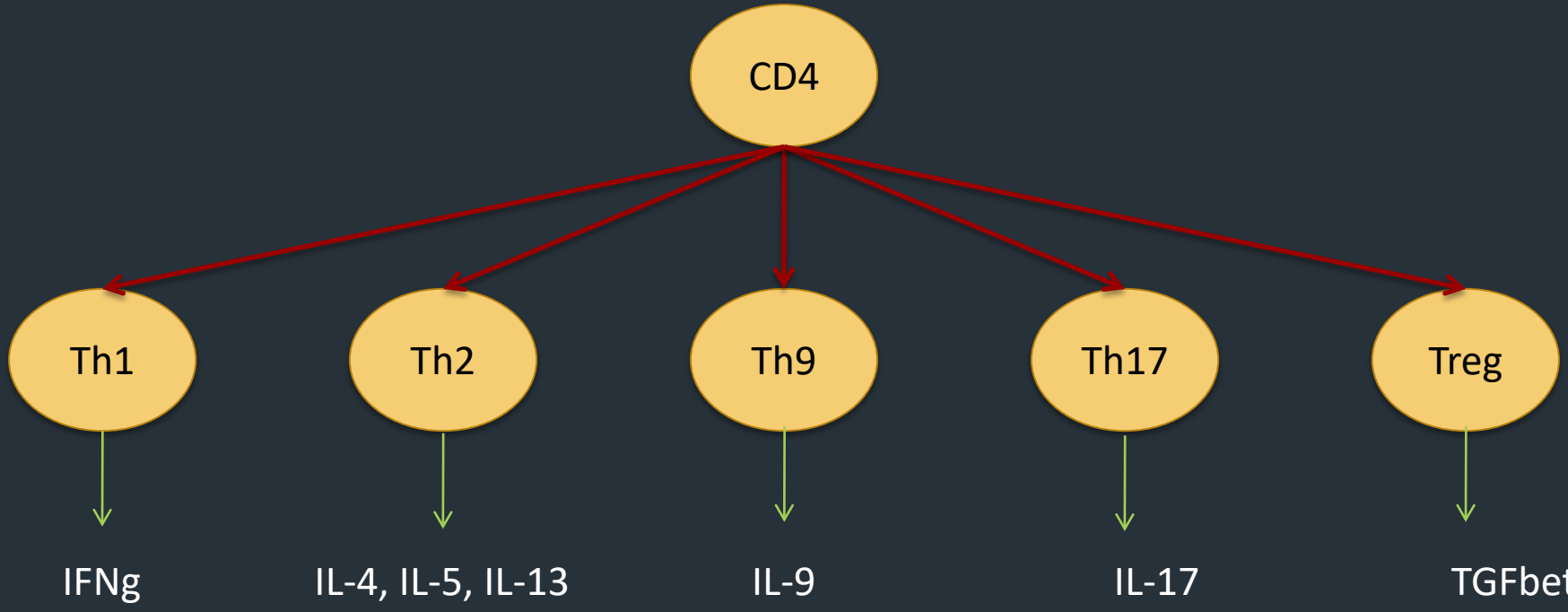
CD8 T cells

- Kill infected cells

Types of Immune Responses

Reactions to:	T Cell Response
Bacteria and Virus	Th1
Worms (some parasites)	Th2
Fungi (some parasites and extracellular bacteria)	Th17
Food	Treg/Th3

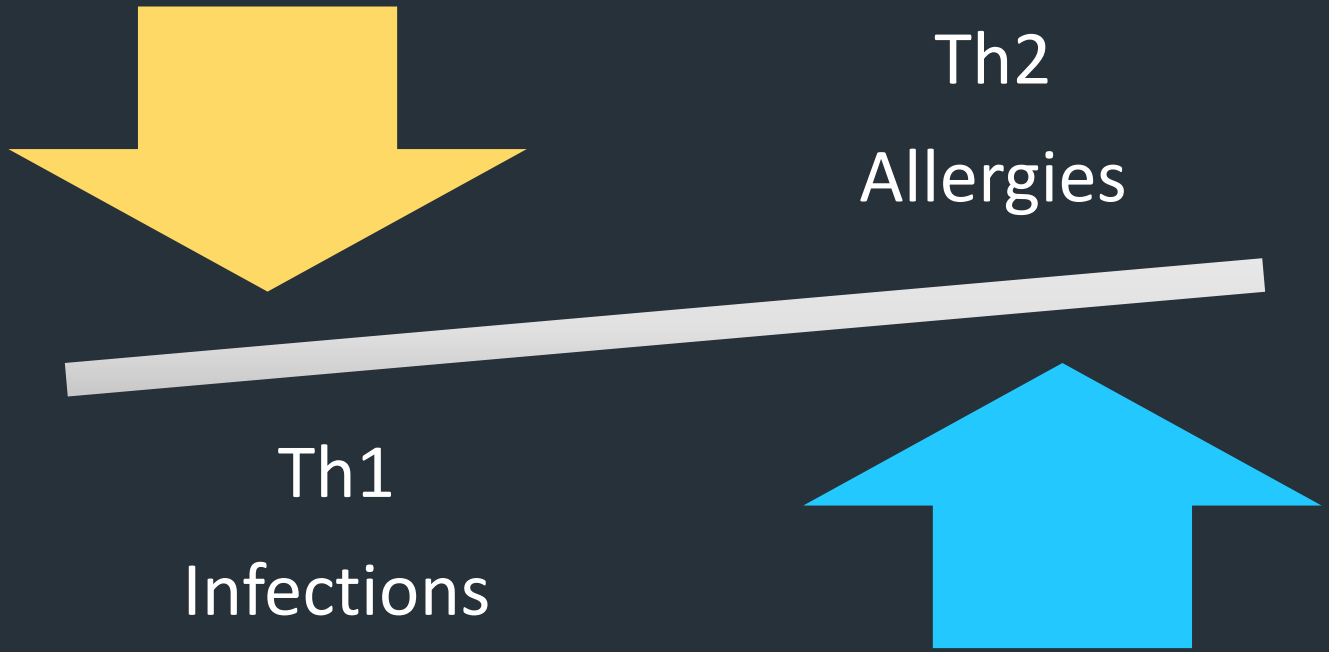
CD4 T cell Subtypes



Antibodies

Reaction to:	T cell	Cytokine	Antibody
Bacteria and Virus	Th1	IFNgamma	IgG
Worms/Allergens	Reaction to	IL-4, IL-5, IL-13	IgE
Food	Th3/Treg	TGFbeta	IgA
Mold/ Autoimmunity	Th17	IL-17	IgG

Balance



Balance

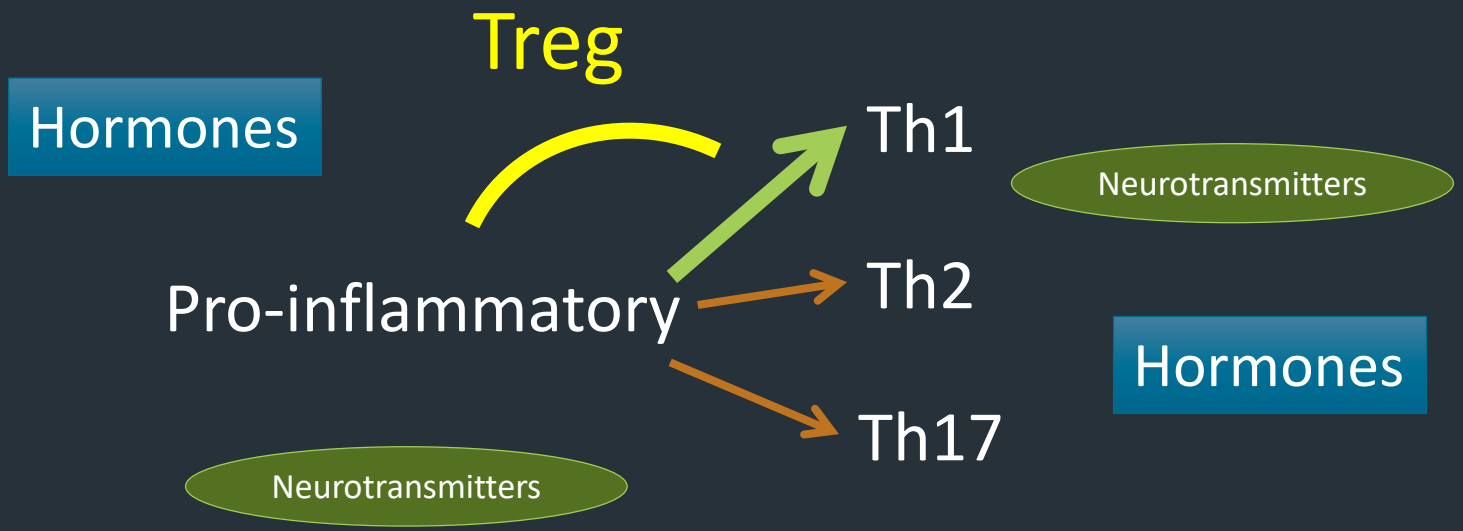
Memory

Initial immune response to pathogen

Expand # of cells specific for pathogen

Next response is faster and more vigorous

Balance


Treg
Hormones
Th1
Neurotransmitters
Pro-inflammatory
Th2
Hormones
Th17
Neurotransmitters

Cell	Express Receptors:
CD4 T cell	β adrenergic receptor Dopamine receptor Acetylcholine receptor 5HT receptor Opioid receptor (?)
CD8 T cell	Dopamine receptor 5HT receptor
B cell	Dopamine receptor
NK cell	Dopmine receptor Opioid receptor
Macrophage	Dopamine receptor α and β adrenergic receptor
Dendritic cell	Dopamine receptor Opioid receptor

Whole system

Constitutional Types

Ayurveda

- Tridosha
  - Pitta, Kapha, Vata

Asian and Oriental Medicine

- 5 Element Theory
  - Wood, Fire, Earth, Metal, Water

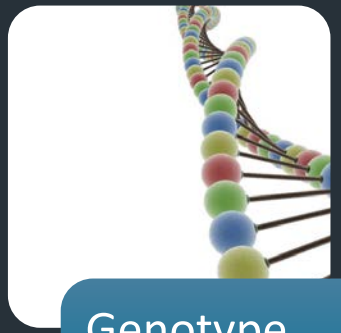
Constitutional Types

- Historically, when a physician took into account someone's constitution, they were using phenotype, to predict genotype.

- While this isn't always accurate, it's much more effective than a one-size-fits-all approach to medicine

Phenotype

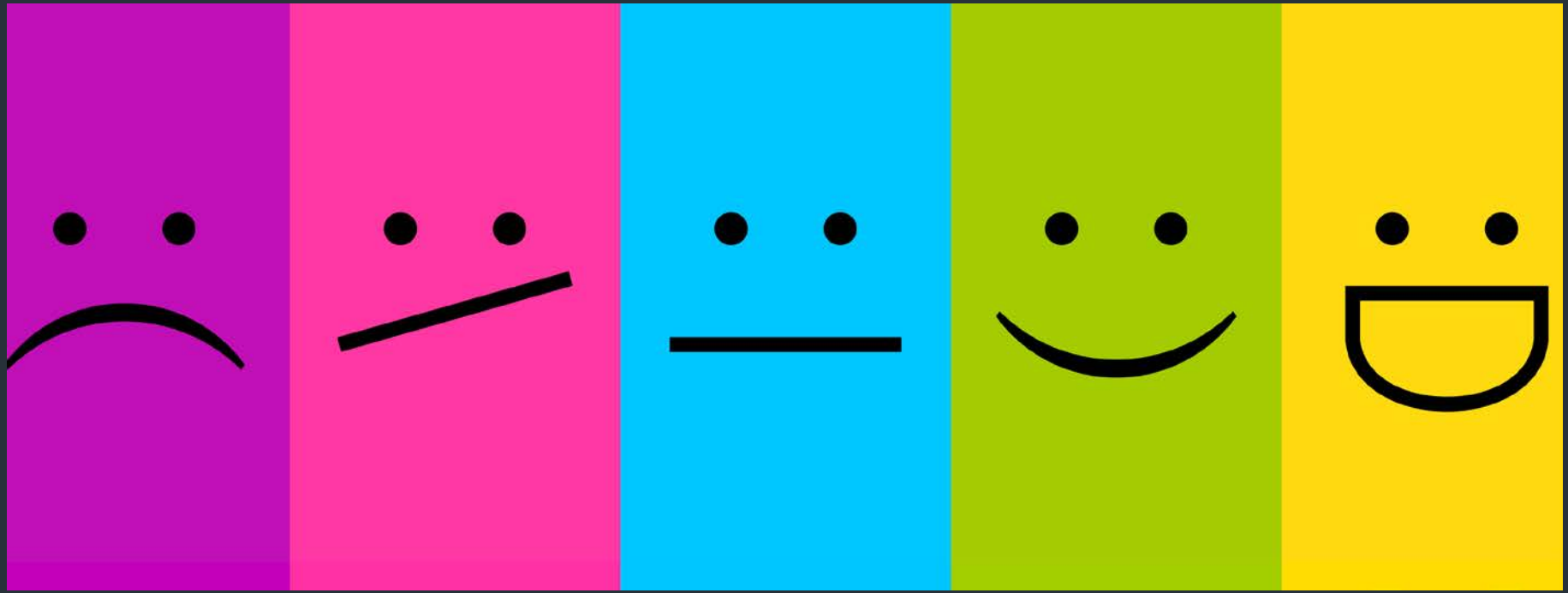
- What is the underlying constitution of the patient?



Genotype

- Which therapy will patient respond to?

Equivalent in Biomedical Medicine



Well-studied Moods

Stress

Bereavement

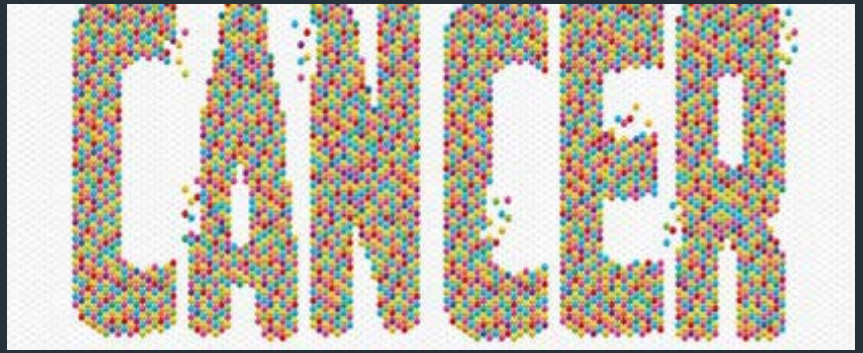
Happiness

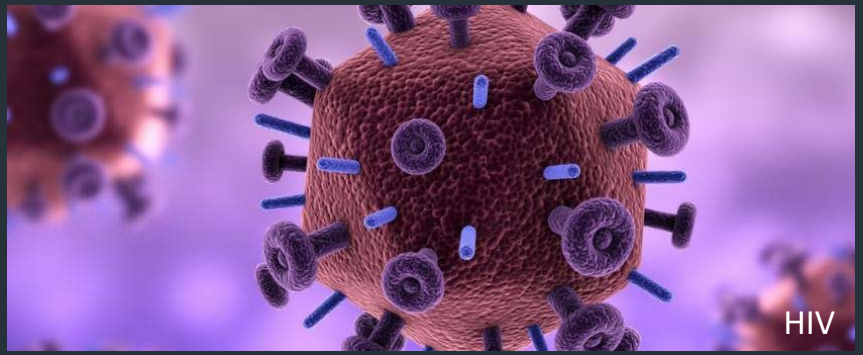
Stress

Why do we know so much about stress?

Bereavement

Why do we know so much about bereavement?





Happiness

Why do we know anything about happiness?

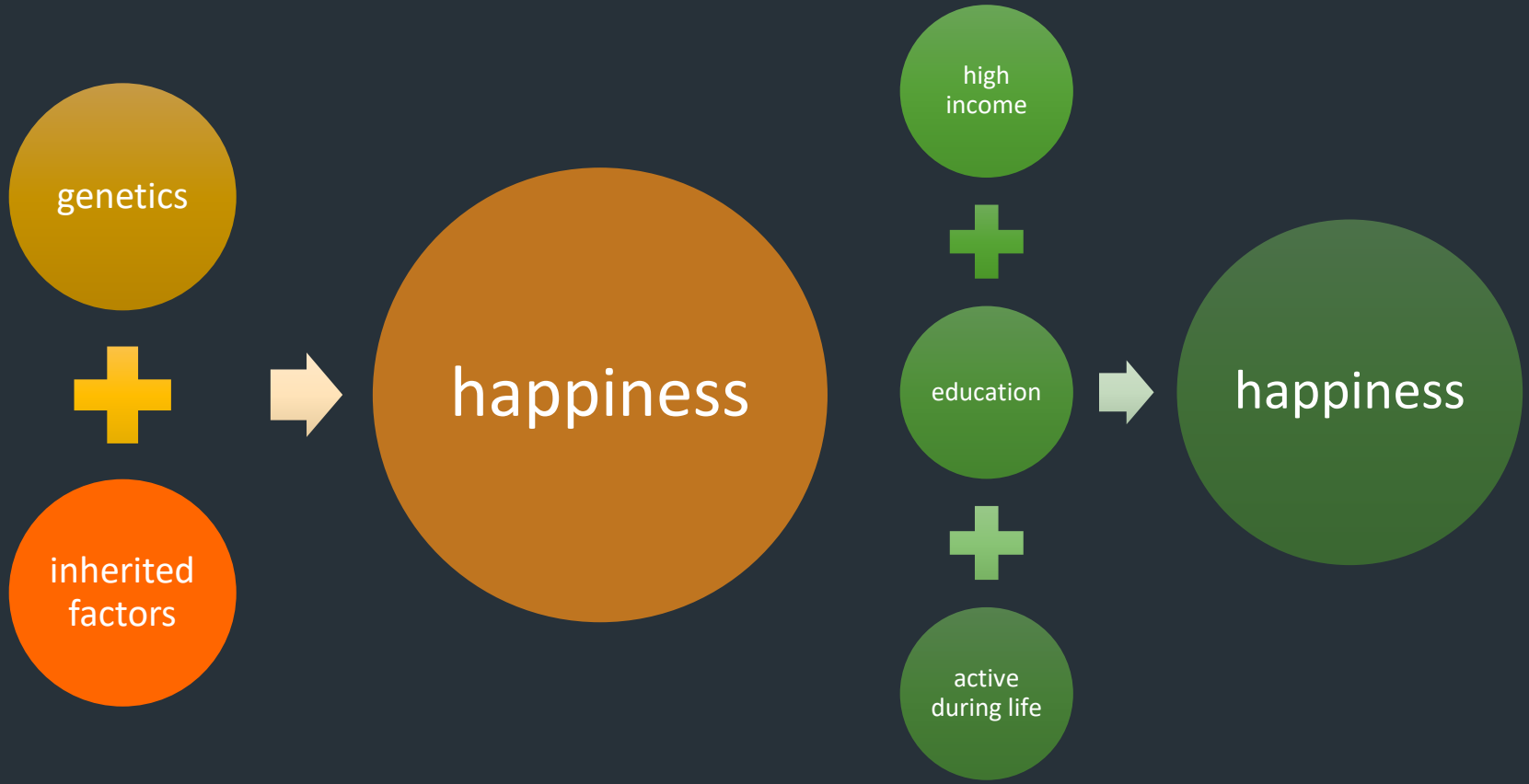
Today's focus

Happiness

Stress

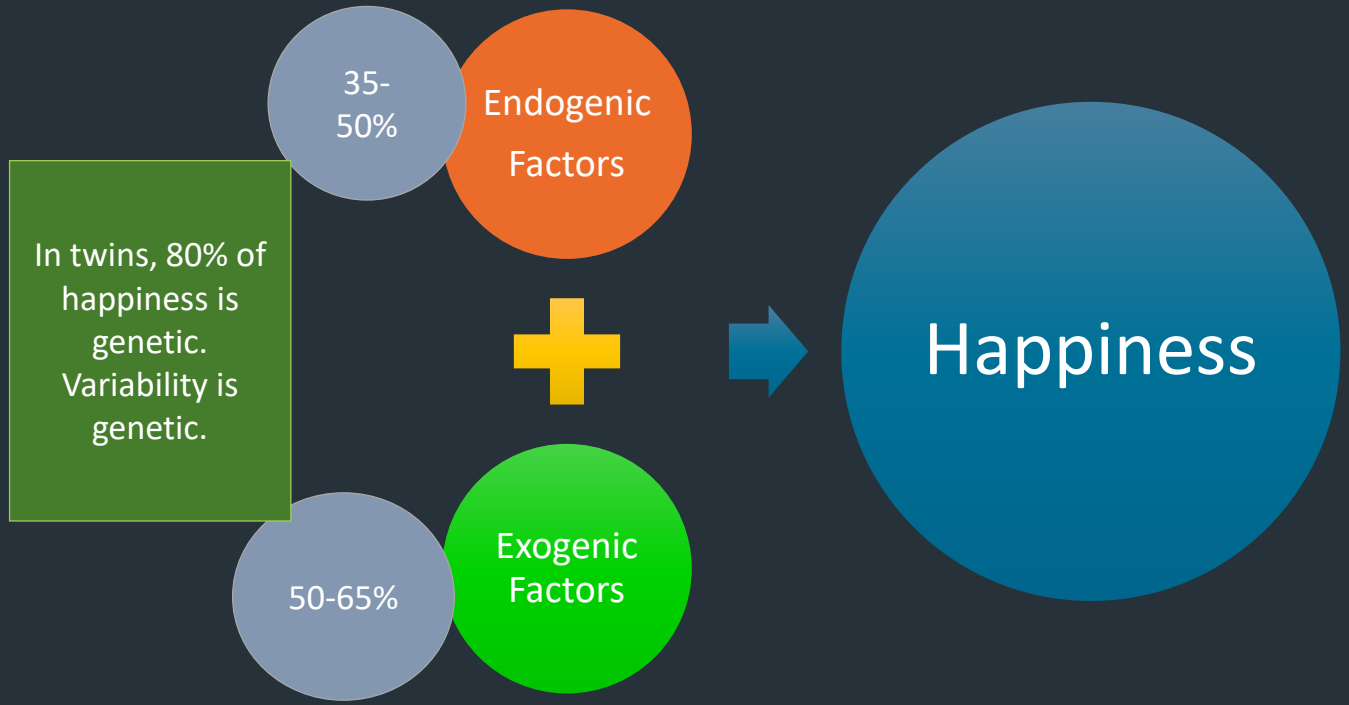
Let’s start with happiness!

Happiness – Historical Debate



Dfarhud D, et al. Happiness and health: the biological factors- systematic review article. *Iran J Public Health*. 2014 Nov;43(11):1468-77.

Happiness – Today



Dfarhud D, et al. Happiness and health: the biological factors- systematic review article. *Iran J Public Health*. 2014 Nov;43(11):1468-77.

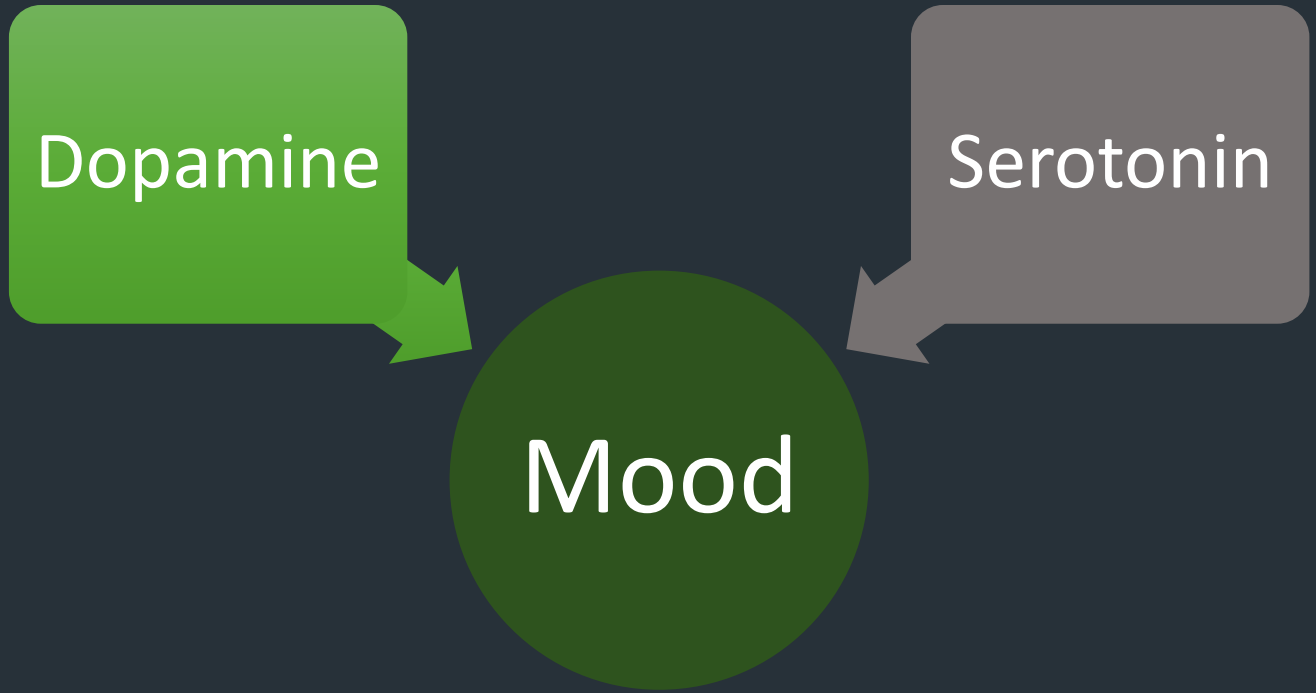
Personality (Positivity/Happiness)

Trait of Emotional Functioning	Test	Measures
Anhedonia	Snaith Hamilton Pleasure Scale	Capacity to experience pleasure
Pleasure Capacity	Temporal Experience of Pleasure	"I look forward to things in my life" (e.g. a good yawn)
Depression-related and Anxiety-related Distress	Mood and Anxiety Symptom Questionnaire	General measure of depression and anxiety
Trait Positive and Negative Affect	PANAS – positive and negative affect scale	20 mood related adjectives and how strongly felt

- Combinations of thoughts, feelings, and behaviors
- Still struggle to define personality scientifically
- Specific combo of neurotransmitters and neuropeptides

Kirkpatrick MG, et al. Emotional traits predict individual differences in amphetamine-induced positive mood in healthy volunteers. *Psychopharmacology* (Berl). 2016 Jan;233(1):89-97.

Neurotransmitters and Genetics



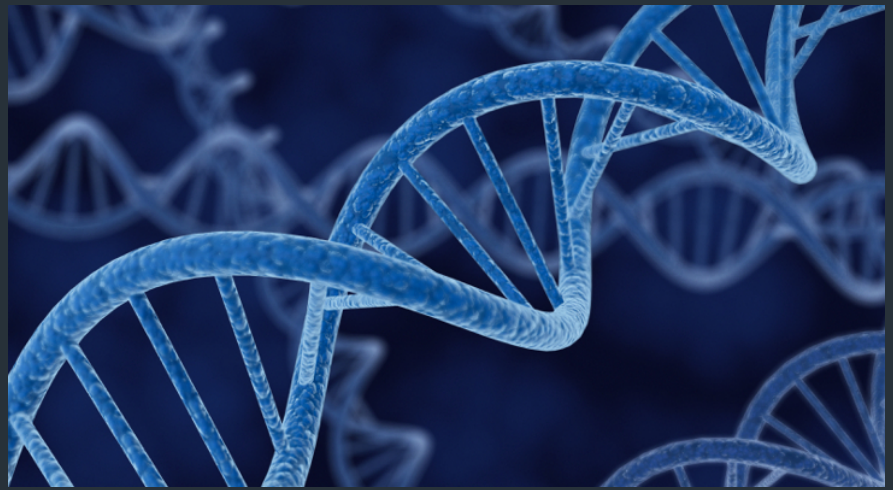
Genes involved

5HTTPR

- Regulates serotonin distribution in the brain

MAO-A

- Catabolic enzyme for serotonin, dopamine, and norepinephrine
- The less you express, the happier you are
- In general, women express less MAO



Dfarhud D, et al. Happiness and health: the biological factors- systematic review article. *Iran J Public Health*. 2014 Nov;43(11):1468-77.

Neurotransmitters

Dopamine

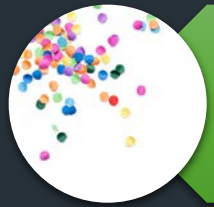
Serotonin

GABA

Source: Brain and gut. Microbes in the gut make neurotransmitters.

Neurotransmitters and Mood

Dopamine



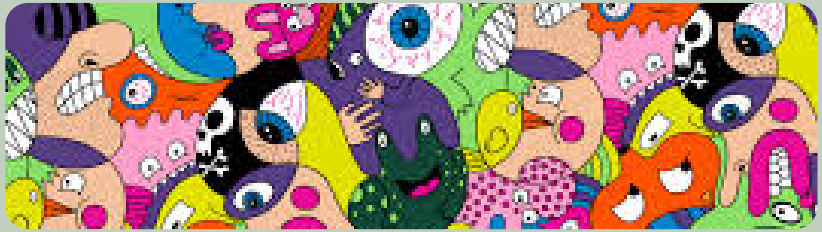
Reward

Positive Affect

Extraversion

Wacker J. Effects of positive emotion, extraversion, and dopamine on cognitive stability-flexibility and frontal EEG asymmetry. *Psychophysiology*. 2017;55(1):12727.

Dopamine



Psychological Effects

- Low – Depression, ADHD, Social Anxiety Disorder
- High – Schizophrenia, Mania

Gut Effects

- Contraction of the colon

Dopamine and Immunity

Increase Allergies – Th2

- Stimulation of D1-D5 decreases IFNgamma (Th1) and increases IL-4 (Th2) in the blood
- IL-4 increases BDNF and learning

Dopamine at certain concentrations drives Treg

- 5 different dopamine receptors
- Effects are concentration dependent

Evidence that dopamine is misregulated in autoimmune disease (MS, Lupus, IBD)

Huang Y1, et al. Roles of dopamine receptor subtypes in mediating modulation of T lymphocyte function. *Neuro Endocrinol Lett*. 2010;31(6):782-91.
Pacheco, et al. The dopaminergic system in autoimmune diseases. *Front Immunol*. 2014 Mar 21;5:117.

Neurotransmitters and Mood

Happiness

Motivation

Calm

Serotonin

Ward R, et al. The role of serotonin in personality inference: tryptophan depletion impairs the identification of neuroticism in the face. *Psychopharmacology* (Berl). 2017 Jul;234(14):2139-2147.

Serotonin

Psychological Effects

- Low – Anxiety, Depression, Mood Impulse Disorders
- High – Agitation, Restlessness

Gut Effects

- Regulates bowel function and appetite

Neurotransmitters and Mood

GABA

(inhibitory)

Relaxation

Sleep

Focus

GABA

Psychological Effects

- Low – Depression, mania, ADHD
- Relaxation – Xanax and Valium target GABA receptors

Gut Effects

- Intestinal motility,
- Relaxation
- Reduce sensation

Granger AJ, et al. *Curr Opin Neurobiol*. 2017;45:85-91.

GABA and Immunity

GABA is Anti-inflammatory

Alzheimer’s disease

- Decreased GABA
- Increased inflammatory cytokines

Autism

- Decreased GABA
- Increased neuroinflammation, increased glutamate excitotoxicity

Ahuja M, et al. Immunological alteration & toxic molecular inductions leading to cognitive impairment & neurotoxicity in transgenic mouse model of Alzheimer's disease. *Life Sci.* 2017 May 15;177:49-59.
El-Ansary A, et al. GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders. *J Neuroinflammation*. 2014 Nov 19;11:189.

Neurotransmitter Summary

Neurotransmitter	Mood Effect	Gut Effect	Immune Effect
Dopamine	Pleasure/ Depression	Colon contraction	Decreases Th1, Increases Th2 Could increase Treg or Th17
Serotonin	Happy/Anxiety	Bowel movements	IFNg decreases serotonin
GABA	Relaxation/ Depression/ Mania	Intestinal motility; Pain reduction	Decreases pro-inflammatory cytokines

Endorphins

Endogenous opioids are released during…


Exercise


Chocolate


Sex

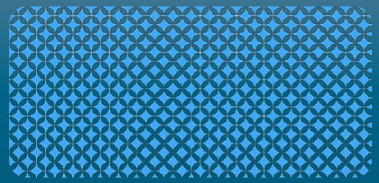

Fear


Love


Music


Laughter

Natural Endorphins and Immunity



mu receptor

- Pro-inflammatory

delta receptor

- T cell proliferation

kappa receptor

- Anti-inflammatory

Taub, D. *Cell Immunol*. 2008; 252(1-2):1-6.

Effects of Endorphins on Immunity

- Opioid abusers have higher incidence of infections
  - Impaired immunity
- Opioid treatment results in reactivation of latent viruses
  - *If you're placing a patient on opioids, consider this*
  - Slows clearance
  - Increases risk of secondary infections

- Influenza
  - Morphine impairs immunity in lungs
  - Opioids decrease NK cell activity
  - Opioids increase risk of pneumonia

Tahamtan A, et al. Opioids and viral infections: a double-edged sword. *Front Microbiol.* 2016;7:970.

Exorphins

Food derived peptides that bind to opioid receptors found in…

Bread

Milk

Corn

Bressan P, et al. Bread and other agents of mental disease. *Front Hum Neurosci*. 2016;10:130.
Ziodrou C, et al. Opioid peptides derived from food proteins. The exorphins. *Journal of Biological Chemistry*. 1979;254(7):2446-2449.

Comfort Food

Exorphins – Autism Relationship

Extreme introversion

Social indifference

Repetitive behaviors

Increase in urinary exorphin peptides

Increase in opioid activity

Increase in antibodies to casein and gluten

Hypotheses:

Exorphins directly bind to opioid receptors

Antibodies increase uptake of gluten and casein in the brain

Reichelt KL, et al. Peptides' role in autism with emphasis on exorphins. *Microb Ecol Health Dis*. 2012 Aug 24;23.

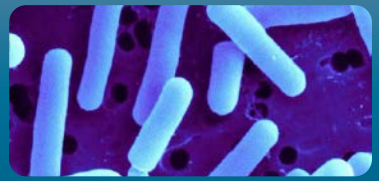
Relationship between Autism and Gut

People with ASD often have food selectivity

- Strong preferences for starches, snack and processed foods

Depleted Bacteroides

- Cause or effect?
- Avoid fruit due to pain?



Lactobacillus and Bifidobacteria improve symptoms of ASD

Endocannabinoids

- Endogenous set of neurochemicals
  - Discovered through effects of *Cannabis sativa*
  - CB1R is expressed in the brain and peripheral tissues
    - Associated with cognition and movement
  - CB2R is on lymphoid cells
    - B, T, Mac, DC, Neuts, and NKs
    - Involved in psychiatric disorders including schizophrenia, depression, and bipolar disorder

Effects of Endocannabinoids on Immunity

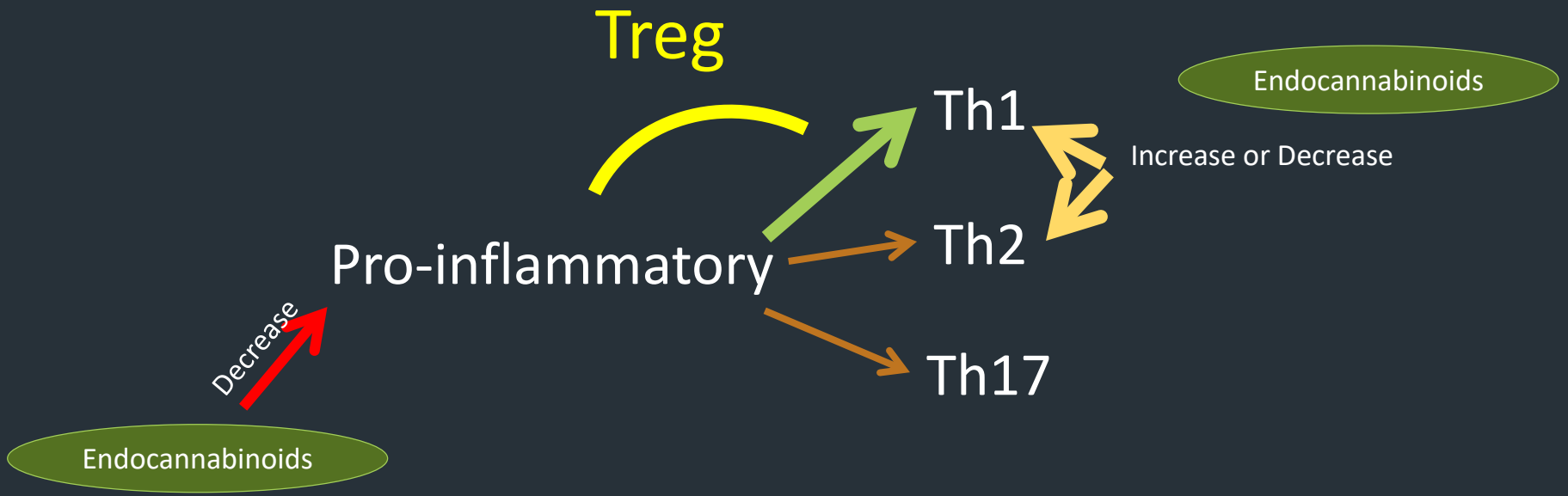
Pathogens stimulate macrophages and DCs

Reduce expression of endocannabinoid-degrading enzymes

Increases endocannabinoids in the body

Increases B cell migration; Shifts cytokine profiles

Endocannabinoids and Immunity



Pandey R, et al. Endocannabinoids and immune regulation. *Pharmacol Res.* 2009;60(2):85–92.

Endocannabinoids ≠ **Cannabis**

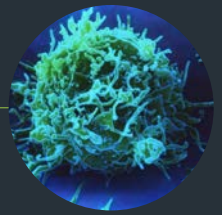
Endo

- Endocannabinoids modulate Th1 and Th2

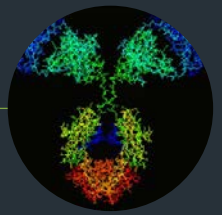
Plant

- Plant derived cannabinoids increase Th2

Laughter



Increase
NKs



Increases
Antibodies

Decreases Pro-
inflammatory Cytokines

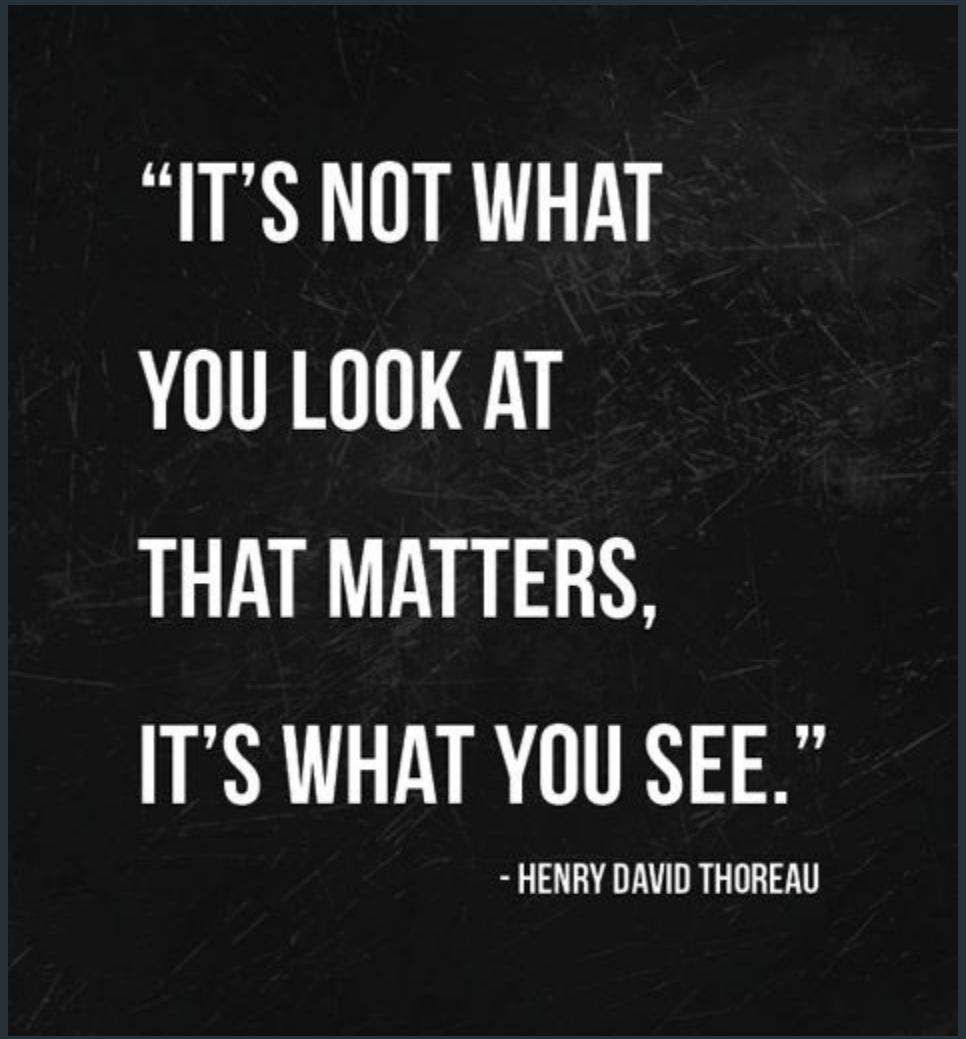
Stress – What is it?

Stress: the perception of threat to the physiological or psychological well being

and

the perception that the individual's responses are inadequate to cope with it.

★ **Abililty to Cope** ★

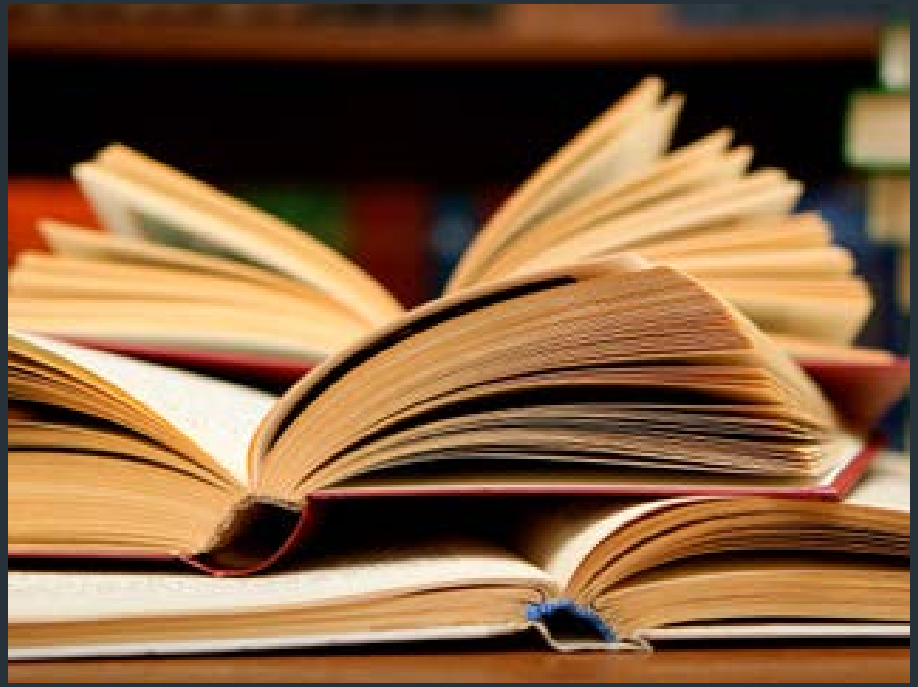

“IT’S NOT WHAT
YOU LOOK AT
THAT MATTERS,
IT’S WHAT YOU SEE.”
- HENRY DAVID THOREAU

Multiple Types of Stress

Sympathetic

Parasympathetic

Sympathetic – Studies with medical students



- Exams and social support effect the response to Hepatitis vaccine response

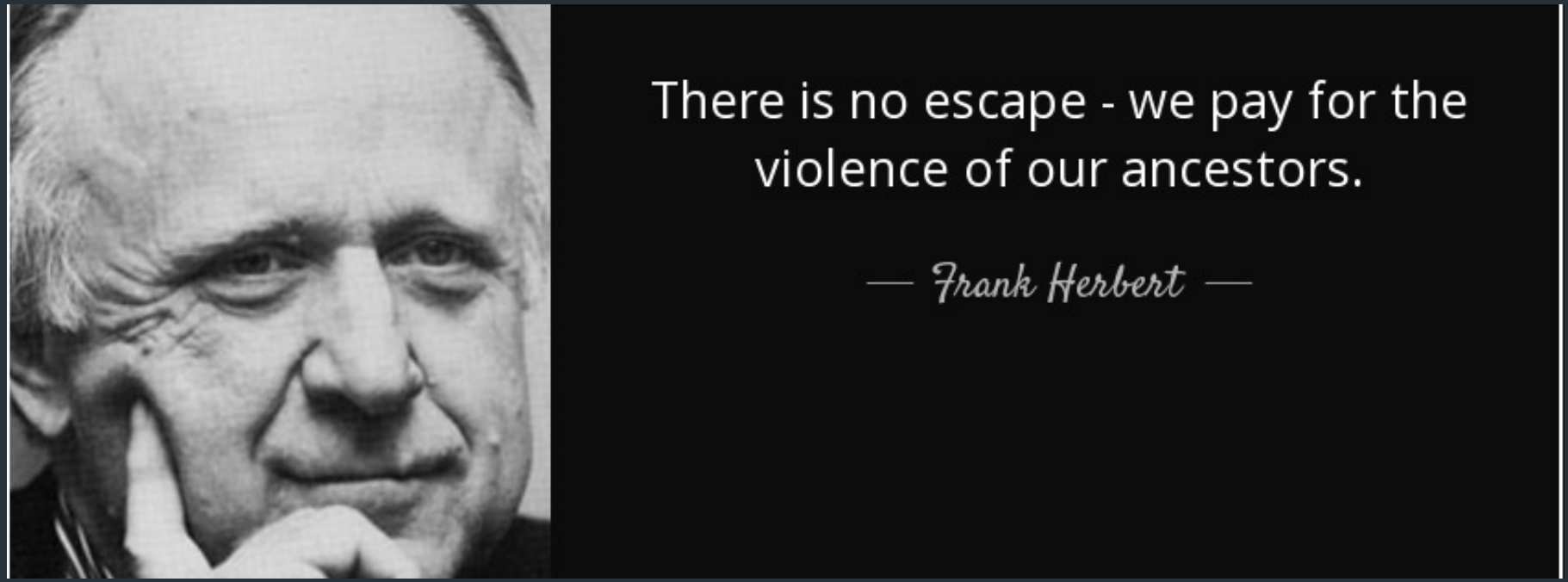
- Isolate peripheral blood leukocytes
  - Treat with catecholamines
  - Shuts down IL-12 production
  - Reduces Th1 which increases Th2
  - Th2 is “allergy”

Other ways we study stress

Hostile marriage increases IL-6 and hs CRP

Increase of IL-1b and TNFalpha

Why?


There is no escape - we pay for the violence of our ancestors.
— Frank Herbert —

HPA Axis

High Cortisol Effects (Acute Stress)

Endocrine

- Increases blood glucose
- Decreases testosterone

Immune

- Blocks T cell proliferation
- Reduces secretion of cytokines

Nervous

- Solidifies a memory
- Decreases overall memory over time

Chronic High Cortisol

Endocrine

- Decreased thyroid function
- Accumulation of abdominal fat

Immune

- Prolonged healing time
- Inability to respond to infections

Nervous

- Impaired cognition

Advocating for vacations and siestas

Endocrine Immune Relationship

Most Studied Endocrine Immune Interaction

- Glucocorticoids
  - “Immunosuppressive”
  - 1950 Nobel Prize to Hench, Kendall, and Reichstein
  - Discovery of Cortisone and its effects on Rheumatoid Arthritis
  - Since then, glucocorticoids are used commonly as anti-inflammatories

Dexamethasone

Prednisone

Hydrocortisone

Effects of Hormones on the Immune System

Cytokines

Cell Trafficking

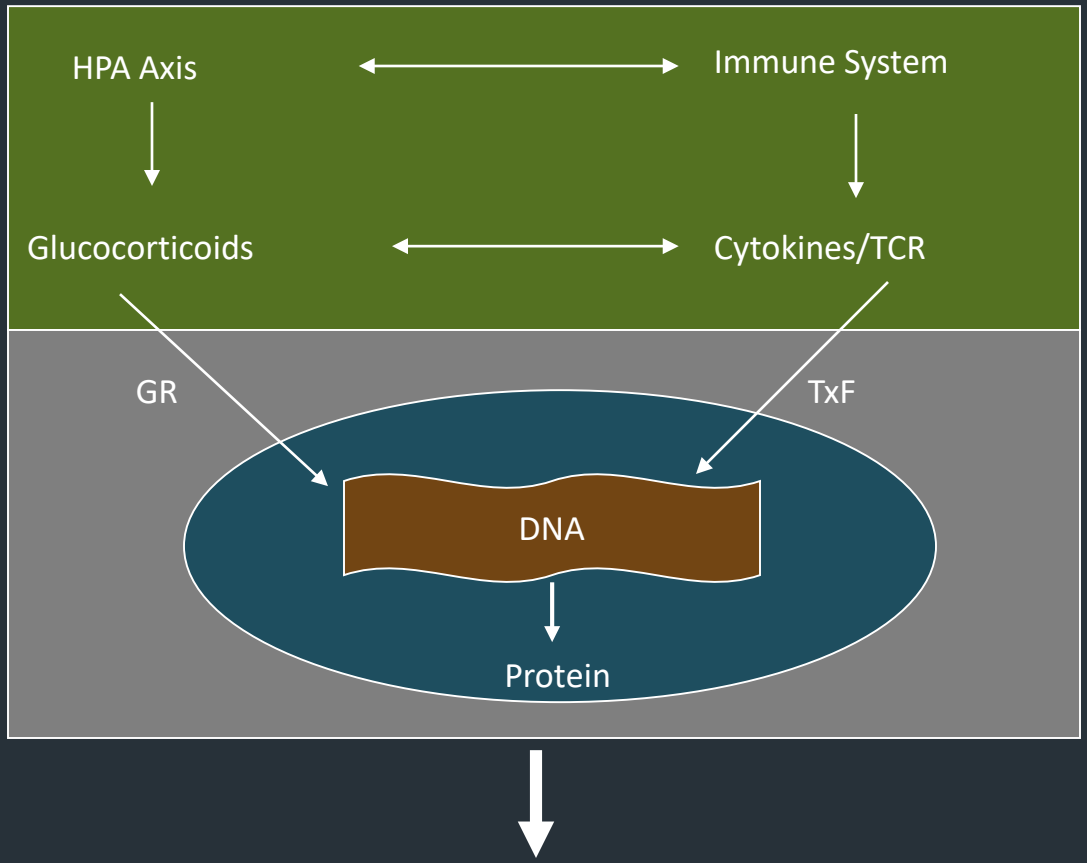
Adhesion Molecules

Proliferation

Effector Functions

Systemic

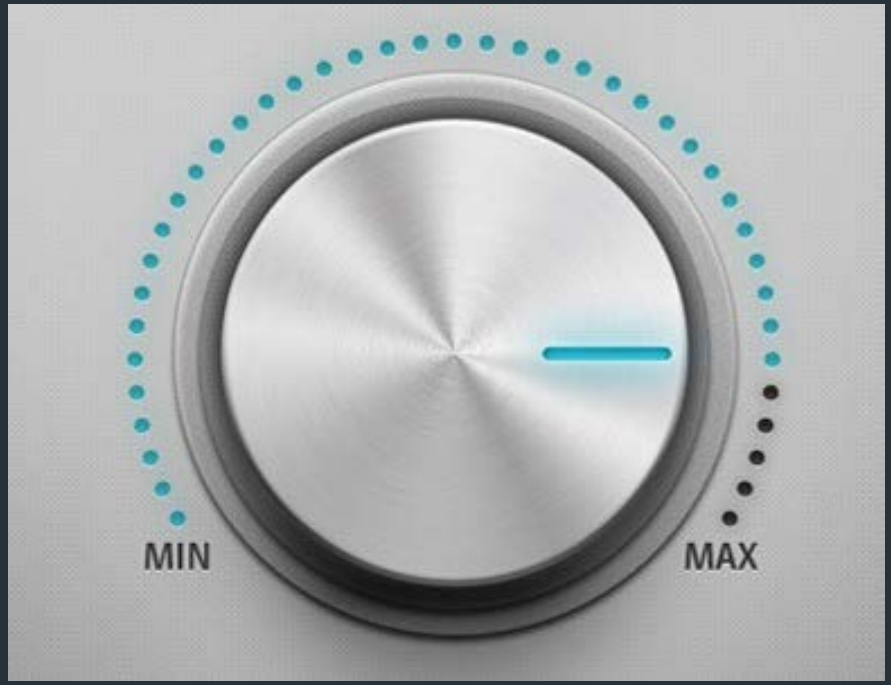
Cellular and Molecular



Adapted from Arzt et al. 2001

Glucocorticoid Mechanism of Action

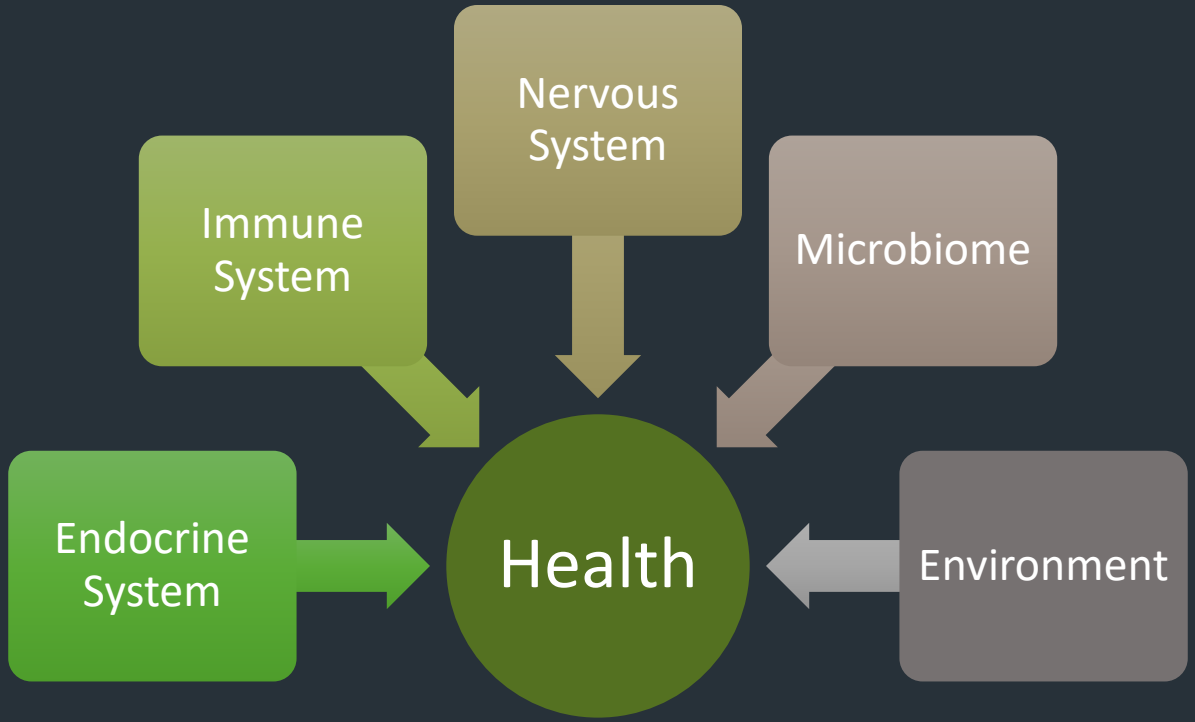
- Glucocorticoid receptors are transcription factors
  - Cytokine genes have Glucocorticoid Response Elements
  - Glucocorticoids can regulate how much cytokine is made



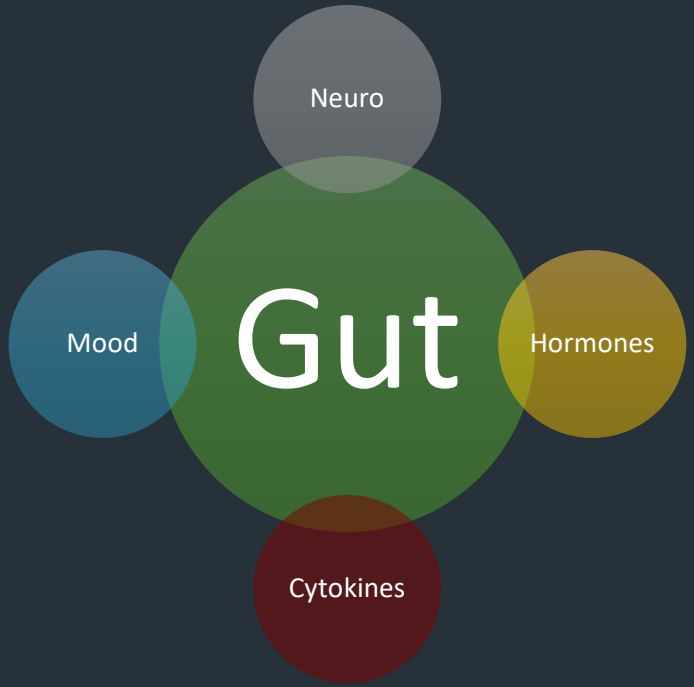
Hormones and Cytokines

Hormone	Endocrine Activity	Immune Effect
Testosterone	Sex steroid hormone	Decreases Th1 (increases Th2) Decreases pro-inflammatory cytokines
Estrogen	Sex steroid hormone	Increases Th1 & Th17 Increases antibody High in RA and SLE
Progesterone	Helps maintain pregnancy; Luteal phase	Shifts from Th1→Th2 Inhibits IL-6, TNF, IFNg Pre-eclampia = high Th1
Prolactin	Lactation; Sexual health in men and women	Increase Th1, Increases antibodies, may increase Th17 (autoimmunity)
Oxytocin	Bonding	Anti-inflammatory, Antibiotic, Wound Healing
DHEAS	Precursor for Testosterone and Estrogen	Decreases IL-6 and IL-12; Increases IL-10

Systems


Nervous System
Immune System
Microbiome
Endocrine System
Health
Environment

Psycho-neuro-endocrin-immunity and Gut



- Gut is huge source of neurotransmitters, cytokines, and hormones
- Microflora can impact immune, neuro, and endocrine outcomes
- Dysbiosis can impact all other diseases

Microbiome

Immune

- Microbiome critical to immune development
- Manipulate cytokine profiles (Th1, Treg, Th17)

Neuro

- Microbiome produces neurotransmitters
- Dopamine, Serotonin, GABA, BDNF

Endocrine

- Influence HPA axis (control ACTH and corticosteroids)
- Can also influence oxytocin, prolactin, and other hormones

What causes dysbiosis?

Food

- SAD has very little prebiotic potential

Antibiotics

- Human
- Food supply

Chemicals

- Pesticides
- Artificial sweeteners

Bian X, et al. The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PLoS One*. 2017;12(6):e0178426.

Example – Parkinson's Disease

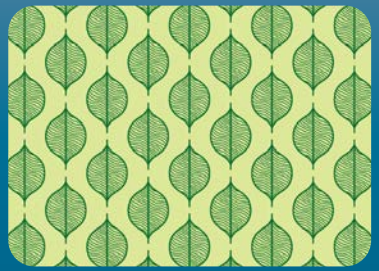
Reduced Prevotella

- Increased Enterobacteriae

Correlates with gait difficulty & postural instability

Eerola-Rautio J, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord*. 2015;30(3):350-8.

What kills the Prevotella?



Glyphosate

- Round Up is an antibiotic
- Kills Lactobacillus
- Used on most soy and wheat

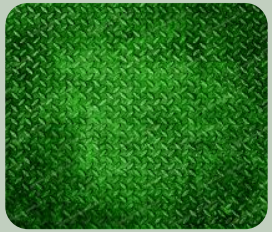
Neonicotinoids

- Bind to nicotinic acetylcholine receptor
- Not thought to cross blood-brain barrier, but gut not studied
- Lactobacillus and Acetobacter
- Used on corn

Daisley BA, et al. Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum* immune stimulation in a Drosophila melanogaster model. *Sci Rep*. 2017 Jun 2;7(1):2703.

Microflora and Brain



Anxiety

Depression

Autism

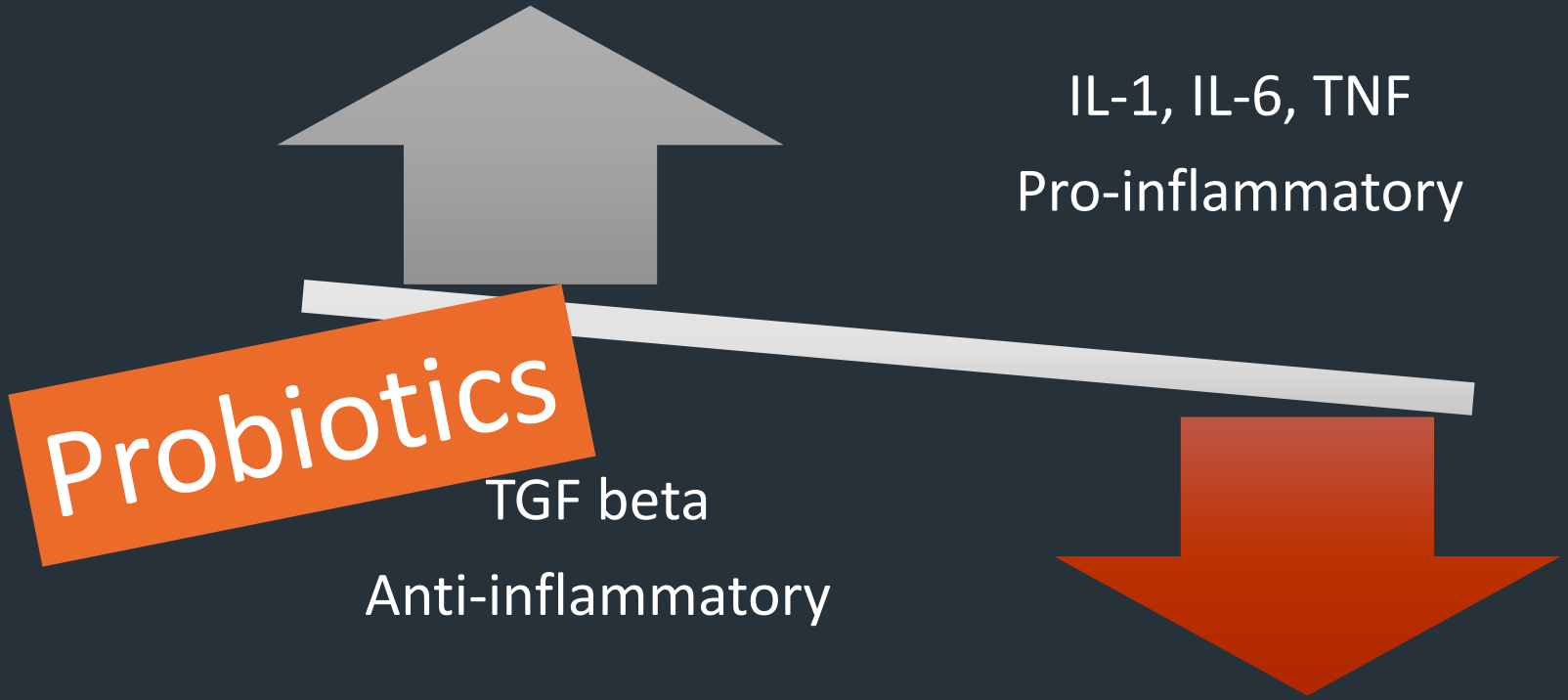
Parkinson's

Multiple Sclerosis

Alzheimer's

Cox LM, et al. Microbiota signaling pathways that influence neurological disease. *Neurotherapeutics.* 2018;15(1):135-145.

Reduce inflammation → Treat the gut



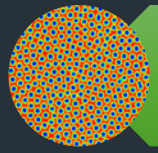
Hur SJ, et al. *Nutr Res*. 2012;32(11):801-16.

Neurotransmitters and Microbes

Neurotransmitter	Microbial Species
GABA	Lactobacillus & Bifidobacterium
Noradrenalin	Escherichia, Bacillus, and Saccharomyces
Serotonin	Candida, Streptococcus, and Escherichia, and Enterococcus
Dopamine	Bacillus
Acetylcholine	Lactobacillus

Cenit MC, et al. Influence of gut microbiota on neuropsychiatric disorder. *World J Gastroenterol*. 2017;23(30):5486–5498.

Treat the Gut



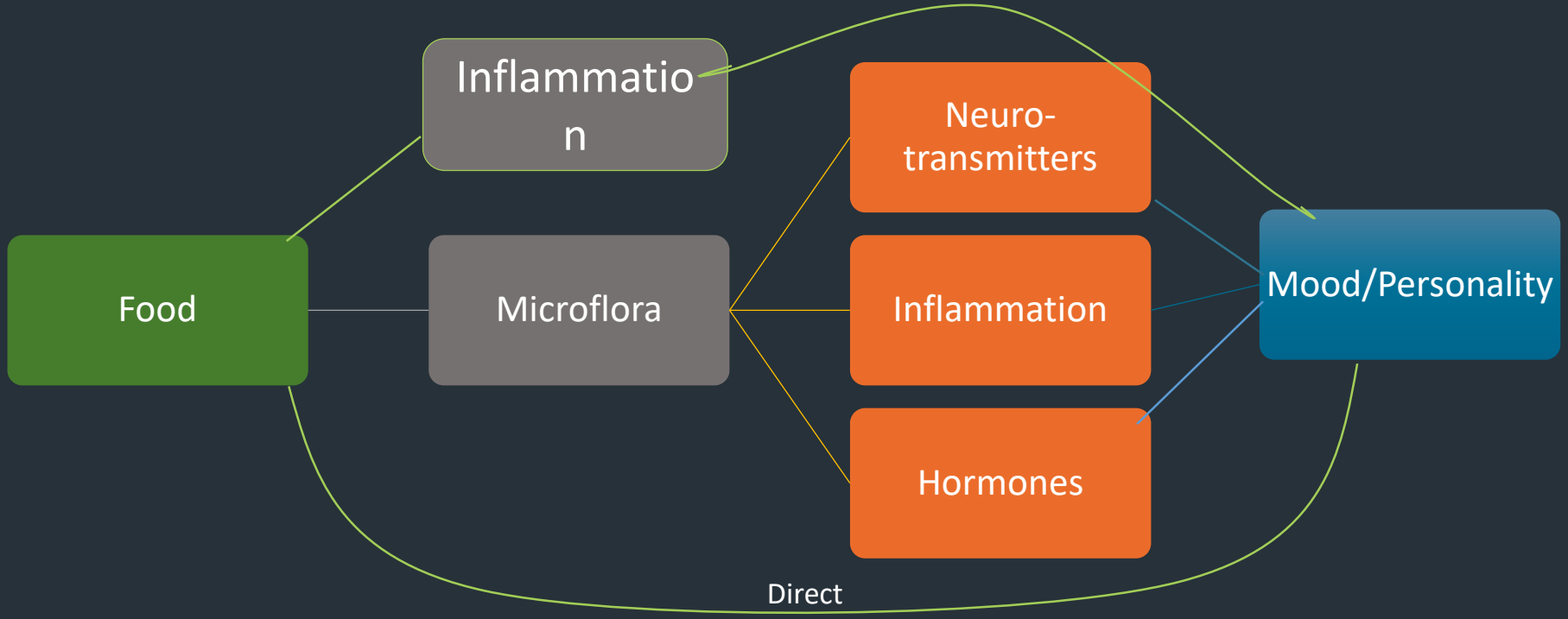
Antibiotics

Probiotics

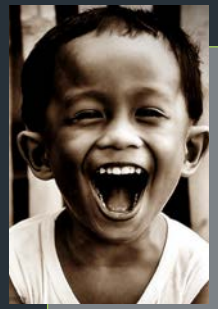
Diet/Prebiotics

Fecal Transplant

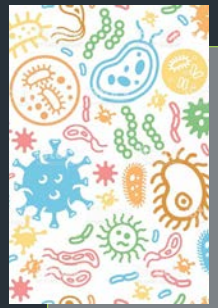
Summary of Immune Response to Food →PNI



Summary

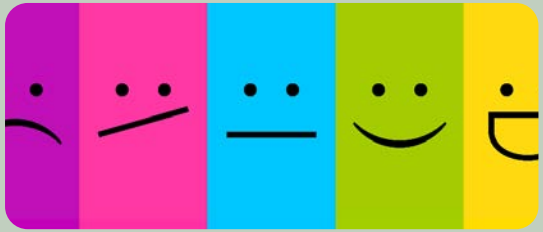


Moods impact the immune, nervous and endocrine system and vice versa



The microbiome impacts the immune, nervous, and endocrine system and vice versa

Summary



Moods can provide biochemical insight

Stress & happiness are well studied

Interventions can happen from multiple directions

Summary: To address psycho-neuro-endocrin-immune outcomes…

Thank you!!!


Metagenics Institute


NIH
National Center for
Complementary and
Integrative Health

Break- 30 min

Please return by 10:30 am

The Microbiome in Neuropsychiatry

The Microbiome in Anxiety, Depression and Cognitive Decline: What Do We Know?

Robert Kachko, ND, LAc

The Gut-Brain Axis

Clinically Relevant Research and Perspectives

What is a "gut feeling" anyway?

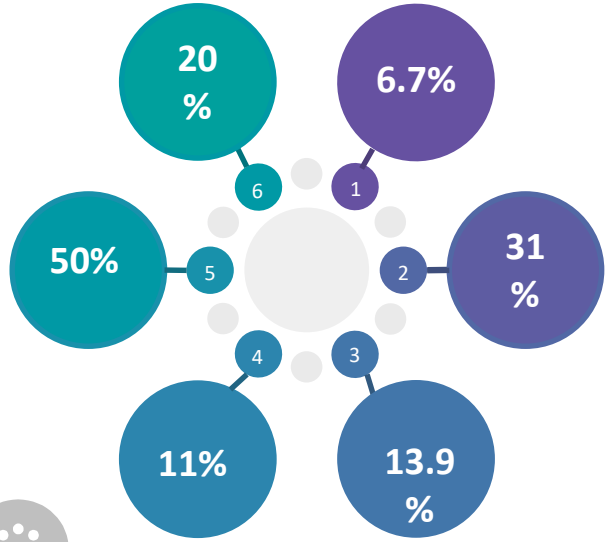
For Context

We are not alone…

The collective genes within the microbiome outnumber genes in the human genome 100:1 (Ref 1)

Gut microbiota is dominated by the phyla *Firmicutes and Bacteroidetes.*
Proteobacteria, Actinobacteria, Fusobacteria, Cyanobacteria, and *Verrucomicrobia* also occur but in much less abundance
Plus a plethora of viruses, archaea, fungi and parasites

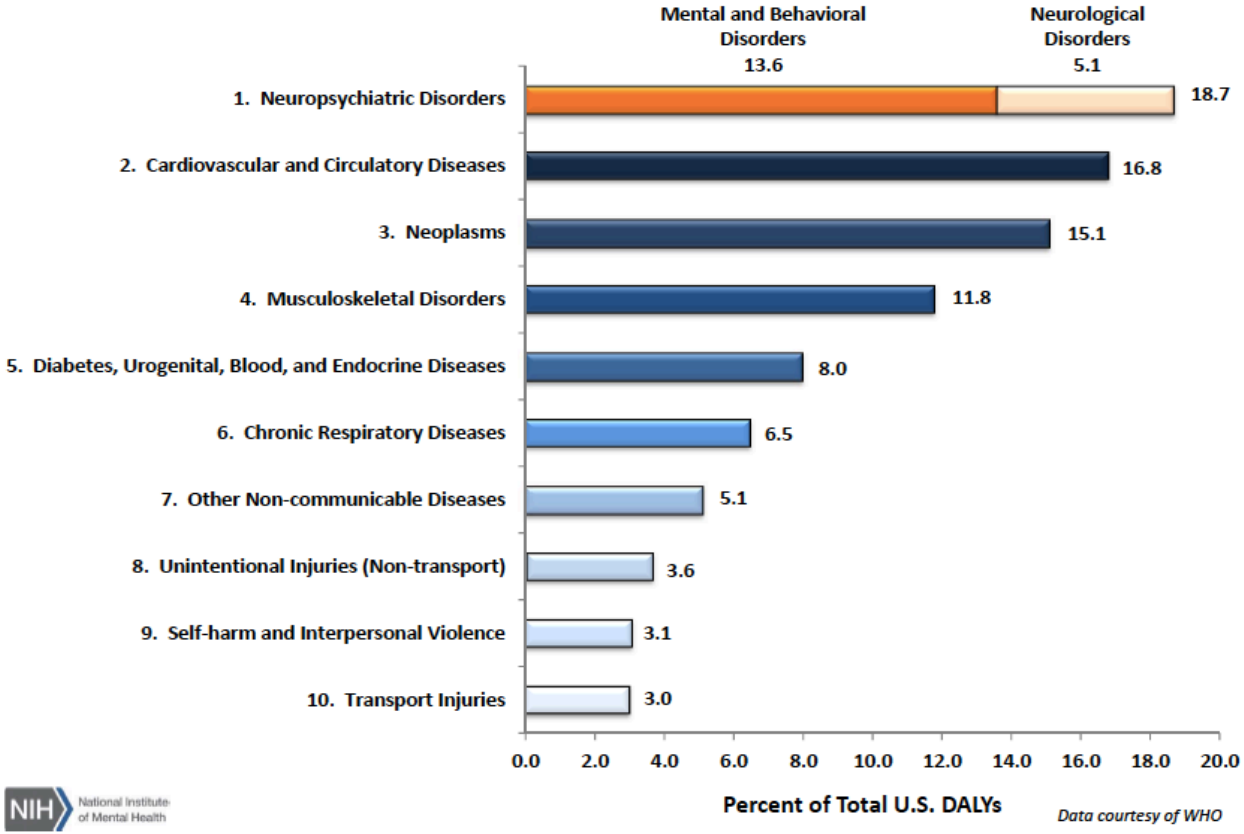
Gut-Brain Epidemiology



Neuropsychiatric disorders are strongly correlated with GI disease, and have major prevalence in the US

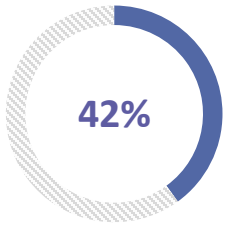
1. **Major *depressive* episodes among 6.7% of adults in past year in US** (2)
2. **Prevalence of any *anxiety* disorder among adults is 31% in US** (3)
3. **Prevalence of dementia in those 70 and older 13.9% in US** (4)
4. **Irritable Bowel Syndrome impacts 11% of the global population** (5)
5. **IBD prevalence in US has increased by 50% since 1999** (6)
6. **Prevalence of chronic constipation in US as high as 20%** (7)

Top 10 Leading Disease/Disorder Categories Contributing to U.S. DALYs (2010)

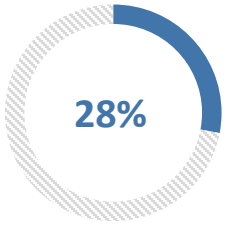


(Ref 8)

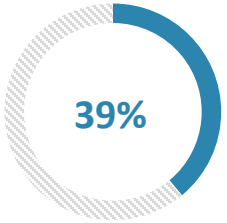
Dementia and Psychiatric Comorbidity



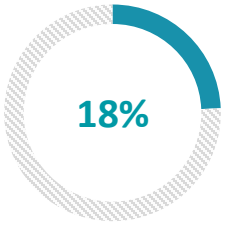
Up to 42% of people with Alzheimer's disease **suffer from significant depression**



28% higher likelihood of **conversion from MCI to dementia with comorbid depression**



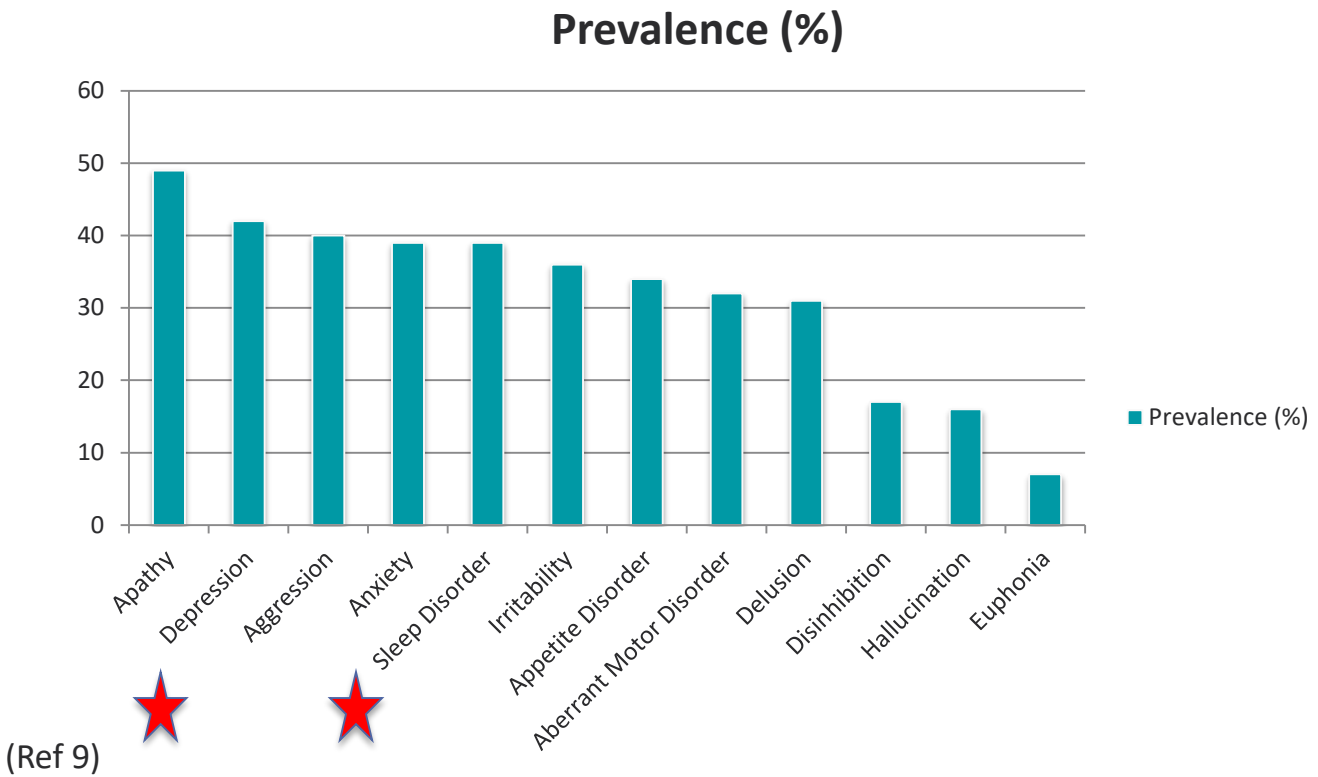
Up to 39% of people with Alzheimer's Disease **suffer from anxiety**



18% higher likelihood of **conversion from MCI to dementia with comorbid anxiety** (Ref 10)

(Ref 9)

The prevalence of neuropsychiatric symptoms in Alzheimer's Disease



(Ref 9)

Functional GI and Neuropsychiatric Disorder Risk

- "…the prevalence of anxiety (37%) and depression (24%) disorders in *constipated* patients is much higher than the general population" (11)
  - "Of patients undergoing psychological assessment for *intractable* constipation, three fifths had evidence of current, and two thirds a previous, affective disorder. " (12)
- "the interaction between psychiatric disorders including generalized anxiety disorder, panic disorder, major depressive disorder, bipolar disorder, and schizophrenia and IBS, which suggests that this association should not be ignored when developing strategies for screening and treatment." (13)
- "The risk ratios are highest for these disorders within 1 year of IBS diagnosis, but the risk remains statistically significant for more than 5 years. Clinicians should pay particular attention to psychiatric comorbidities in IBS patients." (14)
- "In the elderly, all measured psychiatric diagnoses are strongly associated with an increased prevalence of constipation." (15)

YET, THERE HAVE BEEN FEW MAJOR ADVANCES IN PSYCHOPHARMACOLOGY SINCE THE 1950S…

Gut-Brain Axis: Mechanisms

Bidirectional relationship

Neural – Vagus Nerve

Full truncal vagotomy for peptic ulcer shown to reduce risk of some neurological disorders, such as Parkinson's Disease. Effects from *Lactobacillus rhamnosus* eliminated post-vagotomy (16)

Neurotransmitter production

Bifidobacterium infantis has been demonstrated to elevate plasma tryptophan levels and thus influence central 5-HT (17)
In addition to producing precursors, many bacteria can synthesize and release neurotransmitters (18,19)

- *Lactobacillus* and *Bifidobacterium* species can produce γ-aminobutyric acid (GABA)
- *Escheridia, Bacillus*, and *Saccharomyces* species can produce norepinephrine
- *Candida, Streptococcus, Escheridia*, and *Enterococcus* species can produce 5-HT
- *Bacillus* can produce dopamine
- *Lactobacillus* can produce acetylcholine

Immune/Inflammatory

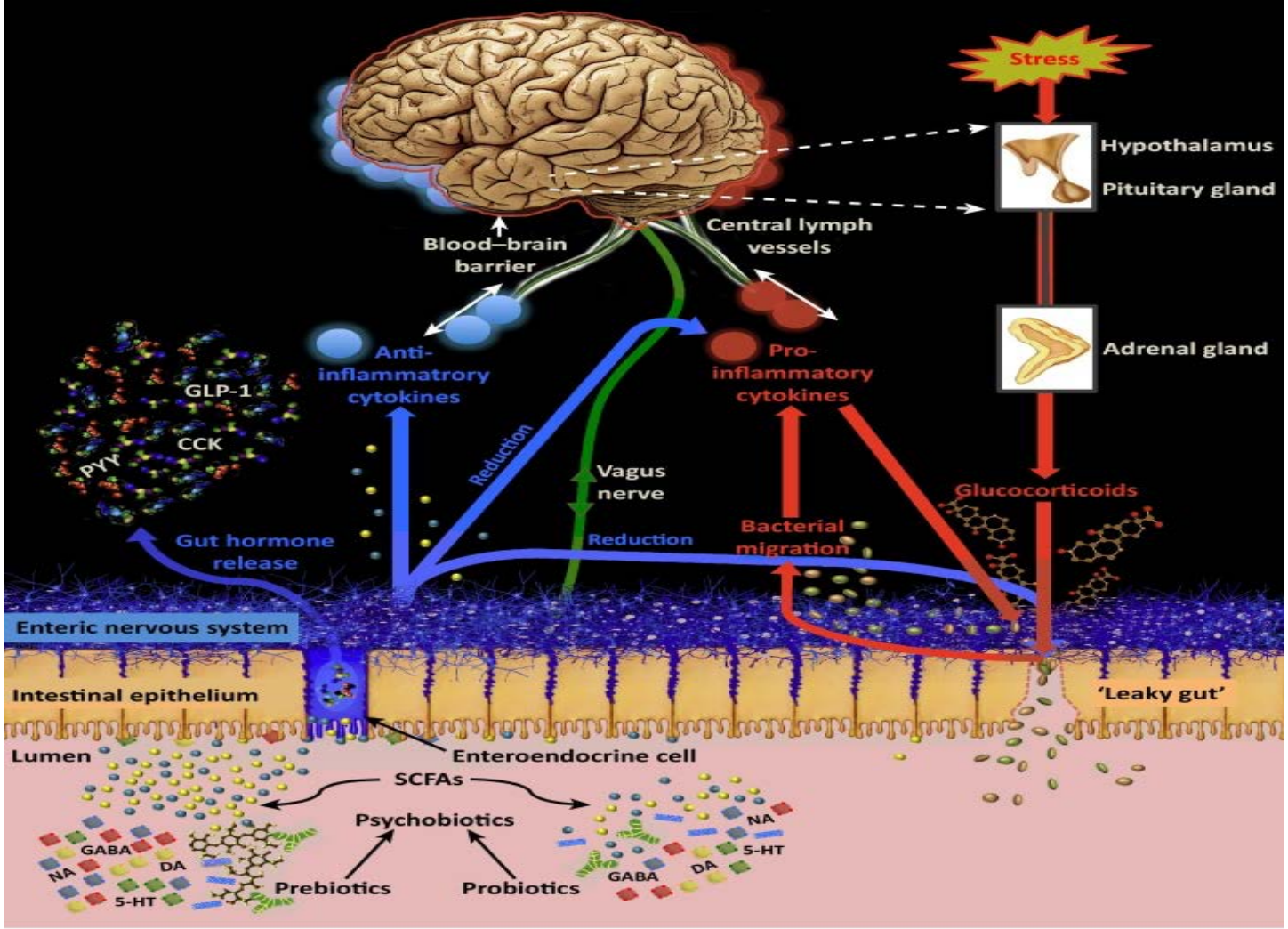
Interleukin 1 and interleukin 6 activate the hypothalamic-pituitary-adrenal axis (HPA) and cause cortisol release (Infections and IBD cause neuropsychiatric sx) (21,22,23)

Metabolic

Short-chain fatty acids (SCFAs), which include butyrate, propionate, and acetate may exert central effects through G protein–coupled receptors (20)

Endocrine

- 5-HT is produced by enterochromaffin cells
- More than 20 signaling molecules, which are modulated by microbiota, released from specialized enteroendocrine cells (EECs) in the GI tract
    - significant endocrine and metabolic functions and are able to communicate with the brain (24)
    - Includes neuropeptide Y, CRF, CCK, Ghrelin, GLP-1, Oxytocin and others



Ref 47

Trends in Neurosciences

Bacteria–Enteric Nervous System Interactions

- There is evidence of direct, bacteria-induced modulation of the enteric nervous system:
  - Gut bacteria play a crucial role in the development and homeostasis of glial populations in the gut (25)
    - myenteric plexus of the jejunum and ileum of Germ Free mice show an unorganized lattice-like appearance, with fewer ganglia, and thinner nerve fibers (26)
  - Myenteric neurons exposed to *Bifidobacterium longum* NCC3001-fermented substances showed reduced generation of action potentials (27)
  - Dorsal root ganglion in the colon do not display hyperexcitability in response to noxious stimulation if they are treated with *Lactobacillus rhamnosus* (28,29)

Vagal Signaling

- The Vagus nerve has more afferent than efferent nerve fibers which have been shown to be modulated by stress, nutrition, exercise etc.
- Antidepressants and anxiolytics may work through vagal effects (30-32)
- Severing the vagus nerve (vagotomy) abolishes responses to psychobiotic administration
  - (at least partially, as this effect is not evident in all relevant trials) (33-35)

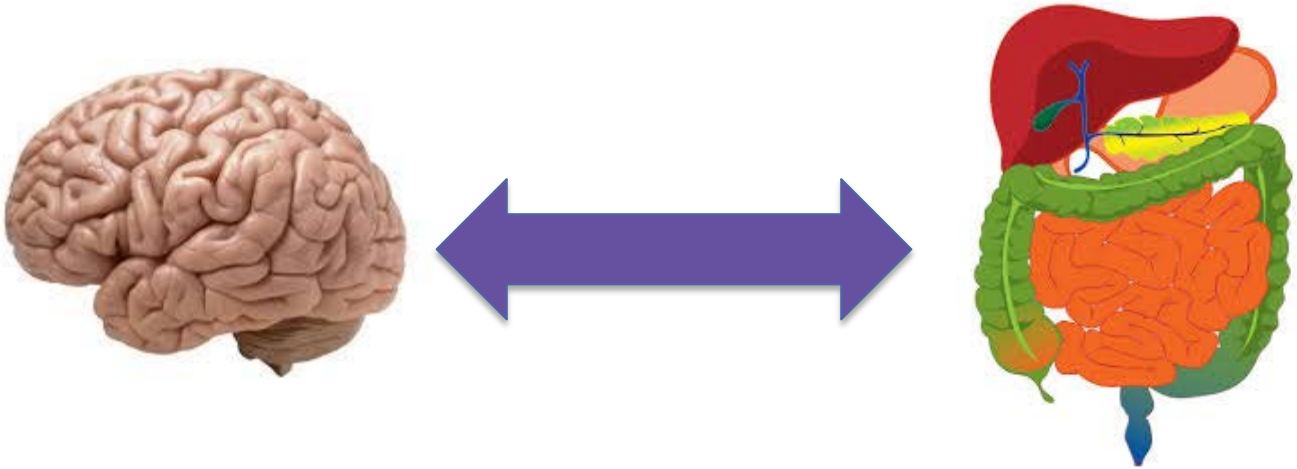
Short-Chain Fatty Acids, Gut Hormones, and Bacteria-Derived Blood Metabolites

- Sodium butyrate injections (200 mg/kg body weight) in rats produce (37):
  - Antidepressant effects
  - Increased central serotonin neurotransmission
  - BDNF expression mechanism for SCFA likely via epigenetic rather than direct agonist effects (few central FFA receptors)
    - Through histone deacetylases
- SCFAs modulate secretion of gut peptides from enteroendocrine cells

Bacteria-Immune Interactions

- MAMPs (microbe-associated molecular pattern) of beneficial bacteria may increase secretion of anti-inflammatory cytokines such as interleukin-10 (37,38)
  - Specifically, *Bifidobacterium infantis* 35624 and *Lactobacillus GG*
  - Proposed Mechanism: competitive inhibition of pro-inflammatory MAMPs via TLR2 and TLR4
- Prebiotics may work by similar inhibition mechanisms (39,40)

A Bidirectional Relationship



The Central Nervous System can control the gut microbiota via adrenergic nerve signaling, primarily affecting:

- intestinal motility
- neurotransmitters activation of immune mediators that shape microbiota composition and function

Ref 41

Nature or Nurture?

Implications from Monozygotic Twin Studies

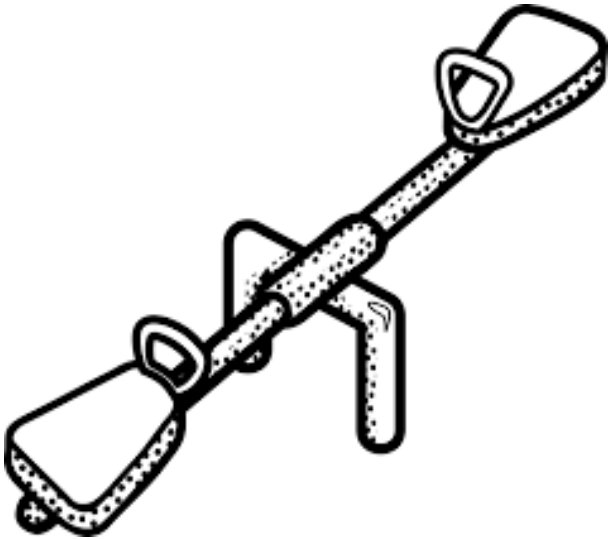
There is considerable discordance in the development of neuropsychiatric disorders among sets of monozygotic twins:

- *Indicates that non-genetic factors are also involved*
- *The microbiome is one such environmental factor (also more readily altered than the human genome)*
- *The microbiome is known to impact the epigenome via metabolites*

(Ref 42-44)

As we age…

1- Healthy aging correlates with a diverse microbiome



2- Reductions in microbial complexity correlate to decreases in neuronal complexity and increased risk of neurodegenerative disorders

(Ref 45-46)

What we learn from germ-free animals

The Pre-Clinical Evidence

- Germ-free mice have:
  - Impaired social behavior (48)
  - Higher anxiety (49)
  - Impaired stress response (50)
  - Trial: 40% greater plasma tryptophan concentrations than normal mice, but the normal mice had 2.8 times greater plasma serotonin levels than the germ-free mice (51)
- Implications
  - *Fecal* transplant has been shown to alter these behaviors (52)
  - Impaired microglial function mitigated by *oral* SCFA (53)
  - *Oral* probiotics in rats and mice improve anxiety and depression (54,55)

Depression

The Role of the Gut-Brain Axis

Animal Trials

- Increase in gut microbiota alpha diversity is associated with depression (57)
  - Alpha diversity (Intra) vs Beta Diversity (Inter)
- Experimentally elevated HPA axis responses and depression have been reversed in rats by administering a single bacterium, *Bifidobacterium infantis* (58)
- Two varieties of *Bifidobacterium* have been more effective than Lexapro (59)

Gut-Brain-Depression Axis

- Review of gut microbiomes of 1135 participants from a Dutch population cohort using deep sequencing showed correlation between gut microbiota diversity and depression (60)
- Increases in the genus *Eggerthella, Holdemania, Gelria, Turicibacter, Paraprevotella and Anaerofilm*
    - reductions in *Prevotella* and *Dialister* have been found in individuals with depression (61)
- Lower numbers of *Bifidobacterium* and *Lactobacillus* have been found in individuals with depression (62)
- A negative correlation between *Faecalibacterium* spp. and severity of depressive symptoms has been reported (61)
- Higher *Firmicutes:Bacteroides* ratio in IBS patients was correlated with clinically significant depression and anxiety (63)

Correlative Stool Samples

- 16S rRNA gene Illumina deep sequencing
  - Microbiome alterations and depression in humans by the analysis of fecal microbiota of 37 patients diagnosed with depressive disorder compared to 18 non-depressed
- The most pronounced result was a general underrepresentation of *Bacteroidetes* in those diagnosed with depression (64)
  - *Alistipes*, a genus in the phylum of *Bacteroidetes* was overrepresented in depressed patients
    - Correlates with chronic fatigue syndrome, IBS

Intervention: Probiotics

- Male and female participants (n = 124)
  - Consumed either a fermented milk drink containing *Lactobacillus casei* Shirota or a placebo for 3 weeks
  - Result: No overall changes in self-reported affect (65)
- **Subgroup analysis**: participants whose baseline mood scores fell in the lowest third of the total range:
  - Probiotic supplementation resulted in significantly more participants self-rating as happy rather than depressed, relative to placebo
  - Potential “ceiling” effects

Intervention: Probiotics

- Study performed by Mohammadi et al.
  - consuming a probiotic yogurt or a multispecies probiotic capsule for *6 weeks* had beneficial effects on the mental health biomarkers of petrochemical workers (66)
- Study performed by Akkasheh et al.
  - *8 weeks* of administration of probiotics to patients with major depressive disorder (MDD) had beneficial effects on Beck Depression Inventory scores (67)

Effect of Probiotics on Depression

- **Systematic Review and Meta-Analysis of Randomized Controlled Trials**
- (5 Trials examined)
  - One of the five - individuals with major depression
  - Remaining four studies examined non-depressed individuals
- **Conclusion**: probiotics were associated with a significant reduction in depression (68)
- *Of note:*
  - Subjects aged 60 and below, oral probiotics effective
  - Aged 65 and older (only 1 trial), no effect was observed

Anxiety

The Role of the Gut-Brain Axis

Intervention: Probiotics

- Double-blind, placebo controlled 30-day trial of a probiotic mixture containing *Lactobacillus helveticus* R0052 and *B. longum* R0175 (69)
- **Outcomes Measures:** Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL) and 24 h urinary free cortisol (UFC)
- **Results**: Improvements in
  - Anxiety
  - Depression
  - Reduced levels of cortisol

Intervention: Prebiotics

- Administered prebiotics (oligosaccharides) to healthy volunteers
- Forty-five healthy volunteers received one of two prebiotics (fructooligosaccharides, FOS, or Bimuno®-galactooligosaccharides, B-GOS) or a placebo (maltodextrin) daily for 3 weeks (70)
  - lower cortisol levels at awakening
  - improved attention to positive stimuli compared to negative stimuli in
    - an emotional categorization task
    - an emotional recognition task

* Effects similar to admin of selective serotonin reuptake inhibitor citalopram or the benzodiazepine diazepam in healthy individuals

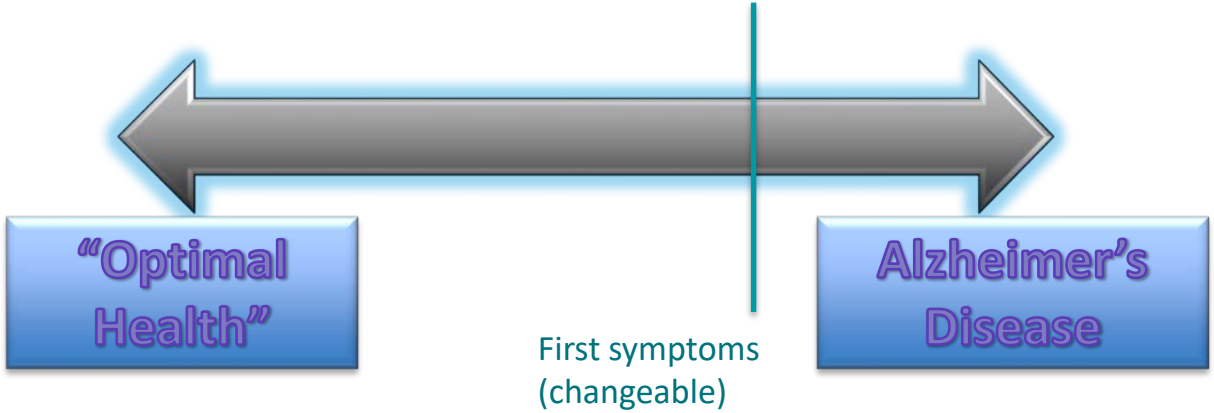
Intervention: Psychobiotic Formulation on Body Composition and Anxiety

- 45 subjects - 3 week intervention
  - 3 Groups: (1) Psychobiotics, (2) Hypocaloric Diet, (3) Combination
  - Primary outcomes: body composition (DXA and BIA)
  - Secondary outcomes: Hamilton Anxiety Scale
- Psychobiotic suspension:
  - *Streptococcus thermophilus*
  - *Lactobacillus bulgaricus*
  - *Lactococcus lactis*
  - *Lactobacillus acidophilus*
  - *Streptococcus thermophiles*
  - *Lactobacillus plantarum*
  - *Bifidobacterium lactis*
  - *Lactobacillus reuteri*
- Results (71)
  - Hypocaloric group had increased HAM-A scores, while Psychobiotic and Combined group had improvements (highest improvement in combined group)

Cognitive Decline

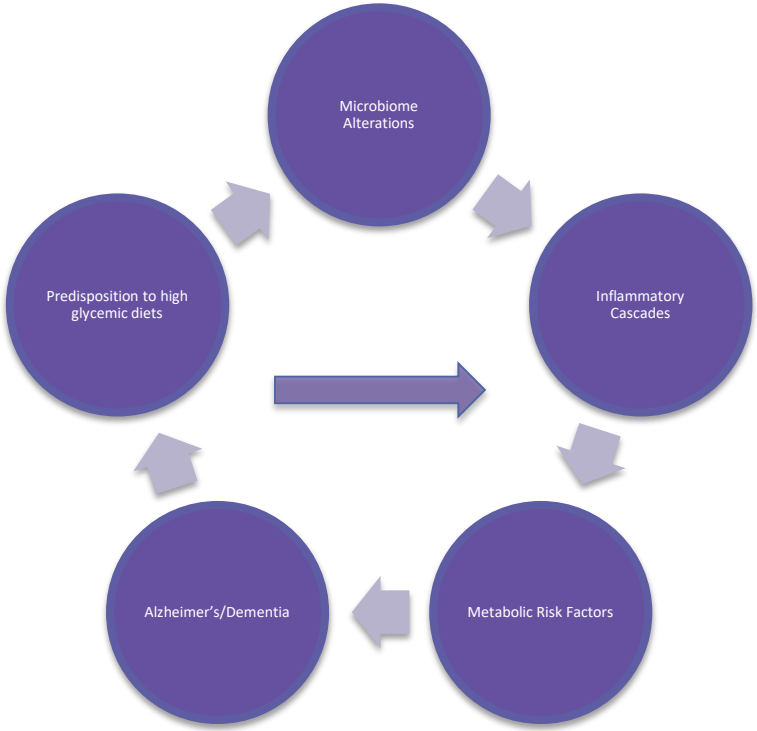
The Role of the Gut-Brain Axis

A Continuum



- Degeneration doesn't occur overnight (decades)
- Diseases of the brain are at their core systemic and multi-factorial
- The growth of new brain cells is normal
- If we don't look at it this way, what we can accomplish will be marginal

A Vicious Cycle



Risk factors for AD such as metabolic syndrome, type 2 diabetes and obesity are associated with gut microbiota alterations (72,73)

“Diabetes of the brain”

- Current Estimated lifetime risk of DM: 38%
  - HbA1c correlates with lower cognitive capacity and changes in hippocampal microstructure
- Risk of AD attributed to hyperinsulinemia: 40%
  - *50–100% increase in overall risk*
- T2DM *and* positive APOE e4: higher neuritic plaques and neurofibrillary tangles in the cortex and hippocampus
- High glucose: Changes in cognitive capacity and hippocampal microstructure even without DM2
- Autopsy studies: correlation between increased pancreatic amyloid deposition and the progression of AD

Ref 74-83

Insulin Resistance → Dementia

- Hallmark of pathology: impaired cerebral glucose *utilization*
  - Impairments in brain insulin/IGF signaling lead to increased expression of amyloid-β precursor protein (AβPP) and accumulation of AβPP-Aβ.
  - Mechanisms:
    1. Activated kinases which phosphorylate tau
    2. Higher APP expression
    3. Oxidative and ER stress
    4. Increased ROS and RNS = DNA, RNA, Lipid, Protein damage
    5. Direct mitochondrial dysfunction
    6. Increased pro-inflammatory and apoptotic cascades
    7. Down-regulation of genes which enhance cholinergic homeostasis

Ref 74-83

Microbiome Contributors to Metabolic Risk Factors

- Dysbiotic signatures in the gut microbiota associated with metabolic disease phenotypes include an increased ratio of *Firmicutes* to *Bacteroidetes* at phylum level (84)
- Other correlations:
  - expansion of *Proteobacteria*
  - reduced abundance of *Akkermansia*
- Insulin Resistance: proliferation of *Prevotella copri* and *Bacteroides vulgatus*
    - Specifically Insulin-resistant phenotypes with elevated circulating levels of branch chain amino acids
- Obesity predisposition: associated with augmented serum glutamate levels due to the reduced abundance of *Bacteroides thetaiotaomicron* that converts glutamate
- NAFLD: Increased abundance of *Proteobacteria* and *Escherichia coli* with a reduction in the population of *Firmicutes*
  - associated with advanced fibrosis in human non-alcoholic fatty liver disease (NAFLD)

Alzheimer's Disease and the Microbiome

Gut microbiota seems to be involved in the direct accumulation of amyloid plaques according to the results of a study using a mouse model of AD (85)

AD Microbiome changes parallel changes observed in other conditions linked to gut microbiome alterations, including obesity, diabetes, IBD, and Parkinson's disease (86-89)

Alzheimer's Disease and the Microbiome

- "the gut microbiome of AD participants has decreased microbial richness and diversity and a distinct composition compared to asymptomatic age- and sex-matched control participants" (90)
- Correlations to CSF p-tau/ Aβ42, a composite measure of AD pathology. AD Patients have….
  - Reduction in phylum *Firmicutes* (also in T2DM and obesity) (88,91)
  - Increase in the phylum *Bacteroidetes* (also in T2DM and Parkinson's) (86, 88)
  - Increase in LPS exposure
  - Reduced *Actinobacteria*, specifically *Bifidobacterium* genus (longevity, anti-inflammatory properties, gut permeability) (92)

Bifidobacterium: Intervention Trial

- Randomized, double-blind controlled trial (93)
- A small study of probiotics that included *Bifidobacterium* demonstrated a change in Mini-Mental State Examination scores after a 12-week intervention among participants with severe dementia

A Fungal Etiology?

- “The present findings demonstrate that fungi can be detected in brain tissue from different regions of the AD CNS. In all eleven patients (plus three additional CP samples) described in this study, as well as in four patients previously analysed, there is clear evidence for fungal cells inside neurons or extracellularly. Therefore, 100% of the AD patients analysed thus far by our laboratory present fungal cells and fungal material in brain sections.” (94)
- Specific Brain regions:
  - External frontal cortex; Cerebellar hemisphere; Entorhinal cortex/hippocampus; Choroid plexus
- Increased chitinase levels are found in blood serum and cerebrospinal fluid from AD patients (95-98)

The Oral Microbiome and AD

- Retrospective cohort study using the National Health Insurance Research Database (NHIRD) of Taiwan (99)
- 9291 patients with Chronic Peridontitis (CP) compared to 18,672 matched controls without CP
- 10-year CP exposure was associated with a 1.707-fold increase in the risk of developing AD
  - Most likely mediated through activated pro-inflammatory cascades

ECOLOGY OF THE GUT MEETS ECOLOGY OF OUR ENVIRONMENTS

Social Engagement

- Meta-analysis: 148 studies, involving 308,849 people:
  - Decreased mortality is associated with increased social engagement
- Rate of global cognitive decline was reduced by an average of 70% in persons who were frequently socially active (90th percentile) as compared to persons who were infrequently socially active
  - *Especially important beyond the age of 70*

Isolation

- Remaining or becoming socially/physically active over a 10 year period had up to a 49% decrease in dementia risk over the subsequent 10 years
- Key take-away: Quality of relationships more important than quantity

Cortisol

- In middle-aged adults, cortisol increases have been related to:
  - worse executive functioning
  - decreased prefrontal cortical volume
- In older adults without dementia, increases have been related to impaired episodic memory and hippocampal atrophy
- High Urinary Free Cortisol (UFC) level and high UFC variability increased the risk for AD by a factor of 1.31 and 1.38, respectively.
  - Effects maintained when controlling for well-known AD risk factors such as APOE ε4 and depression symptoms
  - *Ennis GE, An Y, Resnick SM, Ferrucci L, O'Brien RJ, Moffat SD. Long-term cortisol measures predict Alzheimer disease risk. Neurology. 2017;88(4):371-378.*

Trauma

- 336 community-dwelling older Aboriginal Australians
- Participants completed a life course survey of health, well-being, cognition, and social history including the Childhood Trauma Questionnaire (CTQ), with consensus diagnosis of dementia and Alzheimer disease
- Strong association between CTQ scores and dementia remained significant after controlling for depression and anxiety
  - Odds Ratio: 1.61, 95% CI: 1.05–2.45

Depression

- 14-year longitudinal study of 4922 cognitively healthy men aged 71–89 years
  - Linear response, more severe depression = higher likelihood of dementia
  - *Almeida OP, Hankey GJ, Yeap BB, Golledge J, Flicker L. Depression as a modifiable factor to decrease the risk of dementia. Translational Psychiatry. 2017;7(5):e1117.*

Education

- Lower education is associated with a greater risk for dementia in many but not all studies
- Leaving full-time education at an earlier age was associated with an increased risk of dementia death in women but not men
  - for age ⩽14 v. age ⩾16: Hazard ratio = 1.76
  - Meta-analysis 86,508 men and women in UK
- Limited literacy in older adults, as opposed to adequate literacy (≥9th-grade level), was associated with greater incidence of likely dementia (25.5% vs 17.0%)

Diet and Microbiome

Standard American Diet contributes to an altered/impaired Microbiome

An impaired microbiome impacts dietary response to specific foods

“When in doubt, treat the gut”

- Condition specific pre and probiotics (when research is available, and not contradictory)
- Approaches to address intestinal permeability and inflammation
- Liver support
- Digestive/Assimilation support
- Water goal: half body weight in pounds, in ounces
- Fiber: 25-35g/day
- Foot stool hygiene
- Mindful eating/cooking
  - Appropriate food combining
  - Appropriate meal timing
  - Proper mastication
  - Low-heat/intensity cooking methods
- Castor oil packs
- Symptom-Specific Botanicals

THE ALZHEIMER'S DEFENSE PLAN

HEAL YOUR BODY, SAVE YOUR MIND

DR. ROBERT KACHKO ND, LAC
DR. PETER BONGIORNO ND, LAC

Thank you.
Questions?

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Metagenics Institute

KetoNutrition

Science to Emerging Applications

Dominic P. D'Agostino, PhD
Associate Professor
Department of Molecular Pharmacology and Physiology
University of South Florida, Morsani College of Medicine

Hyperbaric Biomedical Research Laboratory
supported in part by:
DEPARTMENT OF THE NAVY
ONR
Science & Technology

USF
UNIVERSITY OF SOUTH FLORIDA

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We Thank our Supporters

they make research possible!

Disclaimer

- Presentation includes data on ketone technologies patented by University of South Florida.
- I am an inventor of patents that have been commercialized. Royalties from products support USF and our research program.
- Information contained in this presentation is not meant to be taken as medical or nutrition advice.

Disclosures:

1. Dominic P. D'Agostino; Patrick Arnold; Jay B. Dean; Raffaele Pilla; "Ketone esters for prevention of CNS oxygen toxicity" (US Patent: 20140073693 A1)
2. Dominic P. D'Agostino; Angela M. Poff; Patrick Arnold; *"Targeting Cancer with Metabolic Therapy and Hyperbaric Oxygen"* (US Patent No. 9,801,903)
3. Dominic P. D'Agostino; Patrick Arnold; "Composition and Methods for Producing Elevated and Sustained Ketosis" (US Patent No. 9,675,577)
4. Ari, C., Arnold P., D`Agostino, D.P. Technology Title: "Exogenous Ketone Supplements for Reducing Anxiety-Related Behavior" USF Ref. No. 16A007
5. Co-owner of Ketone Technologies LLC

Presentation Outline

- Shifting Metabolic Physiology and Brain Metabolism
- Seizure Control and Nutritional Ketosis 101
- Biochemical and Molecular Mechanisms
- Applications: Proven vs Emerging
- Strategies and Tools for Implementation
- Future Directions

Preventing CNS Oxygen Toxicity

Limits SpecOps Diving

Limits Hyperbaric Oxygen Therapy

NASA NEEMO 22 Mission
O2 Pre-Breath prior to
Staged Decompression

No Way to Predict or Prevent

https://www.youtube.com/watch?v=z7Hi0HO24Vk

Hyperbaric Biomedical Research Laboratory
supported in part by:
DEPARTMENT OF THE NAVY
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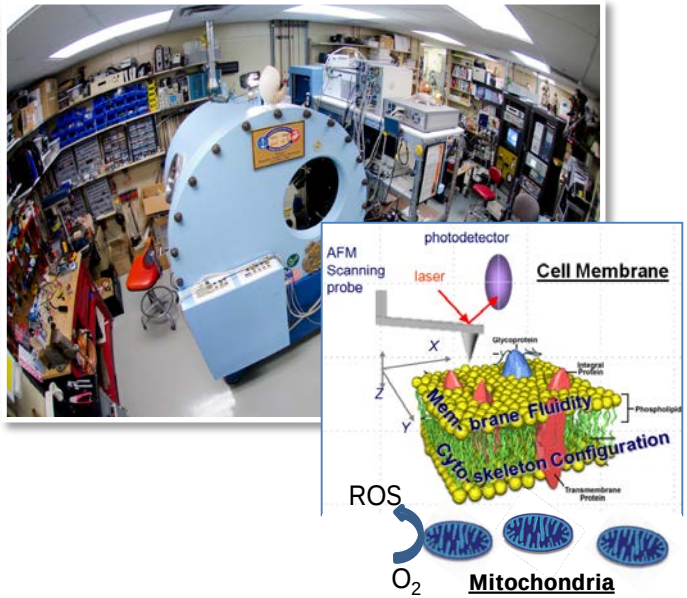
Science & Technology

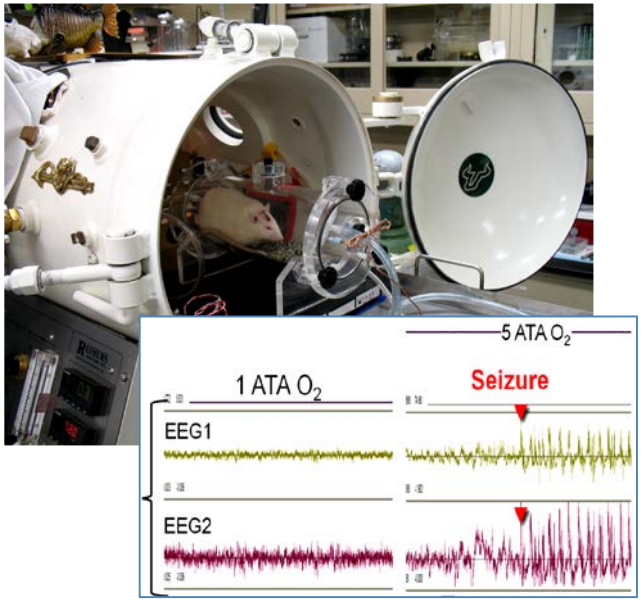
Cellular, Molecular and Physiological Experiments

- ✓ Atomic Force Microscopy (AFM)
- ✓ Laser Scanning Confocal Microscopy
- ✓ Electrophysiology
- ✓ Radio Telemetry (EEG)
- ➢ **Adapted to hyperbaric chambers**

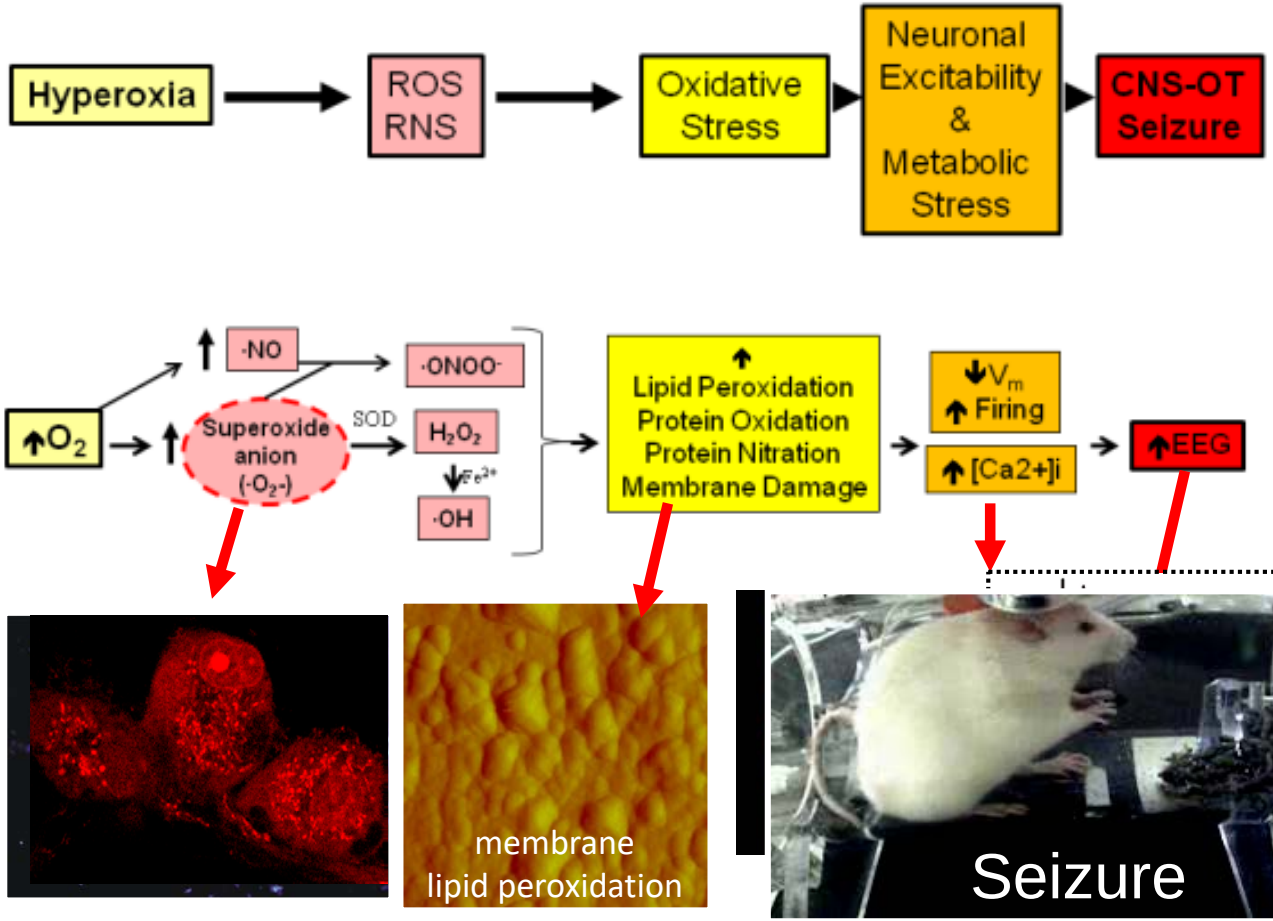
Journal of Microscopy
Journal of Microscopy, Vol. 246, Pt 2 2012, pp. 129–142
doi: 10.1111/j.1365-2818.2011.03599.x
Received 30 July 2011; accepted 12 January 2012

Development and testing of hyperbaric atomic force microscopy (AFM) and fluorescence microscopy for biological applications





Oxidative Stress >> Neurometabolic Impairment



Seizure Prevention Strategies

Depth (fsw)	Length of exposure (mins)
25 or less	240
30	80
35	25
40	15
50	10

- ✖ Limit Exposure
- ✖ Antioxidants
- ✖ Anti-Epileptic Drugs
- ✓ **Fasting Ketosis**
- ✓ **Nutritional Ketosis**

Elevated Ketones (Energy)
Reduced Glucose, Insulin
Reduced Inflammation
Inhibition of HDACs

How Does Fasting Change Brain Metabolism?

Clinical Example
Shifting Metabolic Physiology

Oliver E. Owen, MD

George F. Cahill Jr., MD

BLOOD GLUCOSE, FREE FATTY ACIDS
AND KETONE BODY LEVELS DURING FAST

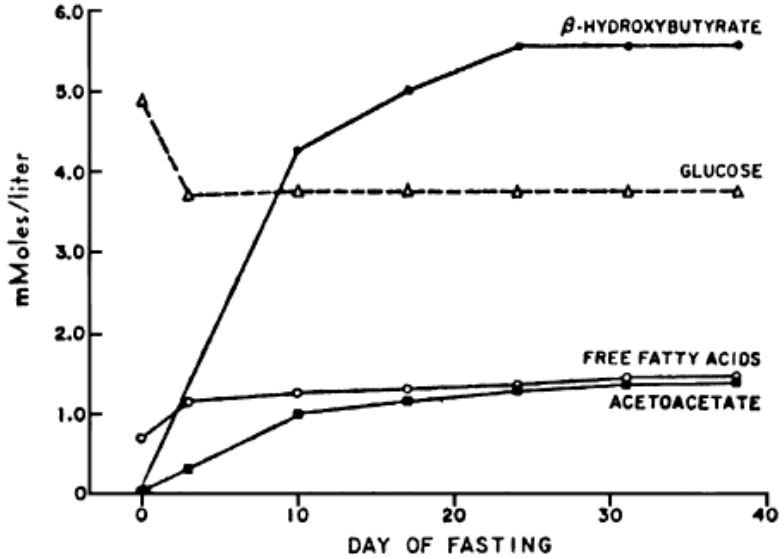
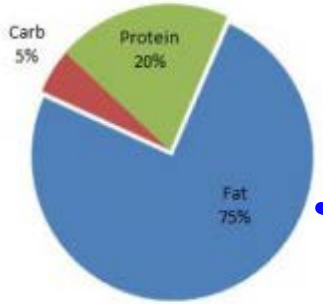


FIG. 2. Circulating concentrations of βOHB, glucose, free fatty acids and acetoacetate in obese but otherwise normal man fasting for 40 days (9).

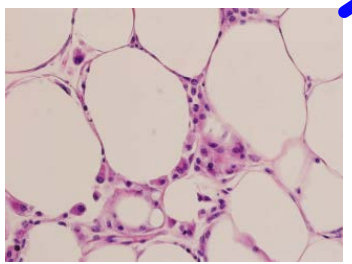
Cahill GF Jr, Veech RL.
Ketoacids? Good medicine?
Trans Am Clin Climatol Assoc.
2003;114:149-61; discussion 162-3.
Review.
PubMed PMID: 12813917; PubMed Central PMCID: PMC2194504.

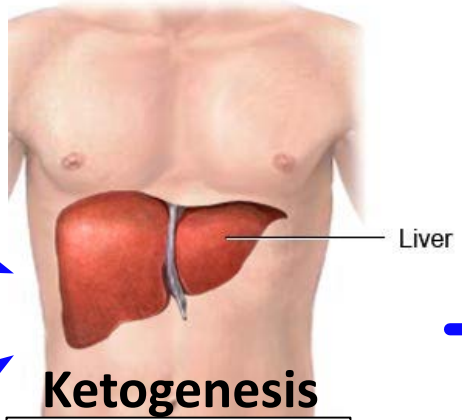
Nutritional Ketosis Mimics Fasting Ketosis

Ketogenic Diet



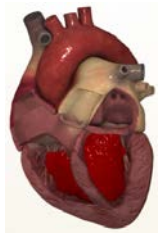
Body Fat

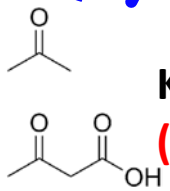




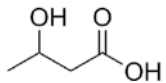
Ketogenesis

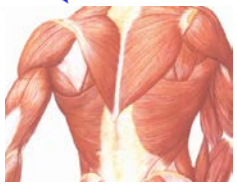
Glucose
Insulin





Ketones
(energy!)





Seizure Control and Nutritional Ketosis 101

Ketogenic Diet is used Clinically for Seizures

(independent of etiology)

- 2/3 of drug-refractory patients respond
- 33% will have a >90% seizure control
- 10-15% are “super-responders” – rapid, total, and permanent seizure control
- Can often stop off diet after 1-2 yrs

Kossoff, E. et al “The Ketogenic And Modified Atkins Diets – Treatments for Epilepsy and Other Disorders”, 6th Edition (2016)

https://www.charliefoundation.org/

THE CHARLIE FOUNDATION

Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats

Dominic P. D'Agostino,[1] Raffaele Pilla,[1] Heather E. Held,[1] Carol S. Landon,[1] Michelle Puchowicz,[2] Henri Brunengraber,[2] Csilla Ari,[3] Patrick Arnold,[4] and Jay B. Dean[1]

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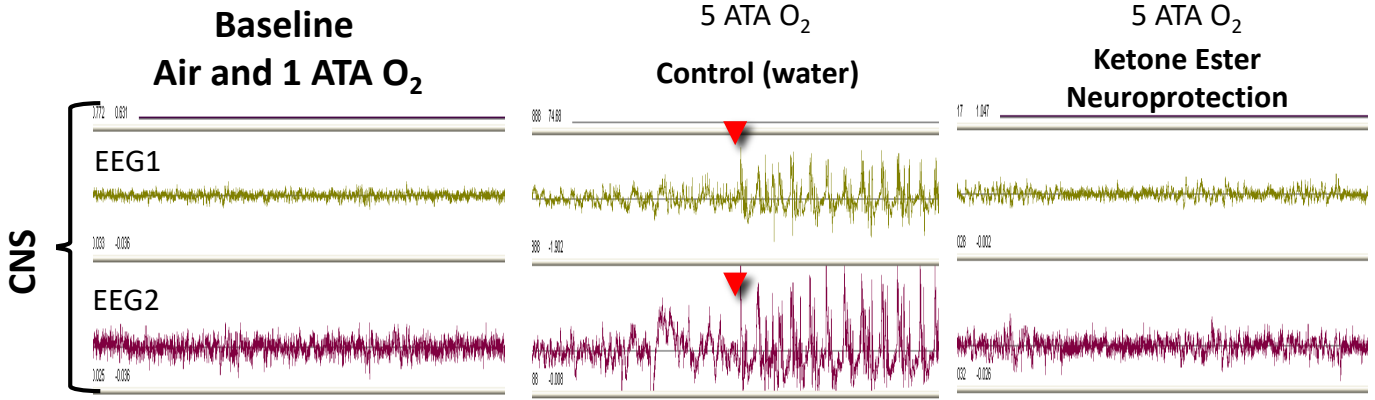
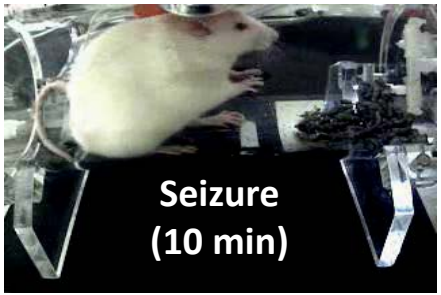


Neuroprotective Effect of Nutritional Ketosis

(Total Ketones: 2-5 mM)



Nutritional Ketosis (NK) 101

Ketone Bodies	Energy substrates from fat oxidation
Ketosis	Blood levels >0.5 mmol/L, Urine > 15 mg/dL
Nutritional Ketosis	Dietary strategy to elevate blood ketones
Keto-acidosis	Pathologically high ketones (>10 mmol/L) *(results from Insulin insufficiency)*
Keto-Adaptation	Physiological shift to metabolizing fat + ketone *(results from reducing Insulin signaling)*
Exogenous Ketones	Synthetic or naturally derived substances to elevate ketone levels

Nutritional Ketosis (NK) 101

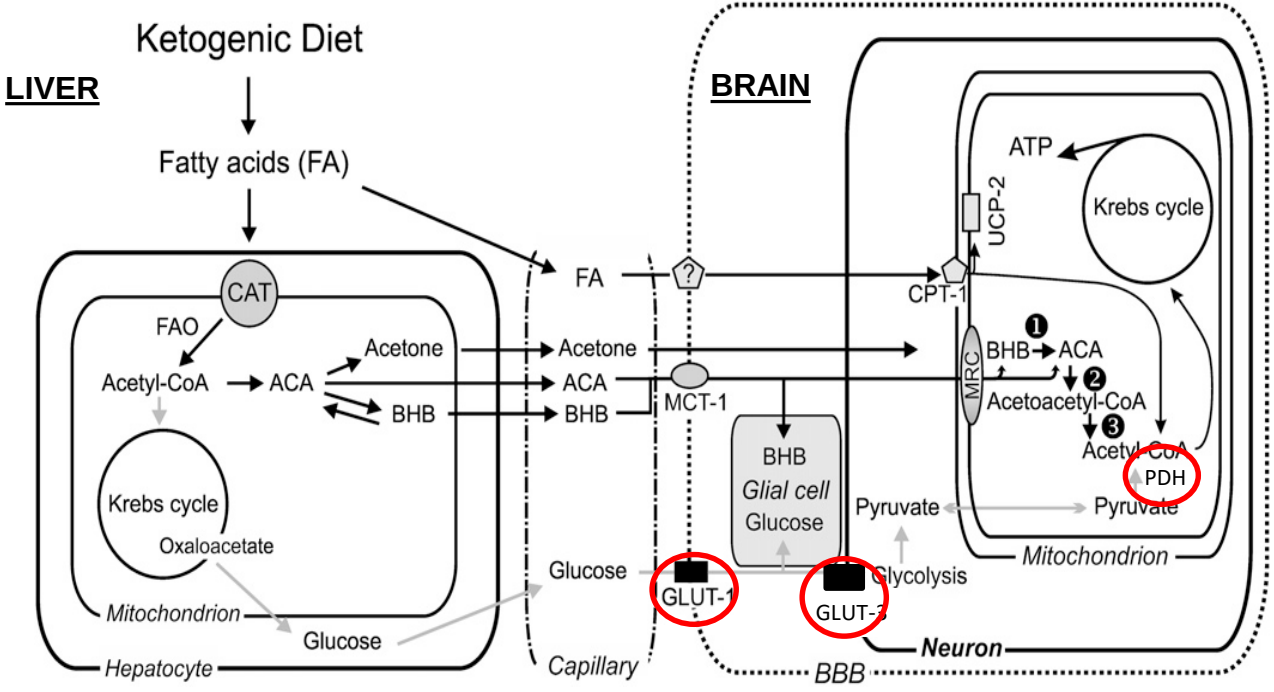
Diabetic Ketoacidosis (DKA) vs Nutritional Ketosis (NK)

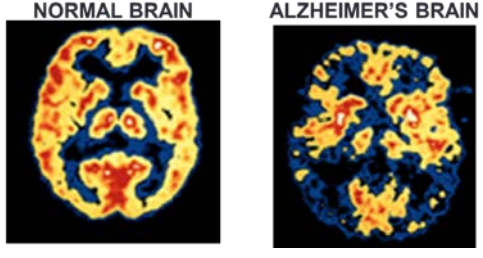
Pathological **Nutritional**

	DKA	NK
Blood Ketones	8 - 30 mmol/L	1 - 3 mmol/L
Insulin	Dysregulated/Absent	Regulated/Low
Glycemia	High	Stable/Low
Renal Metabolism	Ketonuria, Glycosuria, Reduced GFR	Mild Diuresis
Acidosis	Very high	Normal
Inflammation	Elevated	Reduced
Pathology Side Effects	Hypovolemia, Coma, Death	None, Transient or Manageable

Nutritional Ketosis: Biochemical and Molecular Mechanisms

Bioenergetic Effect of Ketones in the Brain





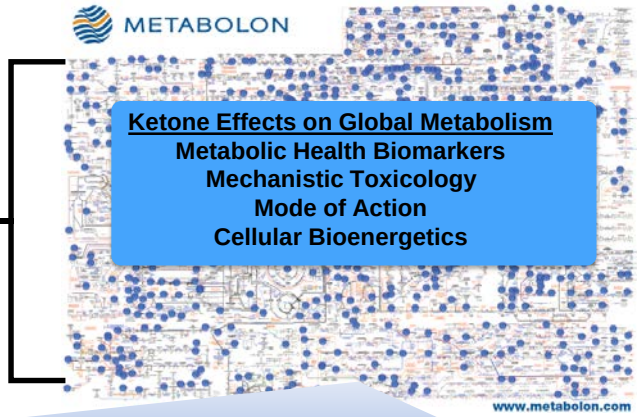
Gano et al. *Journal of Lipid Research*
Volume 55, 2211-2228; 2014

Metabolomic Studies

(>4000 metabolites)

UHPLC-MS/MS

GC-MS



Amino Acid Metabolism

Glutathione metabolism
Inflammatory mediators
Microbiome metabolism
Amino acid catabolism
Bioactive intermediates & trace amines
Polyamines/ornithine metabolism
Urea cycle

Carbohydrate Metabolism

Gluconeogenesis
Glucose metabolism
Glycogen metabolism
Glycosylation pathways
Metabolism of other carbon sources
Metabolism of sugars (fructose, galactose)
Polyol metabolism
Pyruvate metabolism

Cofactor/Vitamin Metabolism

Ascorbate metabolism
CoA metabolism
FAD metabolism
Folate metabolism
NAD/NADP metabolism
PLP metabolism
SAM metabolism
Many other cofactors and vitamins (tocopherol, B12, Biotin)

Energy Metabolism

Ketone Bodies
Beta-oxidation
Mitochondrial function
Acyl-carnitines
Creatine metabolism
FAD metabolism
Glycolysis
Pentose phosphate pathway
TCA cycle

Nucleotide Metabolism

Degradation of nucleotides
Deoxyribonucleotides
DNA damage
FAD metabolism
Modified nucleotides
Nucleotide Coenzymes
Purine and pyrimidine *de novo* synthesis
Purine and pyrimidine salvage synthesis
Ribose metabolism

- **Elevated blood and brain ketones**
- **Increased Krebs/TCA intermediates**
- **2-5x higher antioxidants and adenosine**

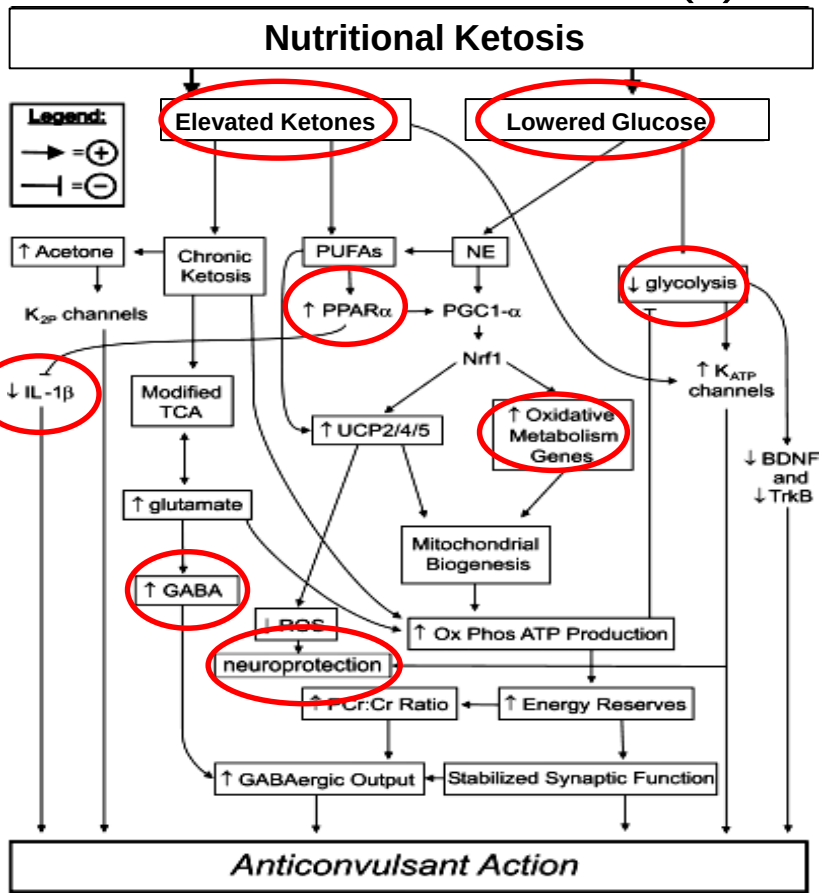
What is the Mechanism(s)?

βHB

AcAc

Acetone

GABA/Glutamate Ratio



Glucose

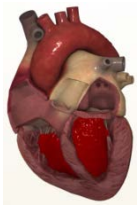
Insulin

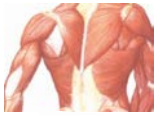
ROS

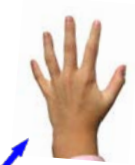
Inflammation

Adapted from: Kristopher Bough and Jong Rho. Anticonvulsant Mechanism of the Ketogenic Diet. *Epilepsia*, 48 (1): 43-58, 2007.

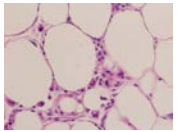
Ketones: Alternative Fuels and Signaling Molecules



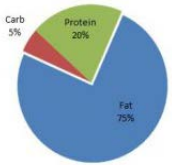




Starvation and Calorie Restriction (Adipose)



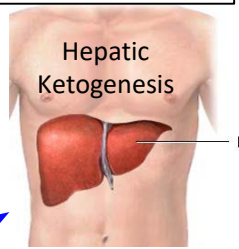
Ketogenic Diet

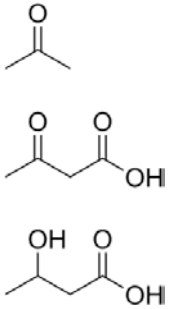


Ketogenic Fat

Difficult to sustain…

- ↓ Glucose
- ↓ Glycogen
- ↓ Insulin





Alternative Fuel

Ketones

Neuroprotective Signaling Metabolites

Exogenous Ketones

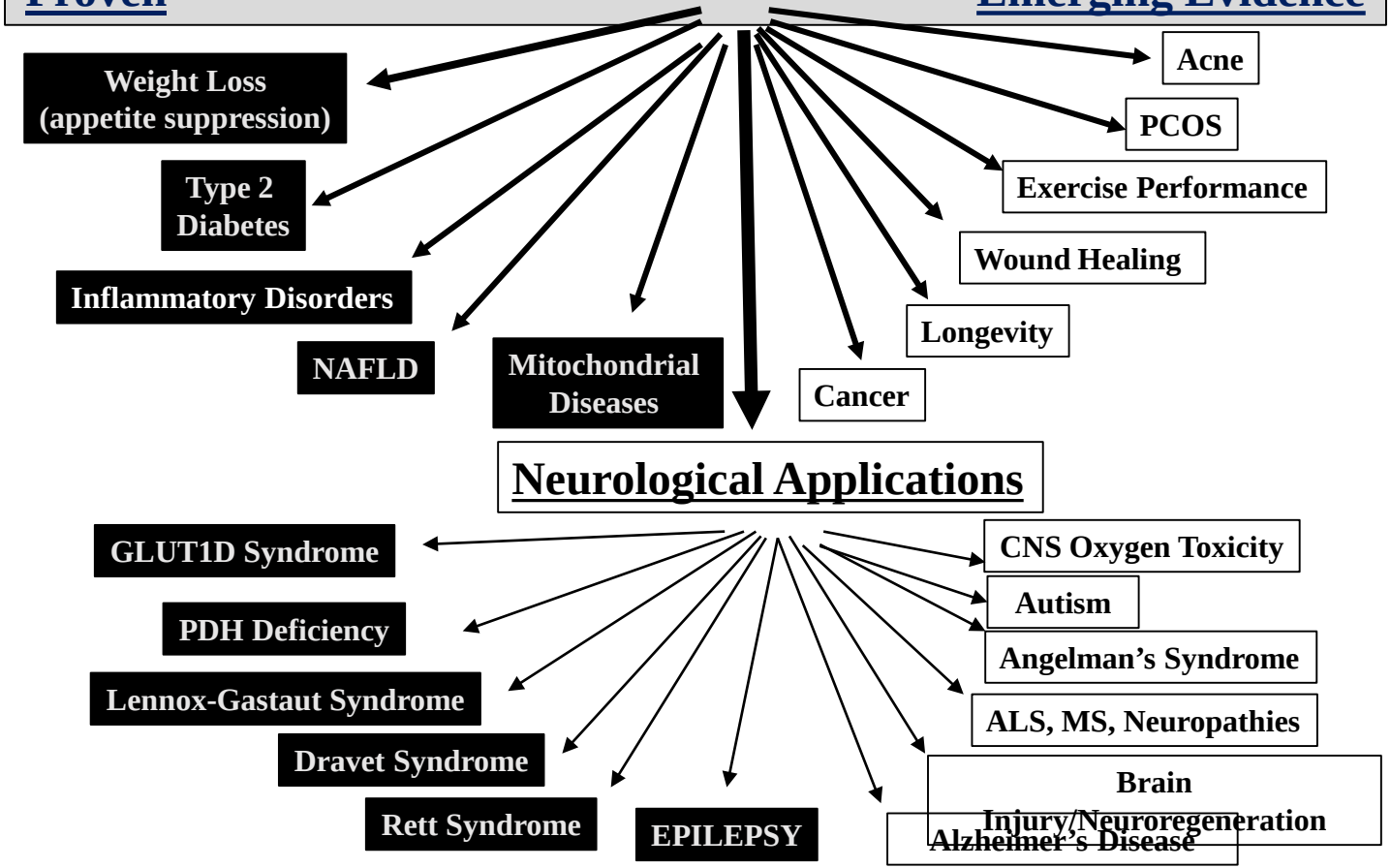
- ↑ **Longevity**
  DAF-16/FOXO
  ↓ IGF-1
  ↓ mTOR
- ↓ **Oxidative Stress**
  Superoxide
  class 1 HDACI
- ↓ **Inflammation**
  NLRP3
  Inflammatory Cytokines
- ↓ Glutamate
  (GAD)
- ↑ GABA
  Transaminase
  Inhibition
- ↑ Anaplerosis
  (TCA
  Intermediates)

Nutritional Ketosis: Support for Emerging Applications

APPLICATIONS OF NUTRITIONAL KETOSIS

Proven

Emerging Evidence



Reduction and Stabilized Blood Glucose

Kesl et al. Nutrition & Metabolism (2016) 13:9
DOI 10.1186/s12986-016-0069-y

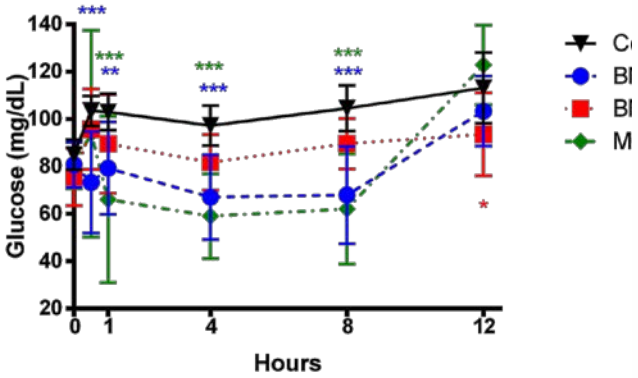
Nutrition & Metabolism

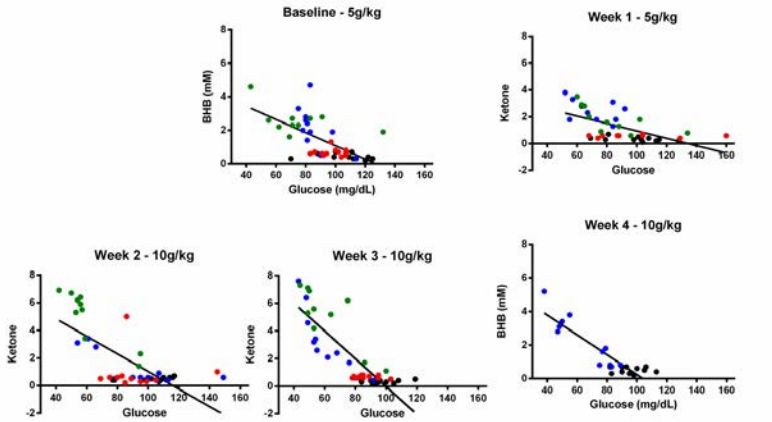
RESEARCH Open Access

Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague–Dawley rats

Shannon L. Kesl[1*], Angela M. Poff[1], Nathan P. Ward[1], Tina N. Fiorelli[1], Csilla Ari[1], Ashley J. Van Putten[1], Jacob W. Sherwood[1], Patrick Arnold[2] and Dominic P. D'Agostino[1]

- Hyperglycemia linked to many chronic illnesses and inflammation
- Lower glucose → lower insulin
- → lower inflammation





Anti-Anxiety and Anti-Convulsant Effects

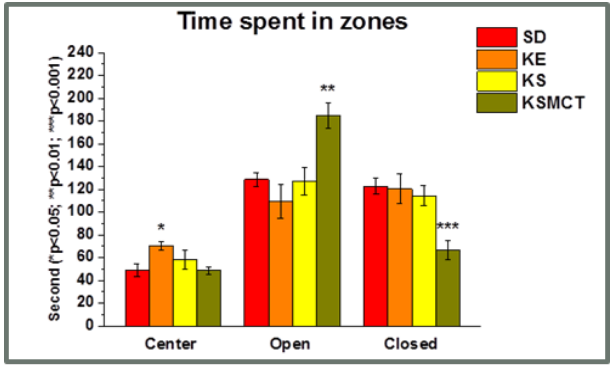
(mediated, in part, through adenosinergic mechanism)

frontiers in Molecular Neuroscience

Exogenous Ketone Supplements Reduce Anxiety-Related Behavior in Sprague-Dawley and Wistar Albino Glaxo/Rijswijk Rats

Csilla Ari[1], Zsolt Kovács[2], Gabor Juhasz[3], Cem Murdun[1], Craig R. Goldhagen[1], Andrew M. Koutnik[1], Angela M. Poff[1], Shannon L. Kesl[1] and Dominic P. D'Agostino[1]*

[1] Department of Molecular Pharmacology and Physiology, Hyperbaric Biomedical Research Laboratory, Morsani College of Medicine, University of South Florida, Tampa, FL, USA, [2] Department of Zoology, University of West Hungary, Szombathely, Hungary, [3] Proteomics Laboratory, Eotvos Lorand University, Budapest, Hungary



ORIGINAL RESEARCH ARTICLE
Front. Mol. Neurosci., 25 July 2017 | https://doi.org/10.3389/fnmol.2017.00235

Adenosine A1 Receptor Antagonism Abolished the Anti-seizure Effects of Exogenous Ketone Supplementation in Wistar Albino Glaxo Rijswijk Rats

Zsolt Kovács[1], Dominic P. D'Agostino[2], Arpád Dobolyi[3,4] and Csilla Ari[2,5]*

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[3]Laboratory of Neuromorphology and Human Brain Tissue Bank, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary
[4]Laboratory of Molecular and Systems Neurobiology, Department of Physiology and Neurobiology, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary
[5]Department of Psychology, University of South Florida, Tampa, FL, United States

frontiers in Behavioral Neuroscience

Anxiolytic Effect of Exogenous Ketone Supplementation Is Abolished by Adenosine A1 Receptor Inhibition in Wistar Albino Glaxo/Rijswijk Rats

*Zsolt Kovács[1], Dominic P. D'Agostino[2,3] and Csilla Ari[2,4]**

[1] Savaria Department of Biology, Eötvös Loránd University (ELTE), Budapest, Hungary, [2] Department of Molecular Pharmacology and Physiology, Metabolic Medicine Research Laboratory, Morsani College of Medicine, University of South Florida, Tampa, FL, United States, [3] Institute for Human and Machine Cognition, Ocala, FL, United States, [4] Department of Psychology, Hyperbaric Neuroscience Research Laboratory, University of South Florida, Tampa, FL, United States

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

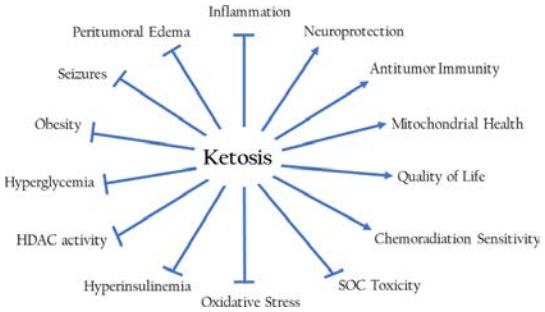
Review

Targeting the Warburg effect for cancer treatment: Ketogenic diets for management of glioma

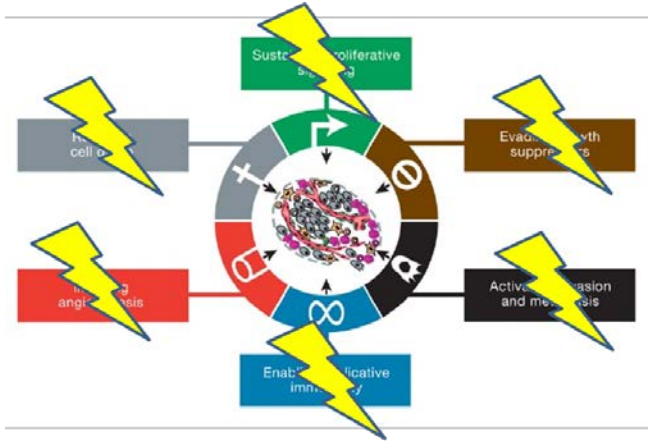
Angela Poff[a], Andrew P. Koutnik[a], Kathleen M. Egan[b], Solmaz Sahebjam[c], Dominic D'Agostino[a], Nagi B. Kumar[b,*]

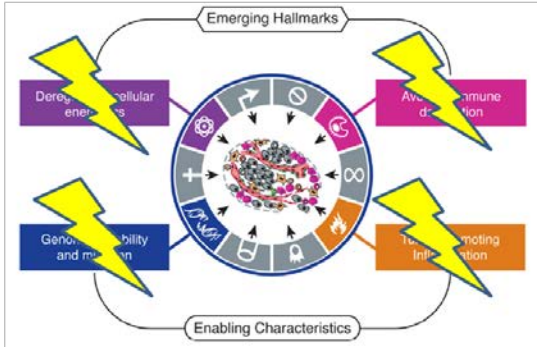
[a] The University of South Florida, Department of Molecular Pharmacology and Physiology, 12901 Bruce B. Downs Blvd, MDC 8, Tampa, FL 33612, United States
[b] Moffitt Cancer Center, H. Lee Moffitt Cancer Center and Research Institute, Department of Cancer Epidemiology, 12902 Magnolia Drive, MRC/CANCONT, Tampa, FL 22612-9497, United States
[c] Department of Neuro-Oncology, H. Lee Moffitt Cancer Center and Research Institute, Department of Cancer Epidemiology, 12902 Magnolia Drive, Tampa, FL 22612-9497, United States

Interconnections between tumor metabolism



Therapeutic Ketosis Targets (directly/indirectly) The Hallmarks of Cancer





Implementing and Defining Nutritional Ketosis

Low Carb

Glucose /Ketones

GKi = 6.6

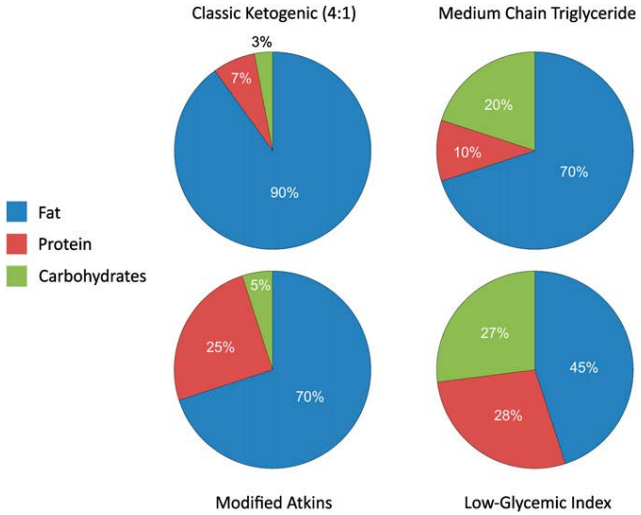
Strict 4:1 Keto

Glucose /Ketones

GKi = 1.0

Strategies for Inducing Nutritional Ketosis

Low Carb/Ketogenic Diet (variants)



Ketone Supplementation

Exogenous Ketones
Products available and in development

2 Day Examples

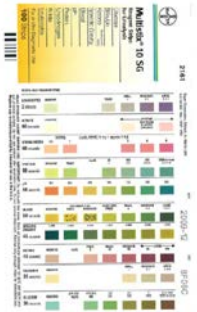
Time-Restricted Eating (TRE)

Normal Diet	24hr Unrestricted		24hr Unrestricted	
ADF ADMF	24hr Unrestricted		24hr Fasting/Restricted	
TRE IF	Fast 20 hr	Eat 4hr	Fast 14 hr	Eat 10hr

<u>Support Supplements</u>:
L-Carnitine*, Potassium Citrate*
MCTs, Mg^{2+}, DHA, D3, Lysine, Leucine, Taurine
*(used with clinical ketogenic diet)

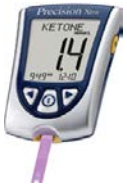
Commercially Available Tools for Assessing Nutritional Ketosis

Urine
Acetoacetate
(AcAc)

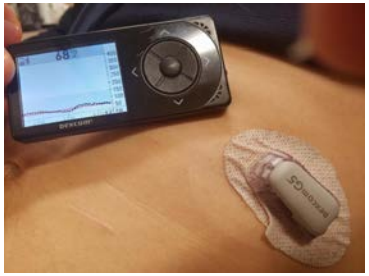


Breath
Acetone

Blood
(BHB)



Future Devices
Continuous BHB/AcAc
(*e.g.* Dexcom)



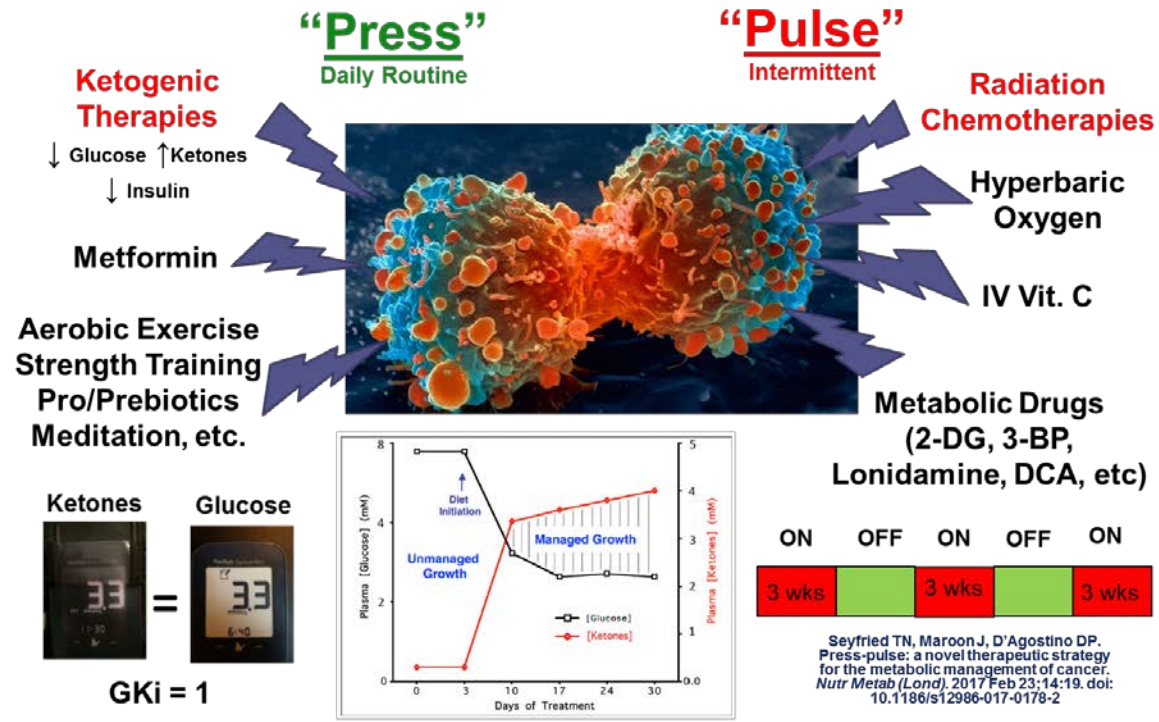
Seyfried *et al. Nutrition & Metabolism* (2017) 14:19
DOI 10.1186/s12986-017-0178-2

Nutrition & Metabolism

METHODOLOGY Open Access

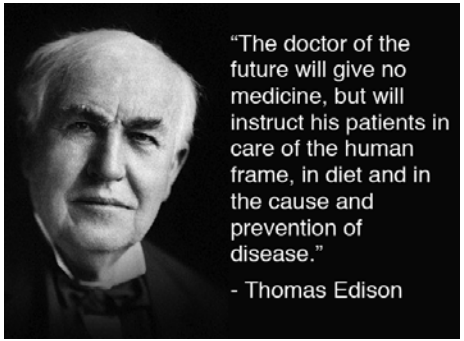
Press-pulse: a novel therapeutic strategy for the metabolic management of cancer

Thomas N. Seyfried[1*], George Yu[2], Joseph C. Maroon[3] and Dominic P. D'Agostino[4]



Seyfried TN, Maroon J, D'Agostino DP. Press-pulse: a novel therapeutic strategy for the metabolic management of cancer. *Nutr Metab (Lond)*. 2017 Feb 23;14:19. doi: 10.1186/s12986-017-0178-2

Future Directions



- Develop and test safe and effective methods to optimize and sustain NK
- Determine effects on cardiometabolic parameters (BP, HbA1c, Trigs), inflammation (*hs*CRP) and longevity
- Human clinical trials for Angelman's syndrome, brain cancer and evaluating for use in operational activities (e.g. NASA NEEMO).
- Research and advocate "Lifestyle Medicine" for treatment, management and prevention of disease

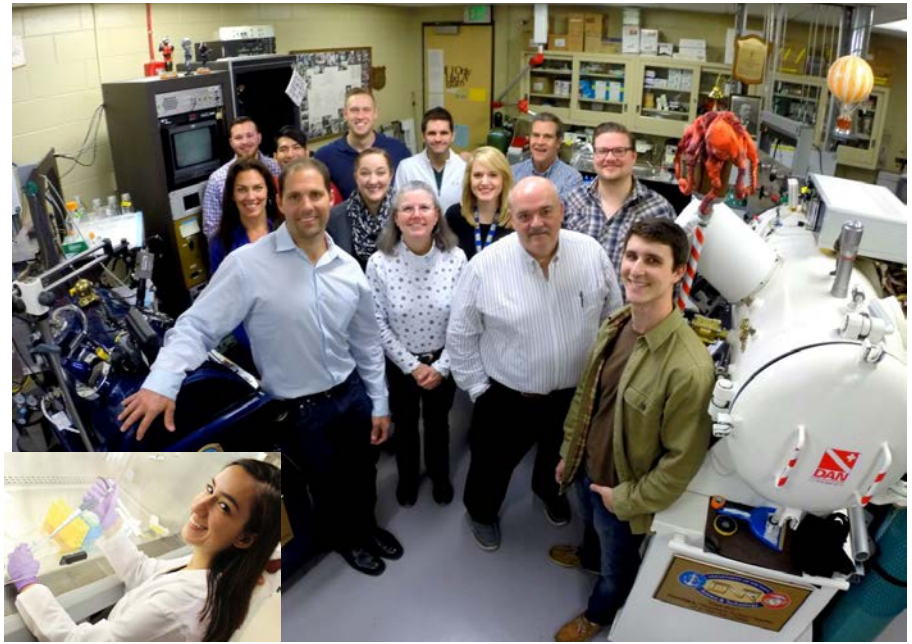
Thank You!

Laboratory of Metabolic Medicine

- Dr. Csilla Ari D'Agostino
- Dr. Angela Poff
- Dr. Chris Rogers
- Dr. Shannon Kesl
- Craig Goldhagen
- Dr. Nate Ward
- Andrew Koutnik
- Janine DeBlasi

Hyperbaric Biomedical Research Lab

- Dr. Jay Dean
- Carol Landon
- Geoffrey Ciarlone
- Jacob Sherwood
- Chris Hinojo



Questions???

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8. Poff A, Ari C, Seyfried TN, D'Agostino, DP. The Ketogenic Diet and Hyperbaric Oxygen Therapy act Synergistically to Prolong Survival in Mice with Systemic Metastatic Cancer. PLoS ONE, 2013; 8 (6): e65522 DOI: 10.1371/journal.pone.0065522
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10. Poff A, Ari C, Seyfried TN, D'Agostino, DP. Ketone Supplementation Decreases Tumor Cell Viability and Prolongs Survival of Mice with Metastatic Cancer. International Journal of Cancer: IJC-13-2481, 2013.
11. Seyfried TN, Marsh J, Mukherjee P, Zuccoli G, D'Agostino DP. Metabolic Therapy: A New Paradigm for Managing Malignant Brain Cancer. Cancer Letters, 2014 Jul 25. pii: S0304-3835(14)00352-8. doi: 10.1016/j.canlet.2014.07.015.
12. Seyfried TN, Yu G, Maroon JC, D'Agostino DP. Press-pulse: a novel therapeutic strategy for the metabolic management of cancer. Nutr Metab (Lond). 2017 Feb 23;14:19. doi: 10.1186/s12986-017-0178-2. eCollection 2017. PubMed PMID:28250801; PubMed Central PMCID: PMC5324220.

Questions?

Break for Lunch till 2 pm

Please return by 2 pm
for afternoon sessions

WOMEN AND COGNITION: INSULIN, MENOPAUSE AND ALZHEIMER'S

FILOMENA TRINDADE, MD, MPH

www.drtrindade.com

University of Miami's Annual Integrative Medicine Conference

April 26, 2018

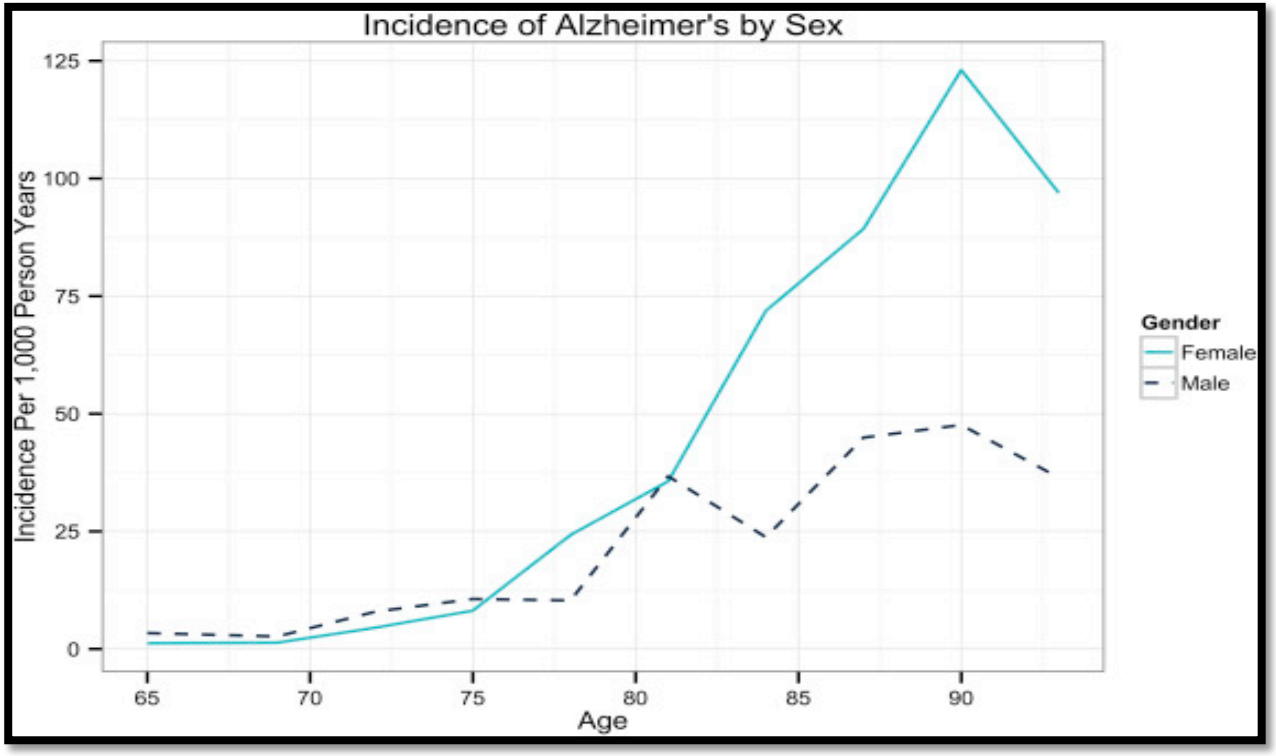
OBJECTIVES

- Gain a basic understanding on the pathophysiology of mild cognitive decline and Alzheimer's and how it relates to insulin resistance and the menopausal transition in women
- Review the potential mechanisms of diabetes type 2 and how it contributes to Alzheimer's disease in women
- Identify the hallmarks of hormone replacement with respect to Alzheimer's disease in women

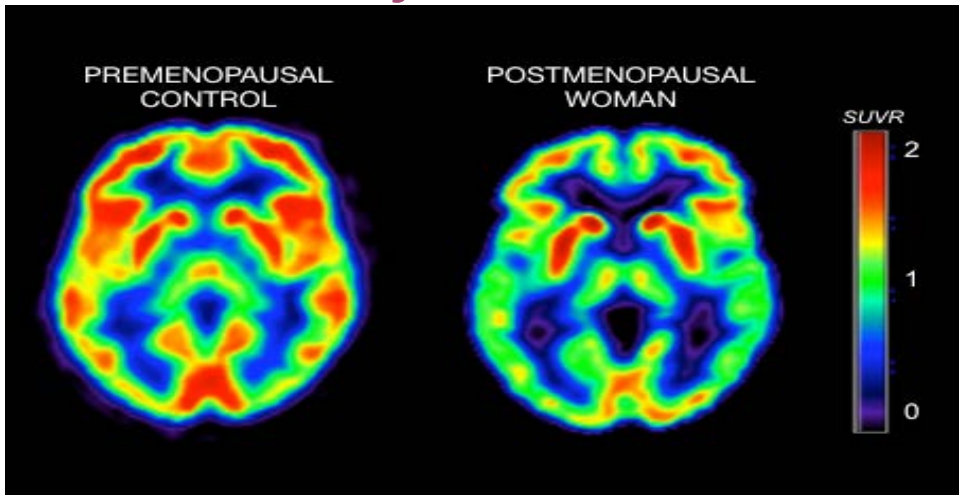
BRANDALYN C, ET AL.

AGE, APOE AND SEX: TRIAD OF RISK OF ALZHEIMER'S DISEASE.

J STEROID BIOCHEM MOL BIOL. 2016;160:134-147.



Menopause Triggers Metabolic Changes in Brain That May Promote Alzheimer's



"The color scale reflects brain activity, with brighter colors indicating more activity, and darker colors indicating lower activity. The scan to the right (menopause) looks 'greener' and overall darker, which means that the woman's brain has substantially lower brain activity (more than 30 percent less) than the one to the left (no signs of menopause)."

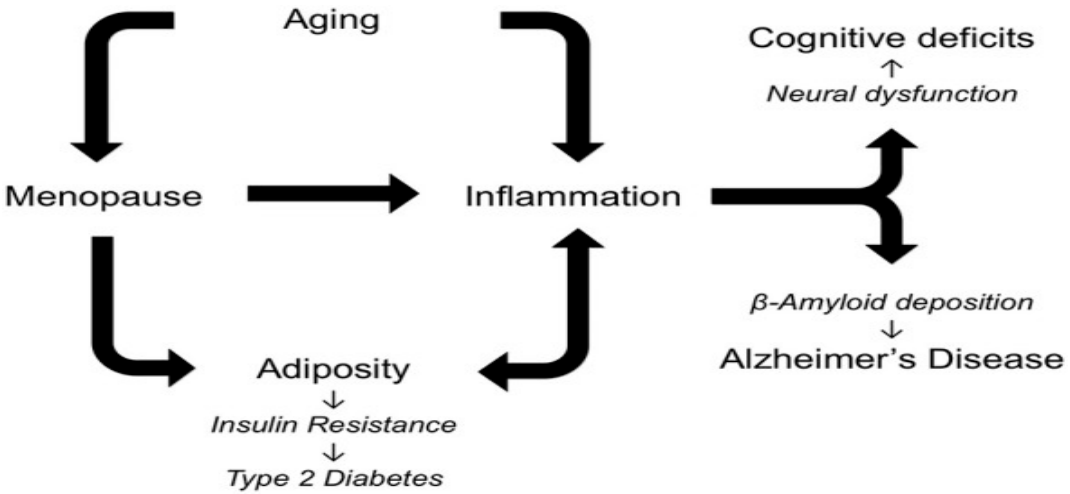
https://news.weill.cornell.edu/news/2017/10/menopause-triggers-metabolic-changes-in-brain-that-may-promote-alzheimers. Accessed April 23, 2018.

MOSCONI L, ET AL.
SEX DIFFERENCES IN ALZHEIMER RISK: BRAIN IMAGING OF ENDOCRINE VS CHRONOLOGIC AGING.
NEUROLOGY. 2017;89(13):1382-1390.

- “This study demonstrated that, in early midlife, women outperformed age-matched men across all memory measures, but sex differences were attenuated for postmenopausal women. Initial learning and memory retrieval were particularly vulnerable, whereas memory consolidation and storage were preserved. Findings underscore the significance of the decline in ovarian estradiol production in midlife and its role in shaping memory function.”

CHRISTENSEN A, ET AL.
MENOPAUSE, OBESITY AND INFLAMMATION: INTERACTIVE RISK FACTORS FOR ALZHEIMER'S DISEASE.
FRONT AGING NEUROSCI. 2015;7:130.

- "The onset of menopause in mid-life elevates the vulnerability of women to AD, an increased risk that is likely associated with the depletion of estrogens. Menopause is also linked with an abundance of additional changes, including increased central adiposity and inflammation."



"Alzheimer's disease (AD) is a multifactorial disorder in which multiple risk factors are theorized to interact in regulating pathogenesis.

As depicted in the diagram an essential factor in AD is increasing age, which is also associated with elevated inflammation and, in women, menopause. The loss of estrogens at menopause increases central adiposity, which in turn increases inflammation and predisposes women to metabolic syndrome, insulin resistance, and AD. Individually and cooperatively, aging, menopause, adiposity, and inflammation lead to cognitive deficits and AD."

Christensen A, et al. *Front Aging Neurosci.* 2015;7:130.

TALBOT K, ET AL.
DEMONSTRATED BRAIN INSULIN RESISTANCE IN ALZHEIMER'S DISEASE PATIENTS IS ASSOCIATED WITH IGF-1 RESISTANCE, IRS-1 DYSREGULATION, AND COGNITIVE DECLINE.
J CLIN INVEST. **2012;122(4):1316-1338.**

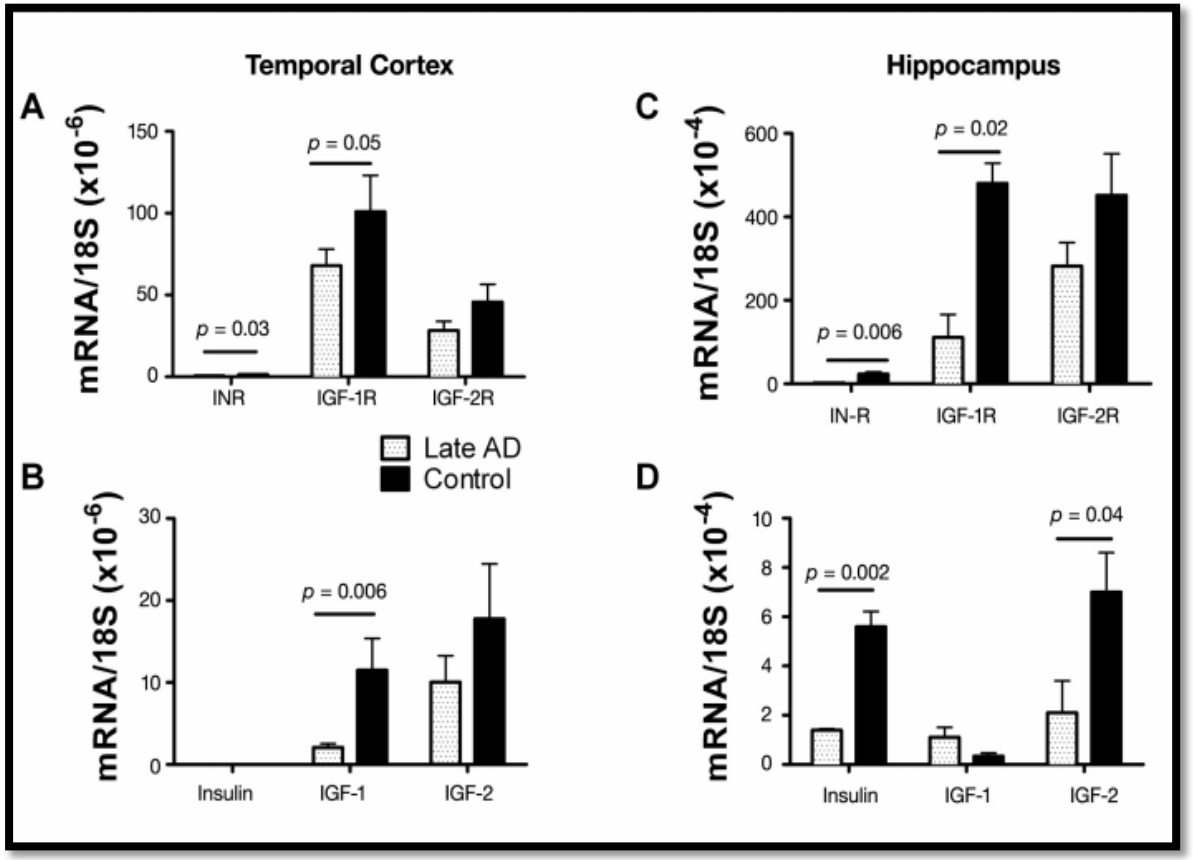
- "Brain insulin resistance thus appears to be an early and common feature of AD, a phenomenon accompanied by IGF-1 resistance and closely associated with IRS-1 dysfunction."

LI W, ET AL.
TYPE 2 DIABETES MELLITUS MIGHT BE A RISK FACTOR FOR MILD COGNITIVE IMPAIRMENT PROGRESSING TO ALZHEIMER'S DISEASE.
NEUROPSYCHIATR DIS TREAT. 2016;12:2489-2495.

- “Type 2 DM might be a risk factor for MCI progressing into AD.”

DE LA MONTE SM, ET AL.
ALZHEIMER'S DISEASE IS TYPE 3 DIABETES-EVIDENCE REVIEWED.
J DIABETES SCI TECHNOL. 2008;2(6):1101-1113.

- “We conclude that the term type 3 diabetes accurately reflects the fact that AD represents a form of diabetes that selectively involves the brain and has molecular and biochemical features that overlap with both type1DM and T2DM.”

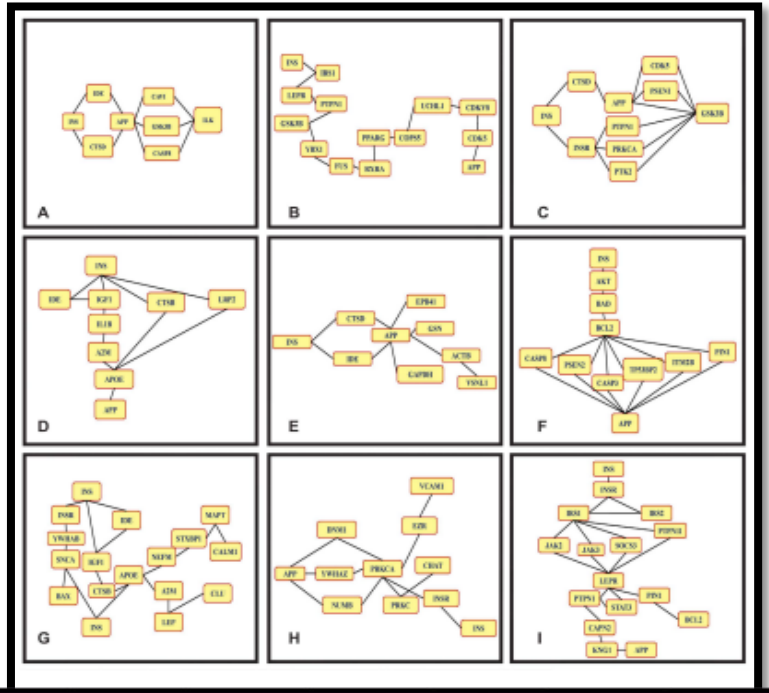


DE LA MONTE SM, ET AL. *J DIABETES SCI TECHNOL*. 2008;2(6):1101-13.

MITTAL K, ET AL.
TYPE 3 DIABETES: CROSS TALK BETWEEN DIFFERENTIALLY REGULATED PROTEINS OF TYPE 2 DIABETES MELLITUS AND ALZHEIMER'S DISEASE.
SCI REP. 2016;6:25589.

- “Type 3 DM is a neuroendocrine disorder that represents the progression of type 2 DM to AD.”

MITTAL K, ET AL.
TYPE 3 DIABETES: CROSS TALK BETWEEN DIFFERENTIALLY REGULATED PROTEINS OF TYPE 2 DIABETES MELLITUS AND ALZHEIMER'S DISEASE.
***SCI REP.* 2016;6:25589.**



Mechanism through which insulin and amyloid beta are linked.

MITTAL K, ET AL.
TYPE 3 DIABETES: CROSS TALK BETWEEN DIFFERENTIALLY REGULATED PROTEINS OF TYPE 2 DIABETES MELLITUS AND ALZHEIMER'S DISEASE.
***SCI REP.* 2016;6:25589.**

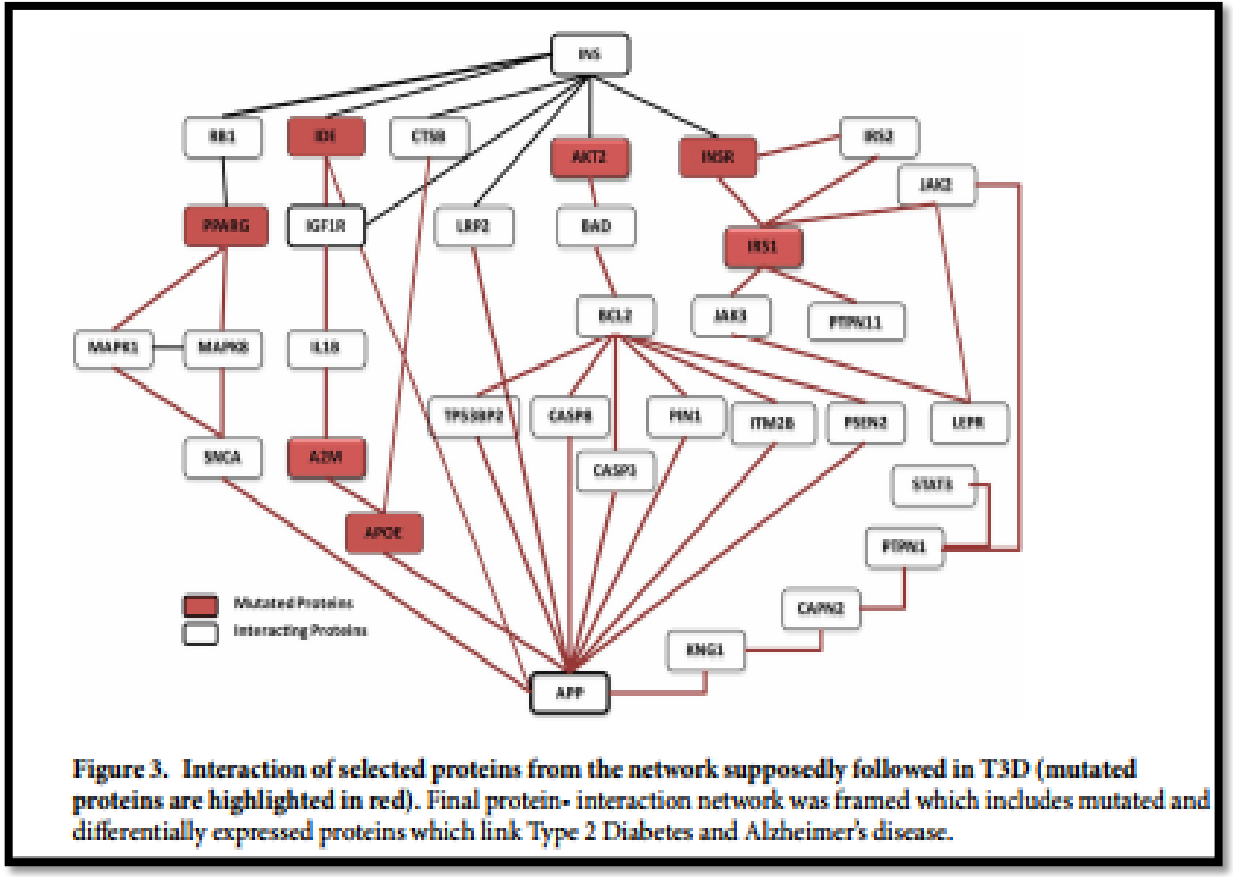


Figure 3. Interaction of selected proteins from the network supposedly followed in T3D (mutated proteins are highlighted in red). Final protein- interaction network was framed which includes mutated and differentially expressed proteins which link Type 2 Diabetes and Alzheimer's disease.

MITTAL K, ET AL.
TYPE 3 DIABETES: CROSS TALK BETWEEN DIFFERENTIALLY REGULATED PROTEINS OF TYPE 2 DIABETES MELLITUS AND ALZHEIMER'S DISEASE.
***SCI REP.* 2016;6:25589.**

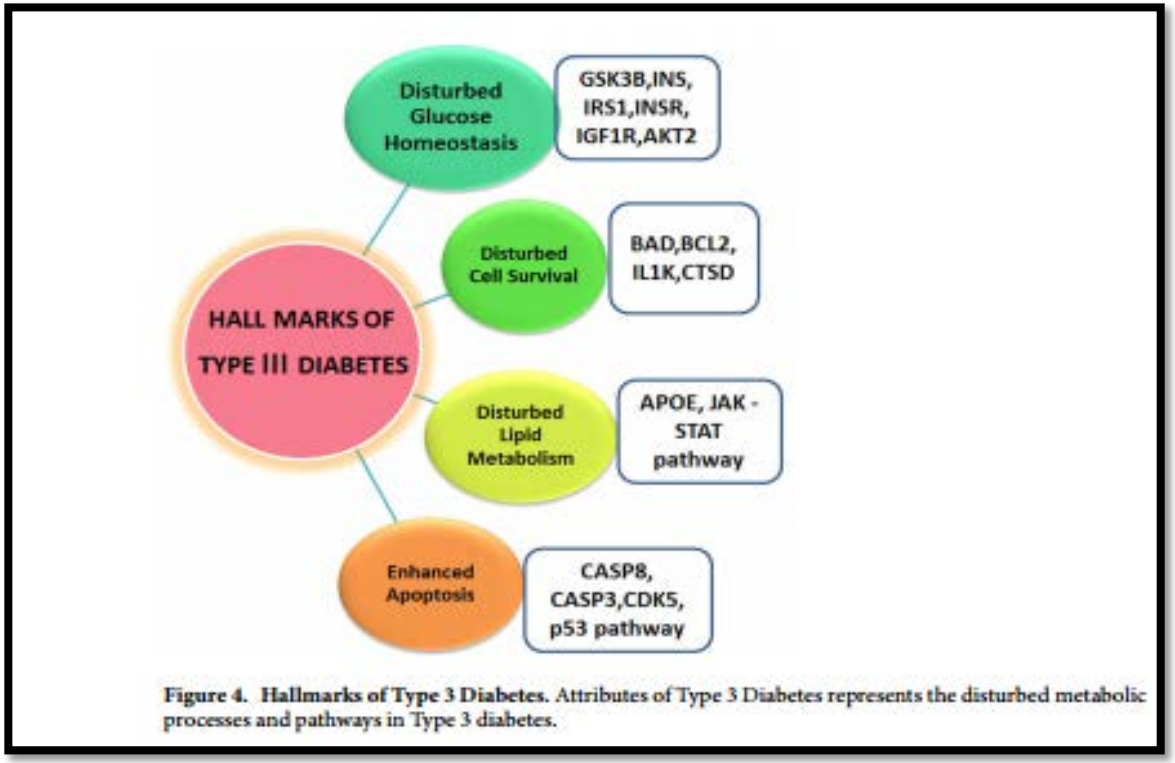


Figure 4. Hallmarks of Type 3 Diabetes. Attributes of Type 3 Diabetes represents the disturbed metabolic processes and pathways in Type 3 diabetes.

GEORGAKIS MK, ET AL.
AGE AT MENOPAUSE AND DURATION OF REPRODUCTIVE PERIOD IN ASSOCIATION WITH DEMENTIA AND COGNITIVE FUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS.
PSYCHONEUROENDOCRINOLOGY. 2016;73:224-243.

- "Existing evidence does not support an association between indices of prolonged exposure to female hormones and lower dementia risk. There are indications, however, for better cognitive performance and delayed cognitive decline, supporting a link between female hormone deficiency and cognitive aging."

HENDERSON VW.

ALZHEIMER'S DISEASE: REVIEW OF HORMONE THERAPY TRIALS AND IMPLICATIONS FOR TREATMENT AND PREVENTION AFTER MENOPAUSE.

***J STEROID BIOCHEM MOL BIOL*. 2014;142:99-106.**

- "Findings of 9 randomized clinical trials of estrogen containing hormone therapy in Alzheimer's disease suggested that hormone therapy does not improve cognitive symptoms of women with Alzheimer's disease."

PINES A.
ALZHEIMER'S DISEASE, MENOPAUSE AND THE IMPACT OF THE ESTROGENIC ENVIRONMENT.
***CLIMACTERIC*. 2016;19(5):430-432.**

- “Recent studies, such as WHIMS-Young, the Kronos Early Estrogen Prevention Study and the Early versus Late Intervention Trial with Estradiol targeted the younger women, and indeed showed that hormone therapy may have positive cognitive outcomes in this age group.”

ZÁRATE S, ET AL.
ROLE OF ESTROGEN AND OTHER SEX HORMONES IN BRAIN AGING. NEUROPROTECTION AND DNA REPAIR.
FRONT AGING NEUROSCI. 2017;9:430.

- "Sex hormones, particularly estrogens possess potent antioxidant properties and play important roles in maintaining normal reproductive and non-reproductive functions. They exert neuroprotective actions and their loss during aging and natural or surgical menopause is associated with mitochondrial dysfunction, neuroinflammation, synaptic decline, cognitive impairment and increased risk of age-related disorders. Moreover, loss of sex hormones has been suggested to promote an accelerated aging phenotype eventually leading to the development of brain hypometabolism, a feature often observed in menopausal women and prodromal Alzheimer's disease (AD)."

OSMANOVIC-BARILAR J, ET AL.
EVALUATING THE ROLE OF HORMONE THERAPY IN POSTMENOPAUSAL WOMEN WITH ALZHEIMER'S DISEASE.
DRUGS AGING. 2016;33(11):787-808.

- “This review points to possible reasons for these mixed data by considering the issues of both preclinical and clinical trials, in particular, the representativeness of animal models, timing of HT initiation, type of HT (different types of estrogen compounds, estrogen monotherapy vs. estrogen-progesterone combined therapy), mode of drug delivery (subcutaneous, transdermal, oral, or intramuscular), and hormone dosage used, as well as the heterogeneity of the postmenopausal population in clinical trials (particularly considering their sAD stage, anti-AD therapy, and hysterectomy status).”

DAVEY DA.

PREVENTION OF ALZHEIMER'S DISEASE, CEREBROVASCULAR DISEASE AND DEMENTIA IN WOMEN: THE CASE FOR MENOPAUSE HORMONE THERAPY.

***NEURODEGENER DIS MANAG*. 2017;7(1):85-94.**

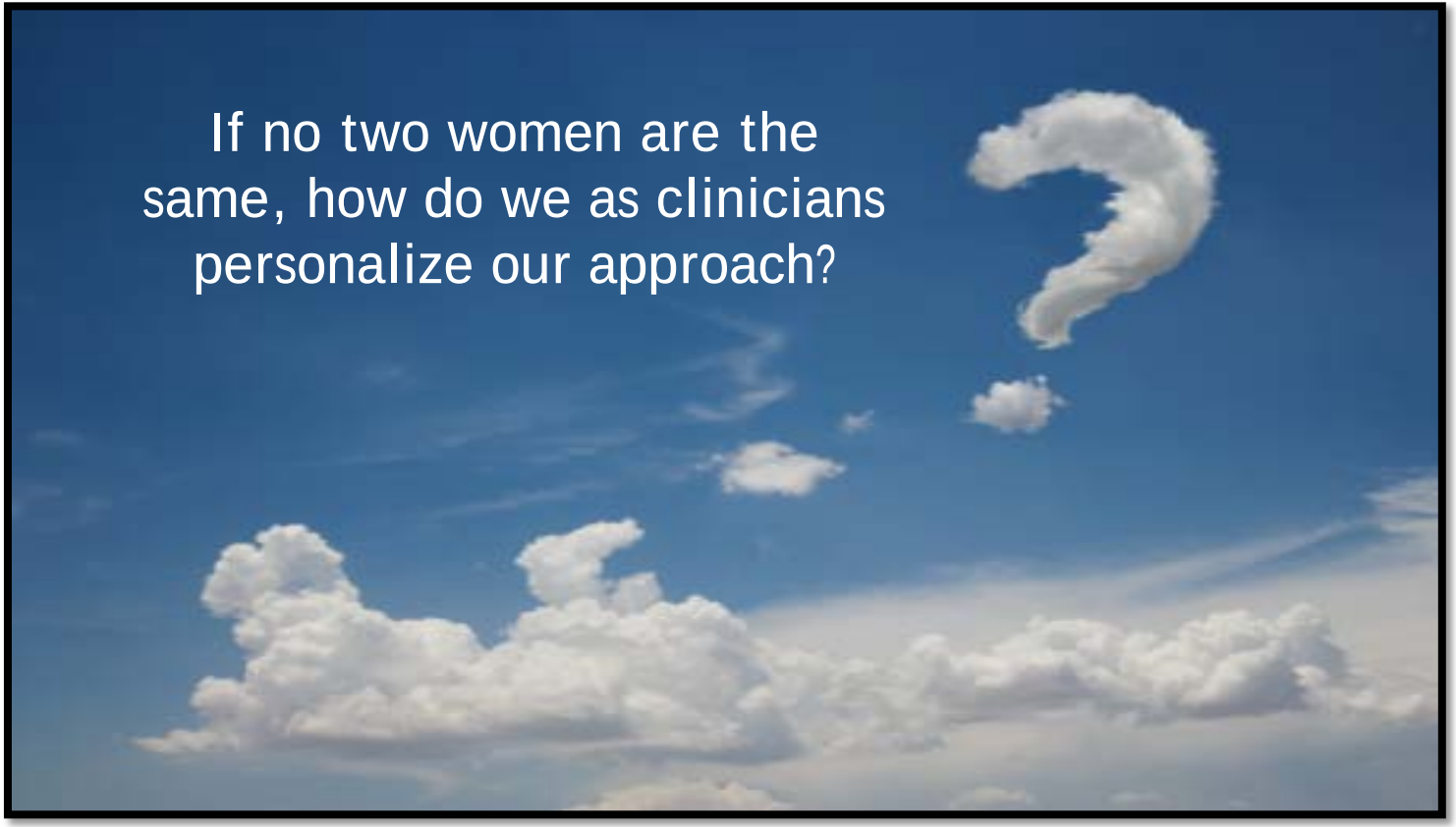
- “Recent advances in menopause hormone therapy including transdermal estrogen therapy have favorably influenced the balance of benefits and risks. A case can be made for menopause hormone therapy in healthy postmenopausal women for 5–10 years starting during the menopausal transition (the ‘window of opportunity’), together with all other protective measures, to delay or prevent the development of ARCID in later life.”

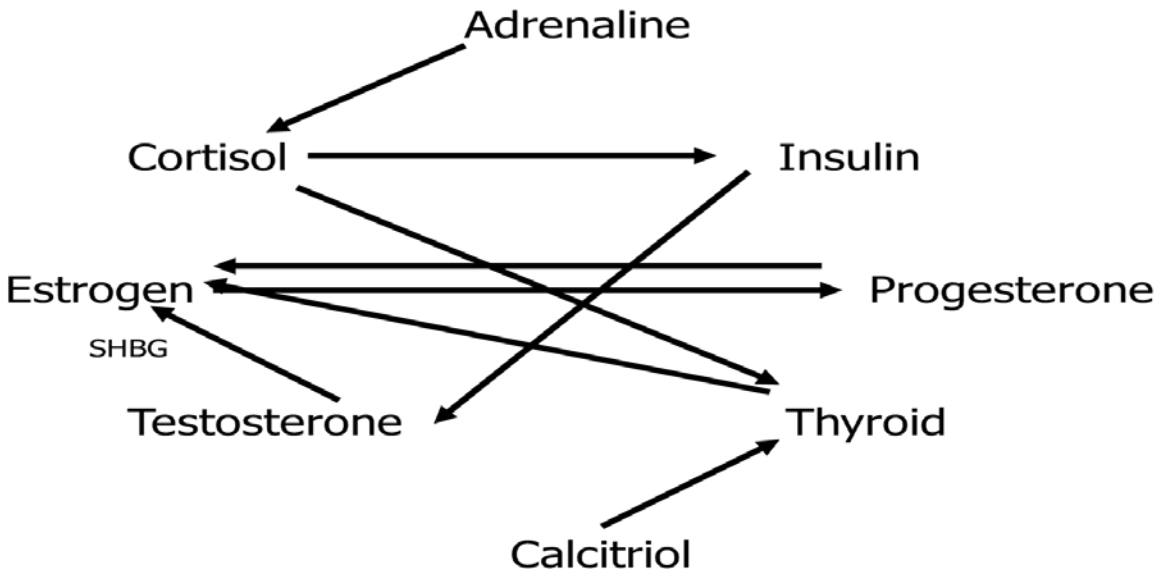
GIVEN ALL THIS, HOW DO YOU APPROACH THE MENOPAUSAL WOMAN?

MOSCONI L, ET AL.
PERIMENOPAUSE AND EMERGENCE OF AN ALZHEIMER'S BIOENERGETIC PHENOTYPE IN BRAIN AND PERIPHERY.
PLOS ONE. 2017;12(10):E0185926.

- "…the optimal window of opportunity for therapeutic intervention in women is early in the endocrine aging process."

No two women are the same

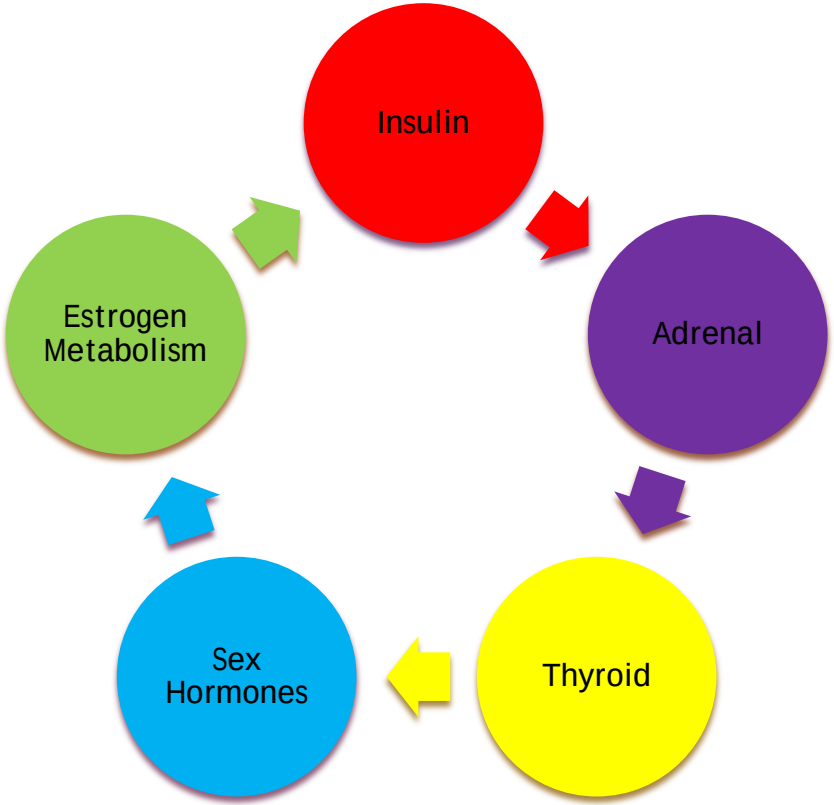

If no two women are the same, how do we as clinicians personalize our approach?


Adrenaline
Cortisol
Insulin
Estrogen
Progesterone
SHBG
Testosterone
Thyroid
Calcitriol

RETTBERG JR, ET AL.
IDENTIFYING POSTMENOPAUSAL WOMEN AT RISK FOR COGNITIVE DECLINE WITHIN A HEALTHY COHORT USING A PANEL OF CLINICAL METABOLIC INDICATORS: POTENTIAL FOR DETECTING AN AT-ALZHEIMER'S RISK METABOLIC PHENOTYPE.
NEUROBIOL AGING. 2016;40:155-163.

- "Detecting at risk individuals within a healthy population is critical for preventing or delaying Alzheimer's disease. Systems biology integration of brain and body metabolism enables peripheral metabolic biomarkers to serve as reporters of brain bioenergetic status."

The Saudade Hormonal Symphony™



DISRUPTORS OF HORMONAL FUNCTION

- Traumatic emotional events
- Physical trauma
- Chronic sleep deprivation
- Infections
- Aging
- Inflammatory diseases
- Single nucleotide polymorphisms
- Exogenous toxins
- Acute physical stress
- Nutritional insufficiencies
- Food allergy, intolerance or sensitivity
- Changes in gut microbiota
- Altered biotransformation
- Pharmaceuticals

"*Listen* to your patient, (s)he is telling you the diagnosis."

-Sir William Osler

THAT STORY IS TYPICALLY TOLD AS…

- Chief Complaint (CC)
- History of Present Illness (HPI)
- Past Medical History (PMH)
- Surgical History
- Family History (FH)
- Dietary History
- Supplement and Medication History
- Lifestyle, Social, and Exercise History
- Physical Exam Findings
- Laboratory Evaluation

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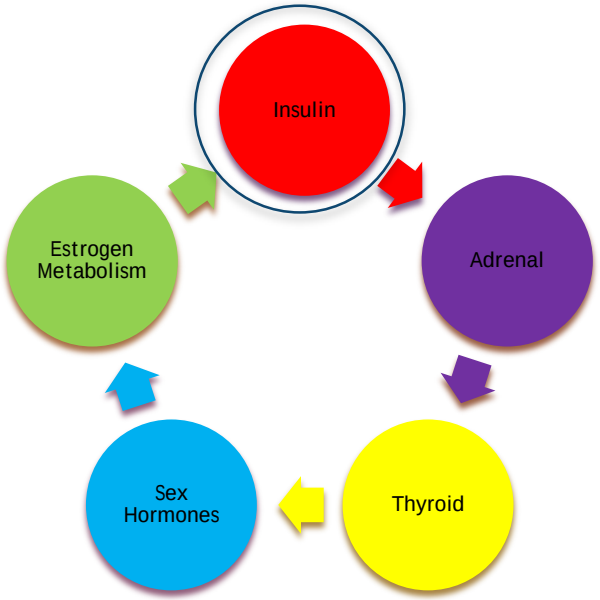


INSULIN'S EFFECTS

- Effects CBO, lipid, Metabolism
- Insulin effects thyroid function…and thyroid function effects insulin production
- Insulin effects endothelial function
- Other hormones….

GAST GC, ET AL.
MENOPAUSAL COMPLAINTS ARE ASSOCIATED WITH CARDIOVASCULAR RISK FACTORS.
***HYPERTENSION*. 2008;51(6):1492-1498.**

- “The findings support the view that menopausal complaints are associated with a less favorable cardiovascular risk profile.”

THURSTON RC, ET AL.
VASOMOTOR SYMPTOMS AND INSULIN RESISTANCE IN THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION.
J CLIN ENDOCRINOL METAB. 2012;97(10):3487-3494.

- "Hot flashes were associated with a higher HOMA index, an estimate of insulin resistance, and to a lesser extent higher glucose. Metabolic factors may be relevant to understanding the link between hot flashes and cardiovascular disease risk."

THURSTON RC, ET AL.
VASOMOTOR SYMPTOMS AND INSULIN RESISTANCE IN THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION.
J CLIN ENDOCRINOL METAB. 2012;97(10):3487-3494.

- "In summary, VMS were associated with insulin resistance, as measured by the HOMA index, over a period of approximately 8 yr. These findings may contribute to ongoing efforts to better understand any mechanisms linking hot flashes to cardiovascular health."

KWON DH, ET AL.

VASOMOTOR SYMPTOMS AND THE HOMEOSTATIC MODEL ASSESSMENT OF INSULIN-RESISTANCE IN KOREAN POSTMENOPAUSAL WOMEN.

***OBSTET GYNECOL SCI*. 2016;59(1):45-49.**

- “Our results suggest that VMS in postmenopausal women are associated with increased insulin resistance.”

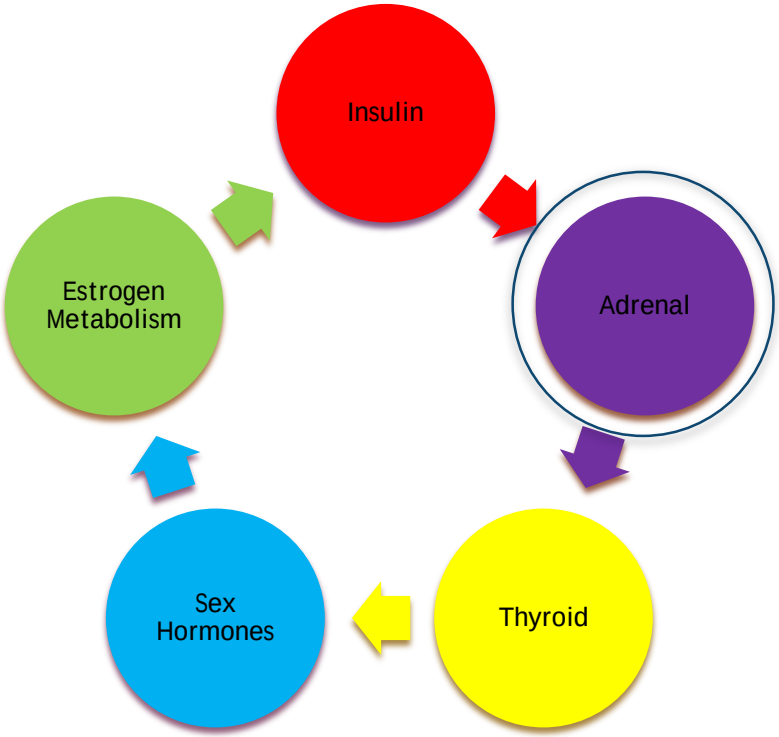
DIETARY MANAGEMENT FOR THE PATIENT WITH INSULIN RESISTANCE

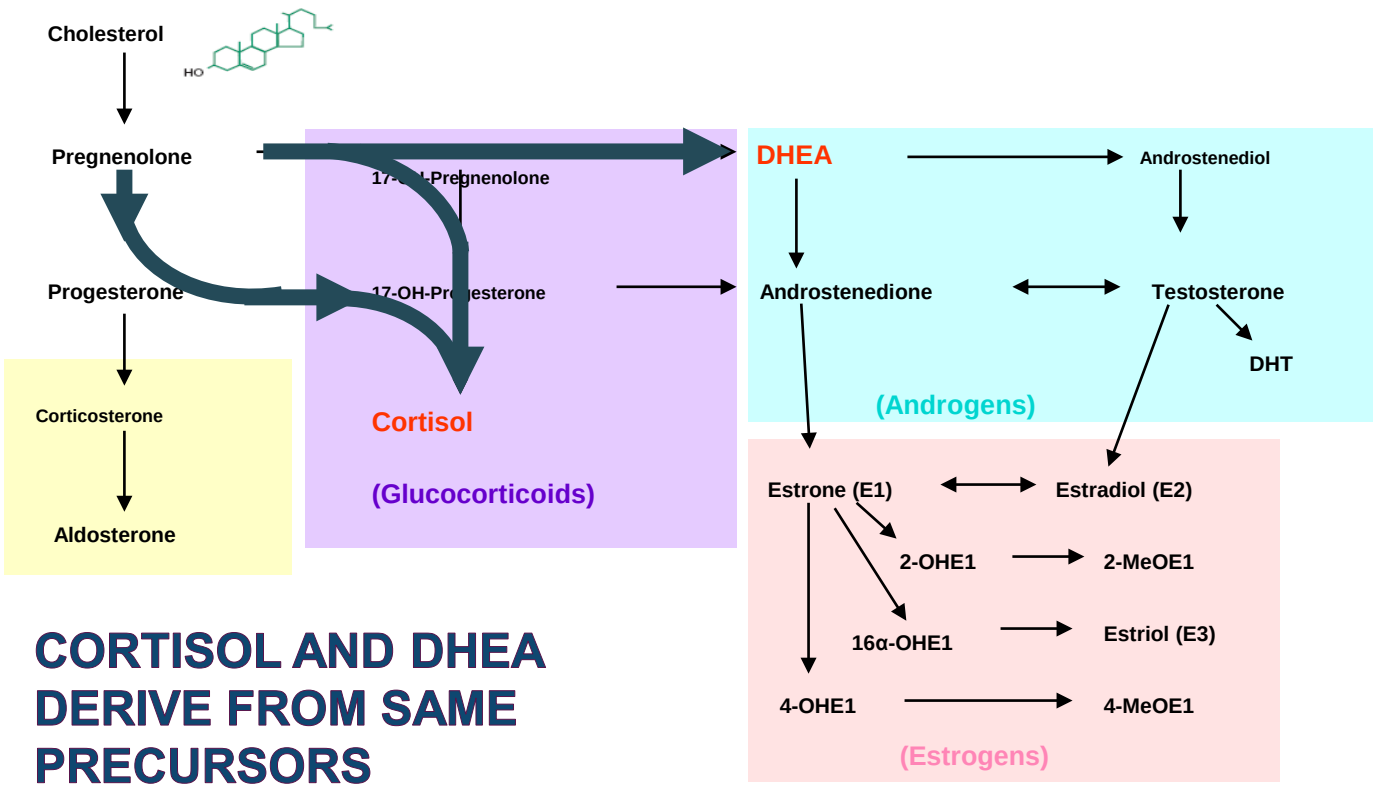
- Decrease insulin stimulation.
  - Dietary modifications which decrease insulin release:
    - Fiber
    - 'Good' (vs. 'bad') fat
    - 'Good' (vs. 'bad') carbohydrates
    - Protein at every meal
    - Elimination of most inflammatory food:
    - Wheat, dairy, soy, corn, nightshades….
- Modify Gut Microbiota
  - Food first
  - Fermented foods
  - Probiotics/prebiotics
- Increase cellular responsiveness to insulin.
  - Agents that modify insulin responsiveness at the cellular level:
    - Spices
    - Herbs
    - Chromium
    - Vitamin D
    - Magnesium
    - Omega-3

DUARTE AI, ET AL.
BRAIN INSULIN SIGNALING, GLUCOSE METABOLISM AND FEMALES' REPRODUCTIVE AGING: A DANGEROUS TRIAD IN ALZHEIMER'S DISEASE. *NEUROPHARMACOLOGY.* 2018;FEB 20.

- "We finally discussed AD as the potential type 3 diabetes, and the potential of restoring brain insulin levels or glucose energy metabolism via administration of intranasal insulin and use of ketogenic diets."

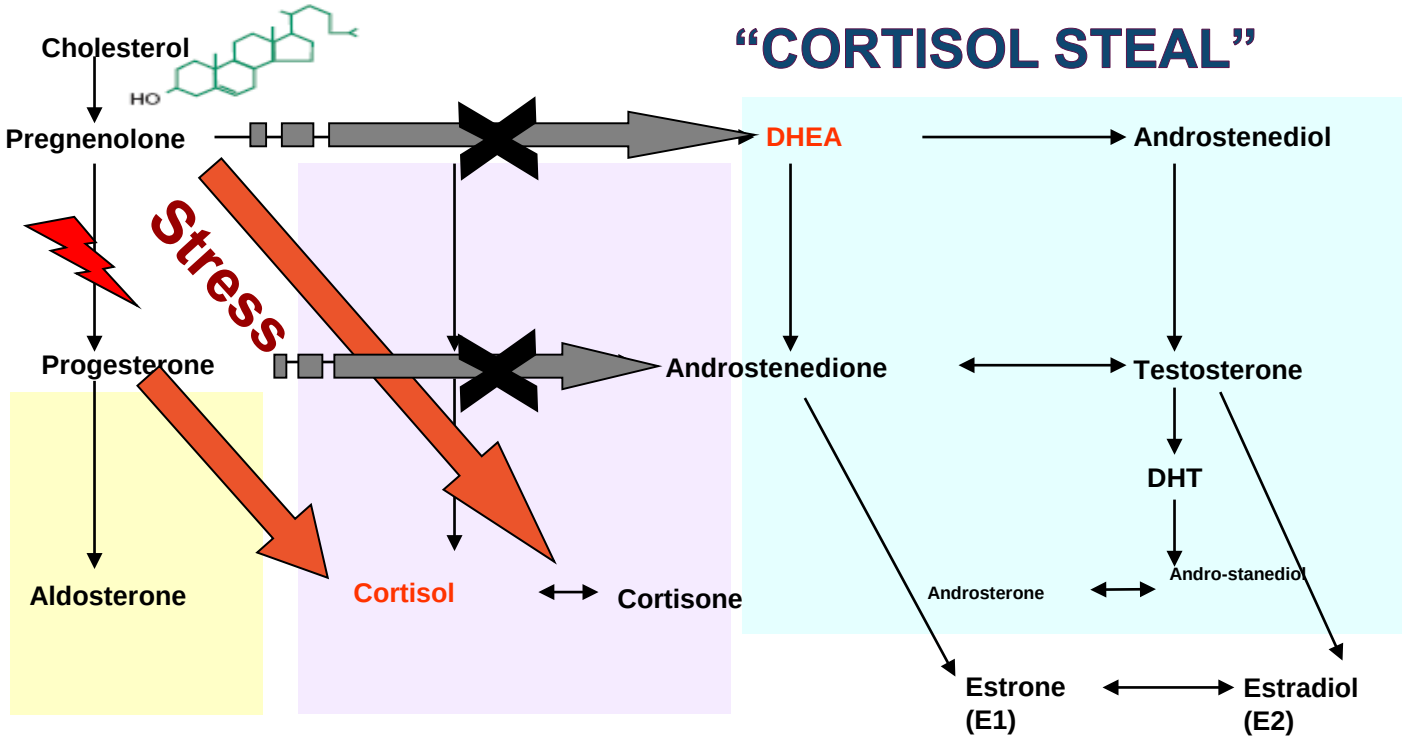
The Saudade Hormonal Symphony™





CORTISOL AND DHEA DERIVE FROM SAME PRECURSORS

“CORTISOL STEAL”



THE BIG PICTURE:
SELYE'S GENERAL ADAPTATION SYNDROME

Stage 1: Arousal

- Both cortisol and DHEA increase with episodic stress, but recovery occurs to baseline
- This may be asymptomatic

Stage 2: Adaptation

- Cortisol chronically elevated, but DHEA declines
- "Stressed," anxiety attacks, mood swings, depression

State 3: Exhaustion

- Adrenal insufficiency / low cortisol and DHEA
- Depression and fatigued

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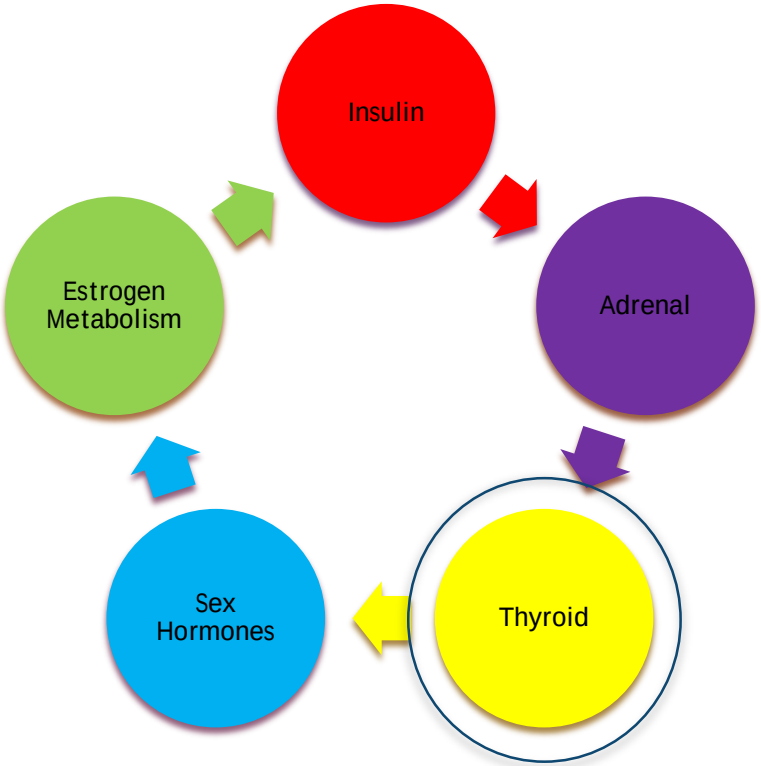
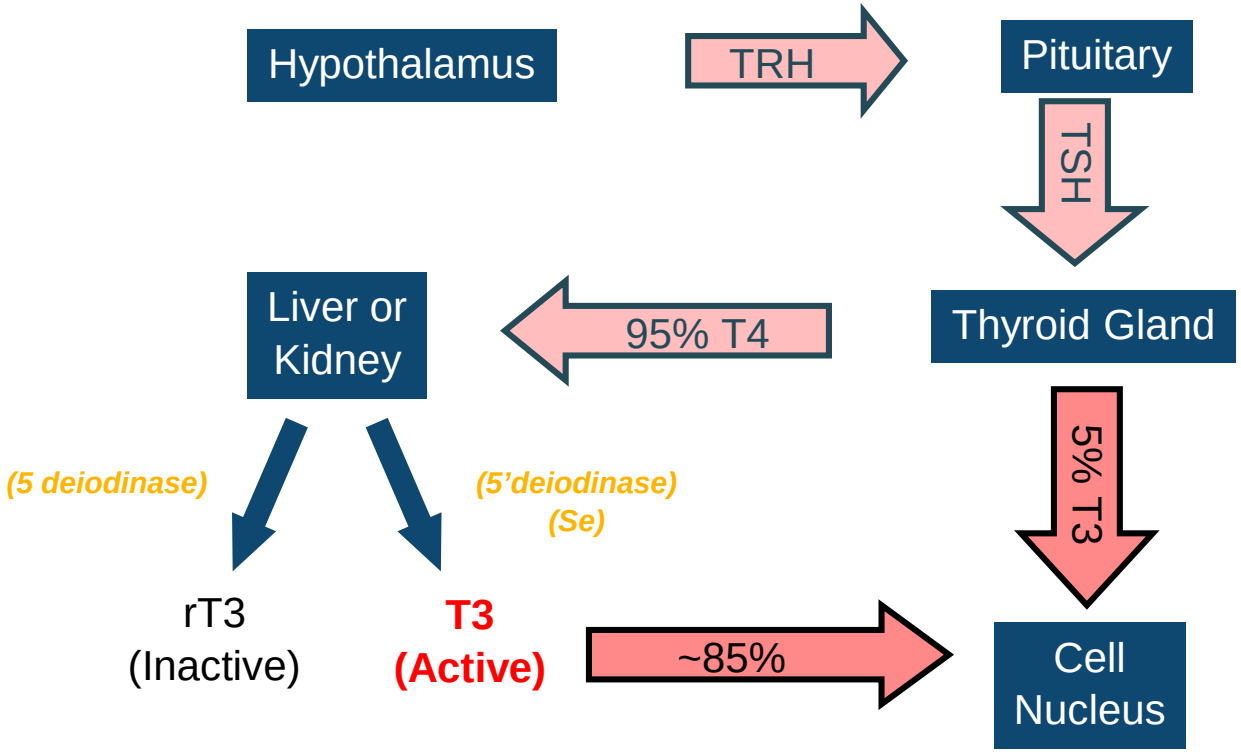
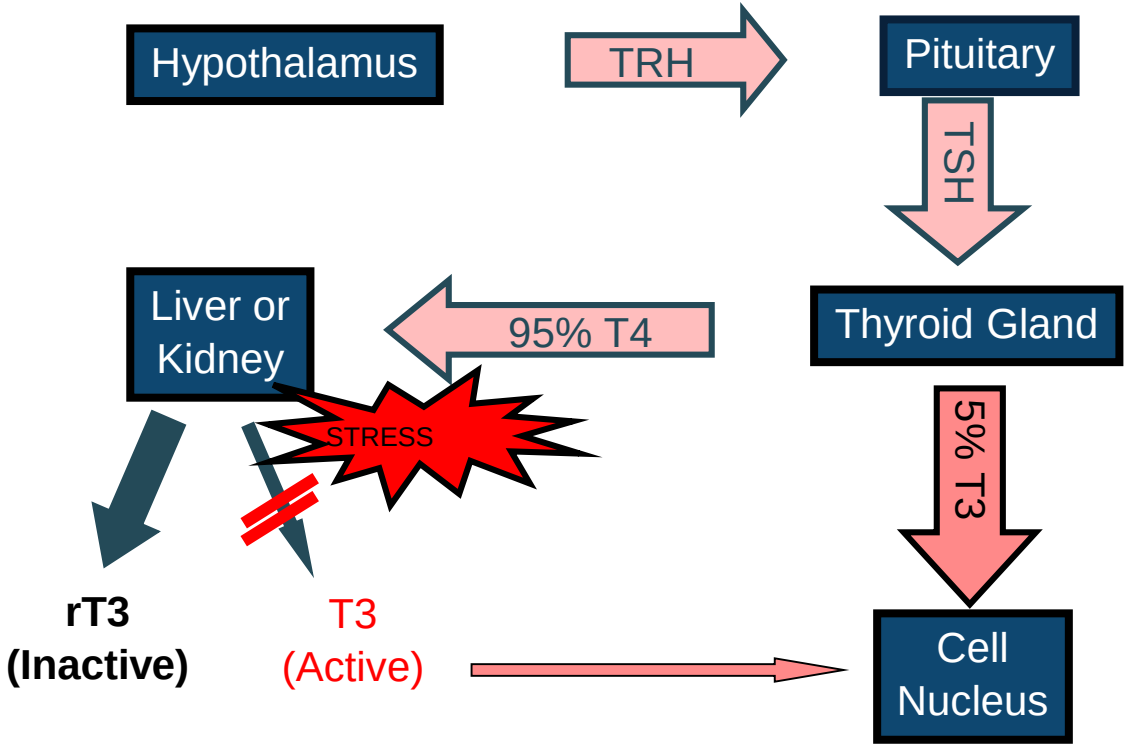
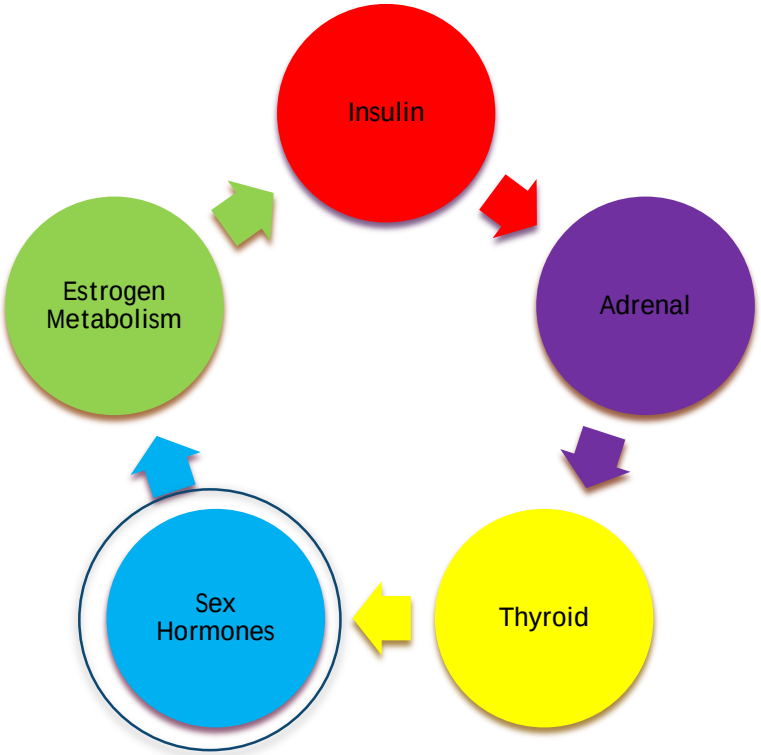
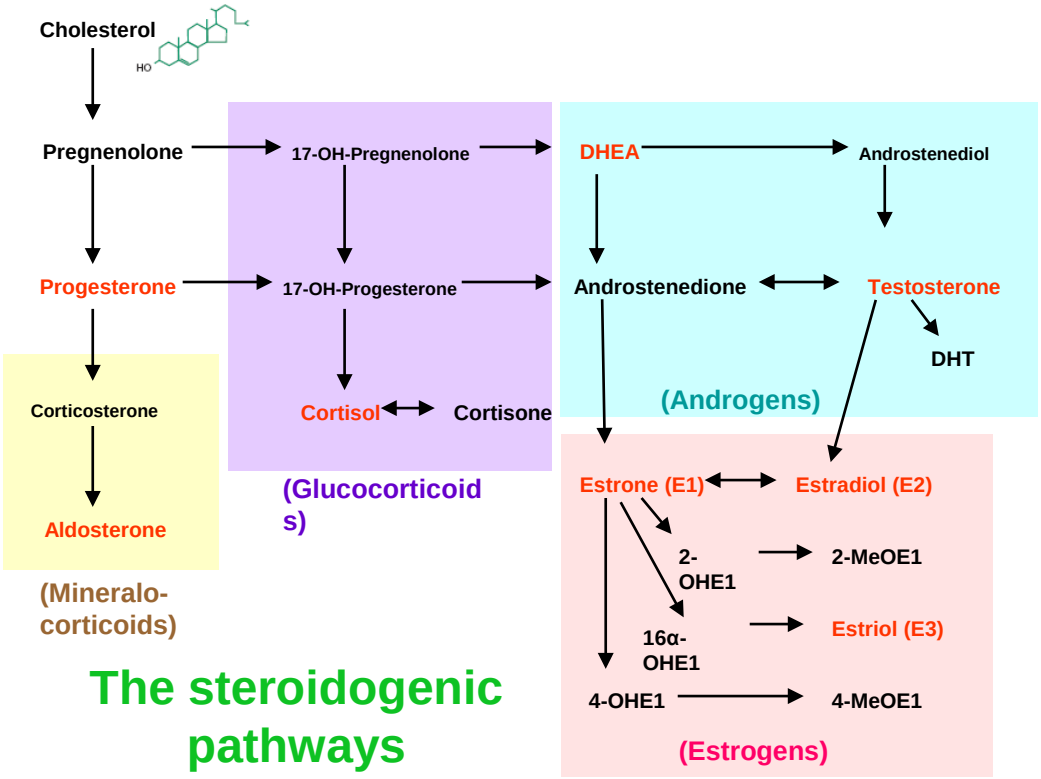



Hypothalamus
TRH
Pituitary
TSH
Thyroid Gland
5% T3
Cell Nucleus
~85%
T3 (Active)
rT3 (Inactive)
(5 deiodinase)
(5'deiodinase) (Se)
Liver or Kidney
95% T4


Hypothalamus
TRH
Pituitary
TSH
Thyroid Gland
5% T3
Cell Nucleus
95% T4
Liver or Kidney
STRESS
rT3 (Inactive)
T3 (Active)

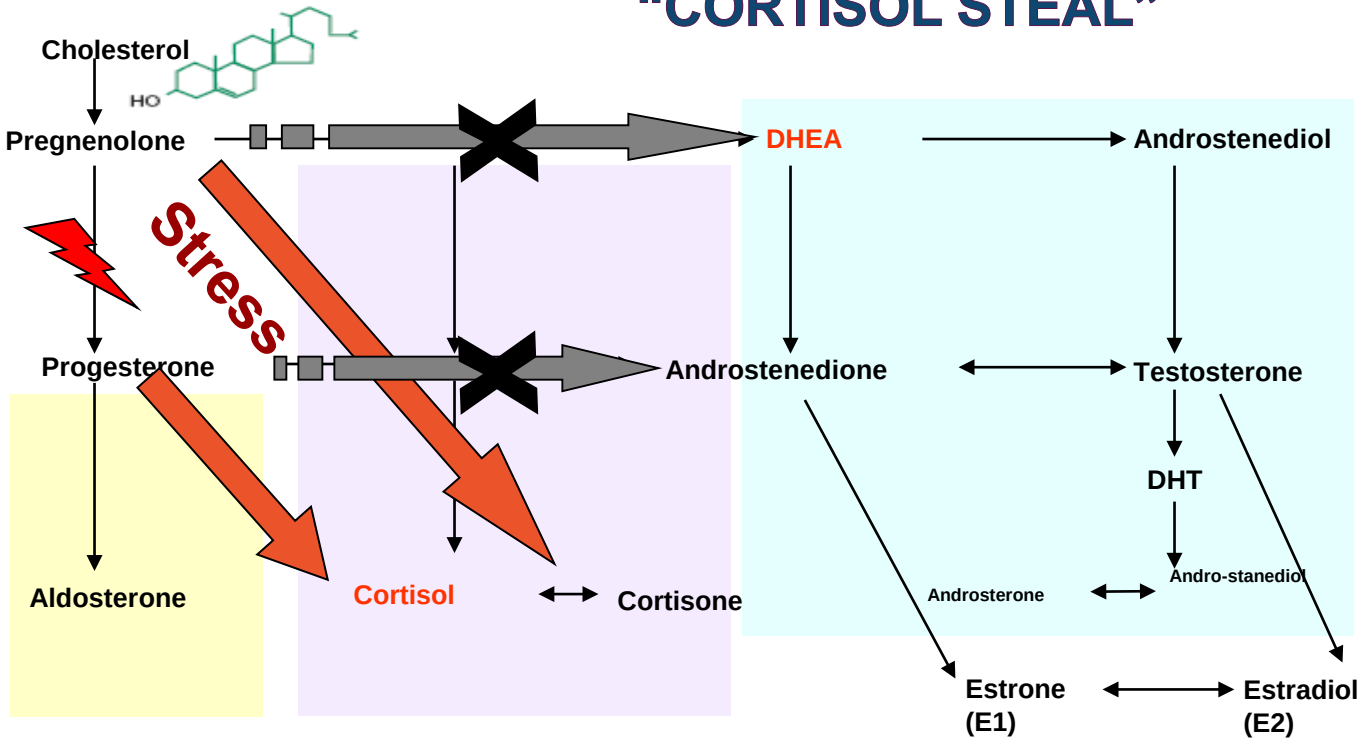
The Saudade Hormonal Symphony™



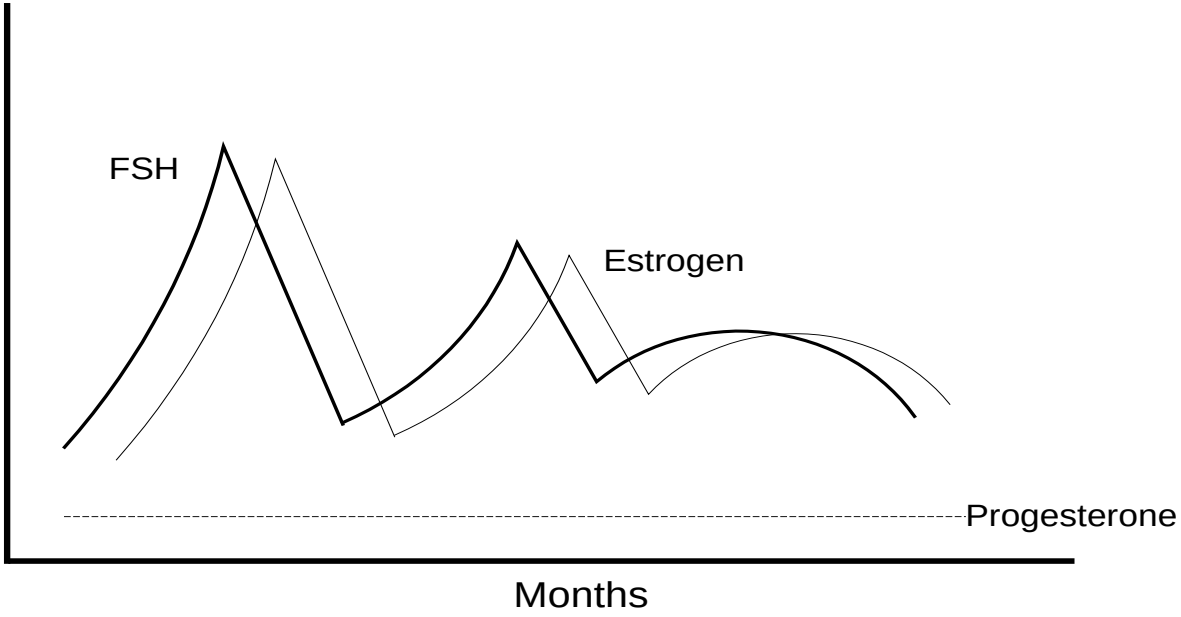


The steroidogenic pathways

“CORTISOL STEAL”



PERIMENOPAUSE



BREAST CANCER RISKS AND HRT

- Follow-up on the French E3N cohort study now 80,377 postmenopausal women found when combined with an estrogen, progesterone has a safer risk profile in the breast compared with some other progestogens.

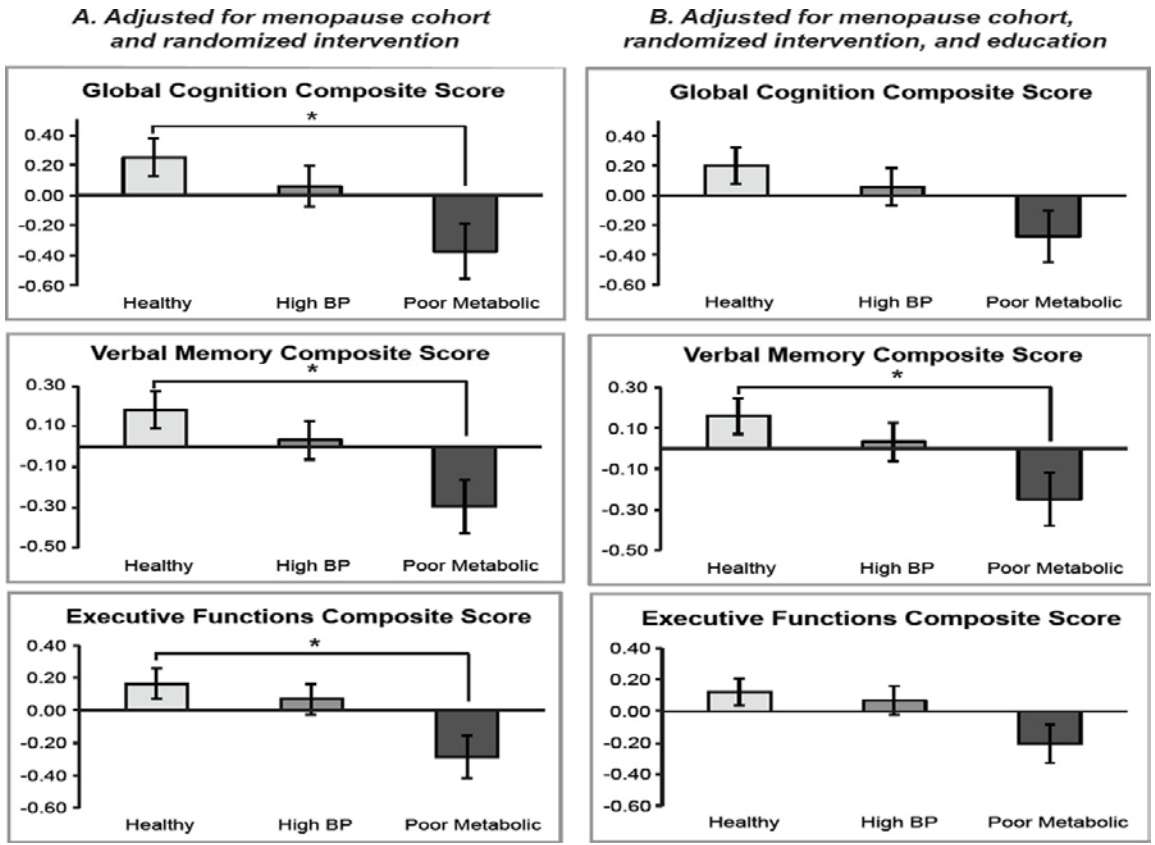
Fournier A. *Breast Cancer Res Treat.* 2008;107(1):103–111.

SHERWIN BB, ET AL.
DIFFERENTIAL EFFECTS OF ESTROGEN AND MICRONIZED PROGESTERONE OR METHOXYPROGESTERONE ACETATE ON COGNITION IN POSTMENOPAUSAL WOMEN.
FERTIL STERIL. 2011;96(2):399-403.

- “Co-administration of CEE with MPA or MP caused differential effects on memory in postmenopausal women."

RETTBERG JR, ET AL.
IDENTIFYING POSTMENOPAUSAL WOMEN AT RISK FOR COGNITIVE DECLINE WITHIN A HEALTHY COHORT USING A PANEL OF CLINICAL METABOLIC INDICATORS: POTENTIAL FOR DETECTING AN AT-ALZHEIMER'S RISK METABOLIC PHENOTYPE.
NEUROBIOL AGING. 2016;40:155-163.

- "Compared with healthy women, poor metabolic women had significantly lower executive, global and memory cognitive performance. Hormone therapy provided metabolic benefit to women in high blood pressure and poor metabolic phenotypes."



RETTBERG JR, ET AL. *NEUROBIOL AGING*. 2016;40:155-163

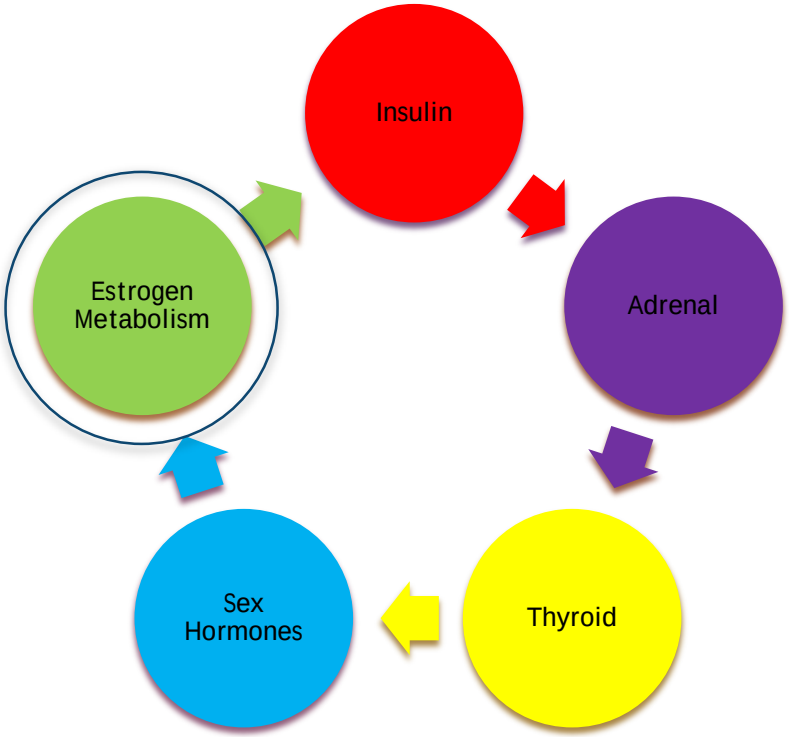
REMES A, ET AL.
WILL MEMORY BE LOST WITH MENOPAUSE- CAN AGEING WOMAN BE PROTECTED FROM MEMORY DISORDER?
DUODECIM. 2015;131(16):1499-1505.

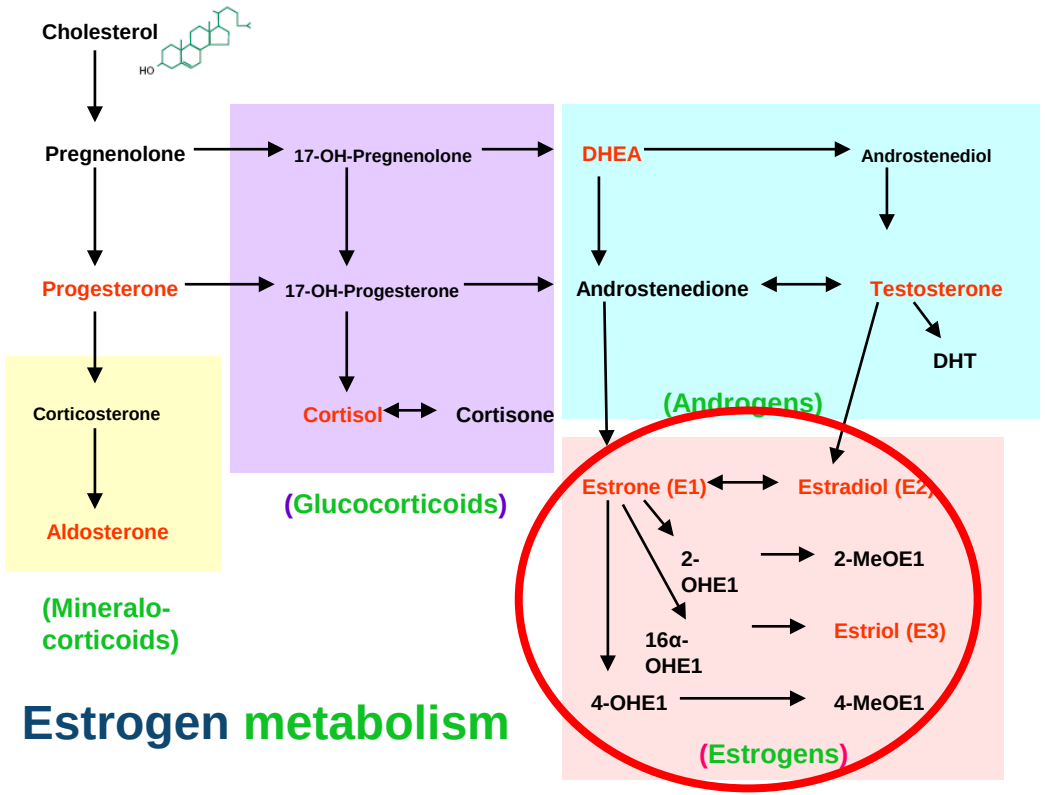
- “It is possible that timing of the start of hormone replacement therapy exactly to the menopause could provide the best benefit of memory and inflammation processing.”

JAMSHED N, ET AL.
ALZHEIMER DISEASE IN POST-MENOPAUSAL WOMEN: INTERVENE IN THE CRITICAL WINDOW PERIOD.
***J MIDLIFE HEALTH*. 2014;5(1):38-40.**

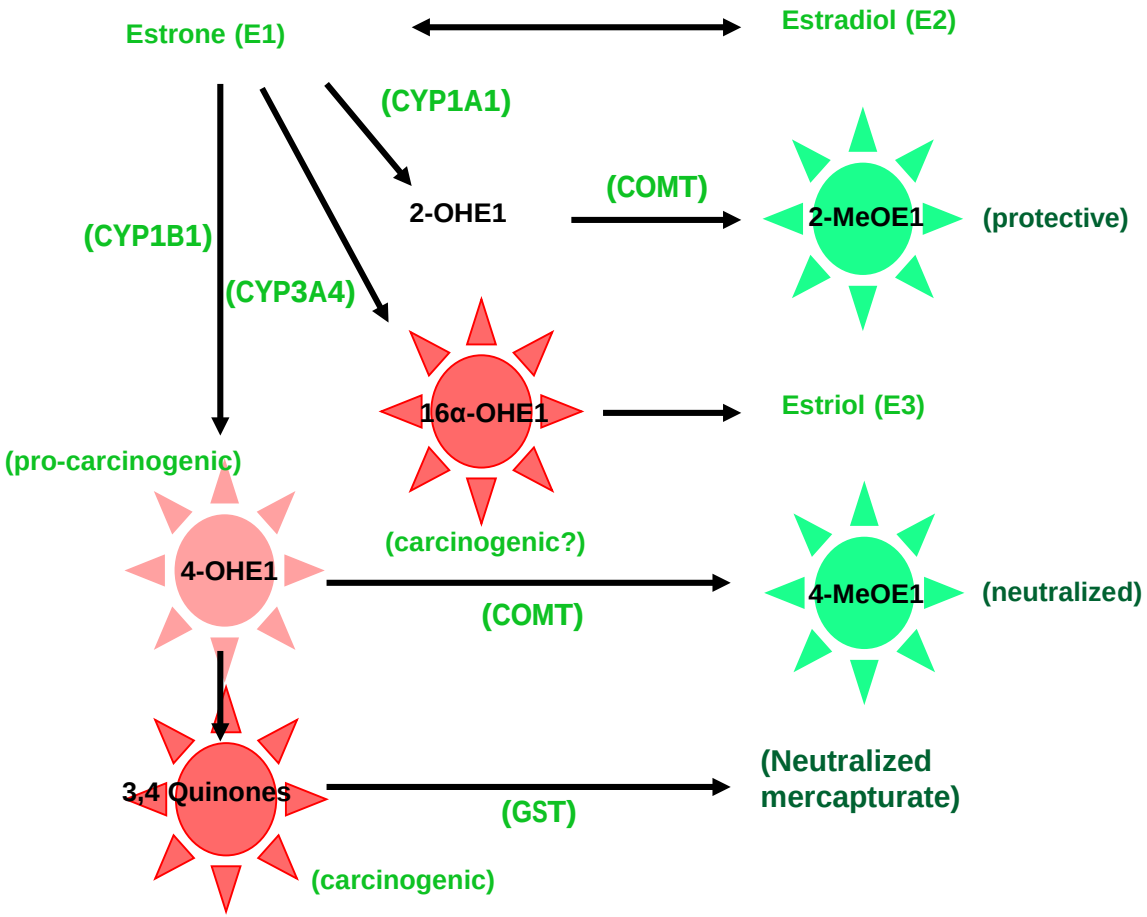
- “Use of 17 ß-estradiol in young and healthy post-menopausal women yields the maximum benefit when the neurons are intact or neuronal stress has just started. Hence intervention in the critical period is key in the prevention or delay of AD in post-menopausal women.”

The Saudade Hormonal Symphony™





Estrogen metabolism


Estrone (E1)
Estradiol (E2)
(CYP1A1)
(CYP1B1)
(CYP3A4)
2-OHE1
(COMT)
2-MeOE1
(protective)
16α-OHE1
Estriol (E3)
(pro-carcinogenic)
(carcinogenic?)
4-OHE1
(COMT)
4-MeOE1
(neutralized)
3,4 Quinones
(GST)
(Neutralized mercapturate)
(carcinogenic)

ENDOCRINE DISRUPTORS

- Environmental xenobiotics act as “endocrine disruptors” that modify intercellular communication and function
- Chemicals commonly detected in people include DDT, Polychlorinated biphenyls (PCB's), Bisphenol A, Polybrominated diphenyl ethers (PBDE's)
- May play role in cancer, obesity
- Changes in DNA methylation (epigenetic modification) which can ultimately change ER activity
- Produce a higher ratio of the 4 and 16 hydroxylated estrogen derivatives that are potentially more genotoxic by modifying members of the CYP450 enzyme family

Latini, et al. *Mini-Reviews in Medicinal Chemistry.* 2010;10;846-855.
Soto AM, et al. *Nat Rev Endocrinol.* 2010;6:363–370.


Metagenics Institute

The Active Brain:
Sports and Cognitive Health

Reviewing Current Research on Neurodegenerative Disorders

Scott Bergman, D.C.

Board Certified Traditional Naturopath
Diplomate, American Association of Integrative Medicine

Objectives

- Discuss the similarities of chronic neurodegenerative conditions, brain trauma and over training syndrome
- Review the triggers and pathways of brain inflammation and their effects on cognitive health
- Evaluate the microbiome-gut-brain axis as an afferent/efferent communication super highway
- Study the ketogenic diet as a viable lifestyle for brain inflammation
- Examine Functional Medicine protocols with a focus on key nutrients to restore neurological health

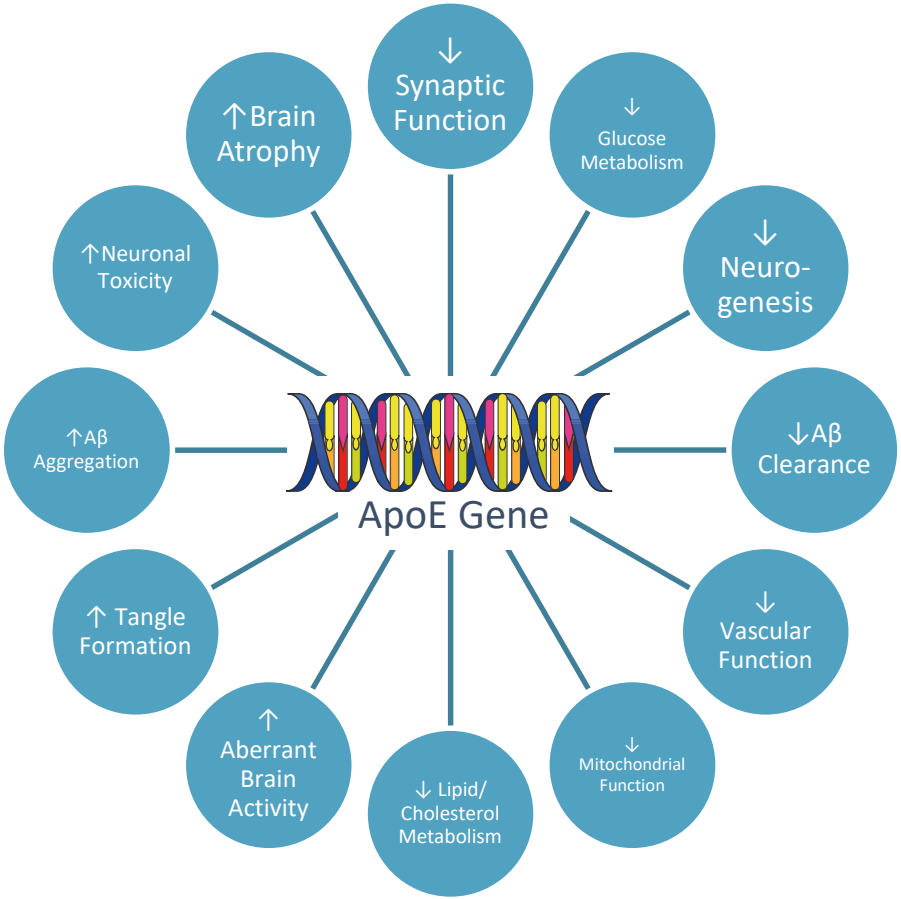
Alzheimer's disease

- Comes from a protective response to inflammatory insults
- Example:
  - Infections, trans fats
  - Suboptimal nutrients
  - Trophic factors
  - Hormone levels
  - Toxic compounds
- All cause amyloid precursor protein (APP) receptor—which protrudes from neurons—to be cut into 4 fragments including amyloid-beta that downsize the neural network and eventually destroy synapses and neurons

Bredesen MD. *The End of Alzheimer's.* August 22, 2017.

ApoE gene

- 5% of AD is considered “familial”
  - *mutations in APP itself are very rare*
- 75 million Americans who are ApoE4 positive have a 30% lifetime risk of developing AD
- 7 million have two copies of the gene, which puts them at a 50% lifetime risk
- ApoE inhibits SIRT1
  - Molecule that has been linked to longevity and has an anti-Alzheimer’s effect
- Associated with activation of nuclear-factor-kappaB (NFkB)



Bredesen MD. *The End of Alzheimer’s.* August 22, 2017.
Image adapted from: Liu CC, et al. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol.* 2013;9(2):106-118.

Alzheimer's types and subtypes (Dale Bredesen, MD)

- **Genetic**
- **Type 1 - Inflammatory**
  - **Type 1.5 - Glycotoxic**
- **Type 2 - Atrophic**
- **Type 3 - Toxic**

Bredesen MD. *The End of Alzheimer's.* August 22, 2017.

Alzheimer's types and subtypes (Dale Bredesen, M.D.)

- **Type 1, inflammatory:**
  - Chronic inflammatory markers
    - hsCRP, IL-6, TNF-α
    - NFkB part alters gene transcription
      - Beta-secretase and gamma-secretase
      - Cleaves amyloid precursor protein (APP)
        - Synaptoclastic processes
- **Type 1.5, Glycotoxic:** Subtype
  - Type III diabetes –
    - Insulin resistance induced inflammation
    - Atrophy processes

Bredesen MD. *The End of Alzheimer's.* August 22, 2017.

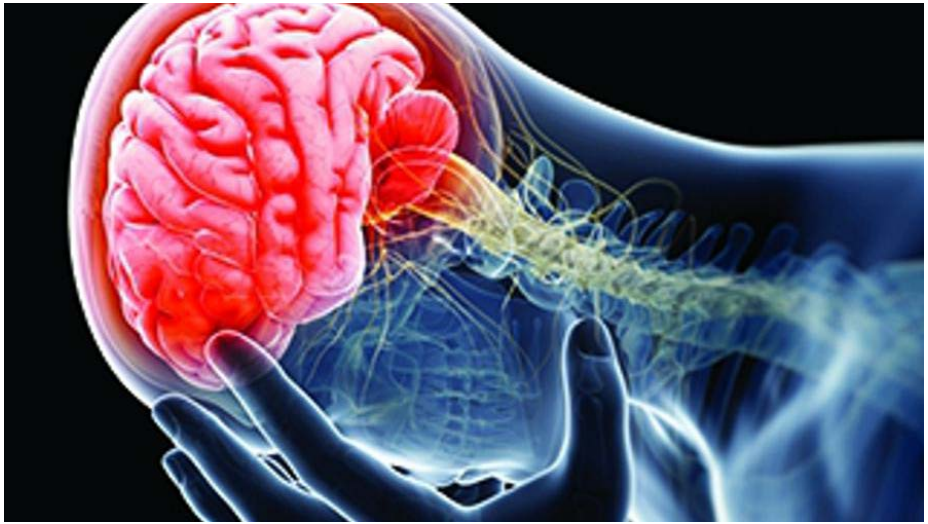
Alzheimer’s Types and Subtypes (Dale Bredesen, M.D.)

- **Type 2, atrophic:**
  - Nerve growth factor resistance
    - Brain-Derived Neurotrophic Factor (BDNF), estradiol, testosterone, vitamin D
      - Any compound that provides atrophic support
  - APP creates amyloid plaques and Alzheimer's cell signaling.
  - Brain responds by blocking synaptogenesis
- **Type 3 Toxic:**
  - These are patients with toxic exposures. Many will have chronic inflammatory response syndrome (CIRS) markers.

Bredesen MD. *The End of Alzheimer’s.* August 22, 2017.

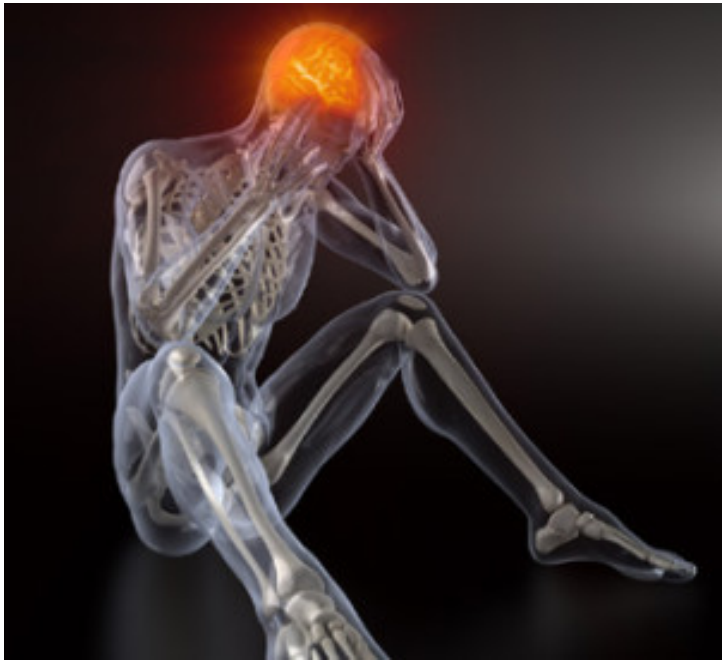
Concussions

- Over 3.8 million concussions reported a year
- Over 500,000 kids every year present to the ER room with concussion from a sport
- Once you receive a concussion, you are 1.5 times more likely to receive a second concussion
- After the 2nd one, you are 3x more likely to have a 3rd one



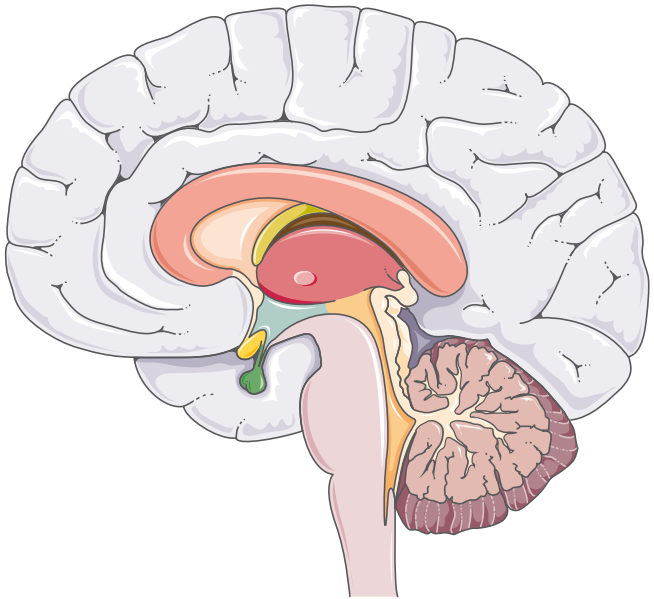
Concussion - traumatic brain injury (TBI) Mimics Alzheimer's

- The brain is the most nutrient dependent, energy dependent, toxin and stress vulnerable organ
- Cerebral blood flow is impaired
  - 7-10 days post concussion
- The brain is starved of glucose
  - 7-10 days post concussion
- TBI alters brain chemistry
- TBI with APOE-e4 variation upregulates beta-amyloid and tau formation



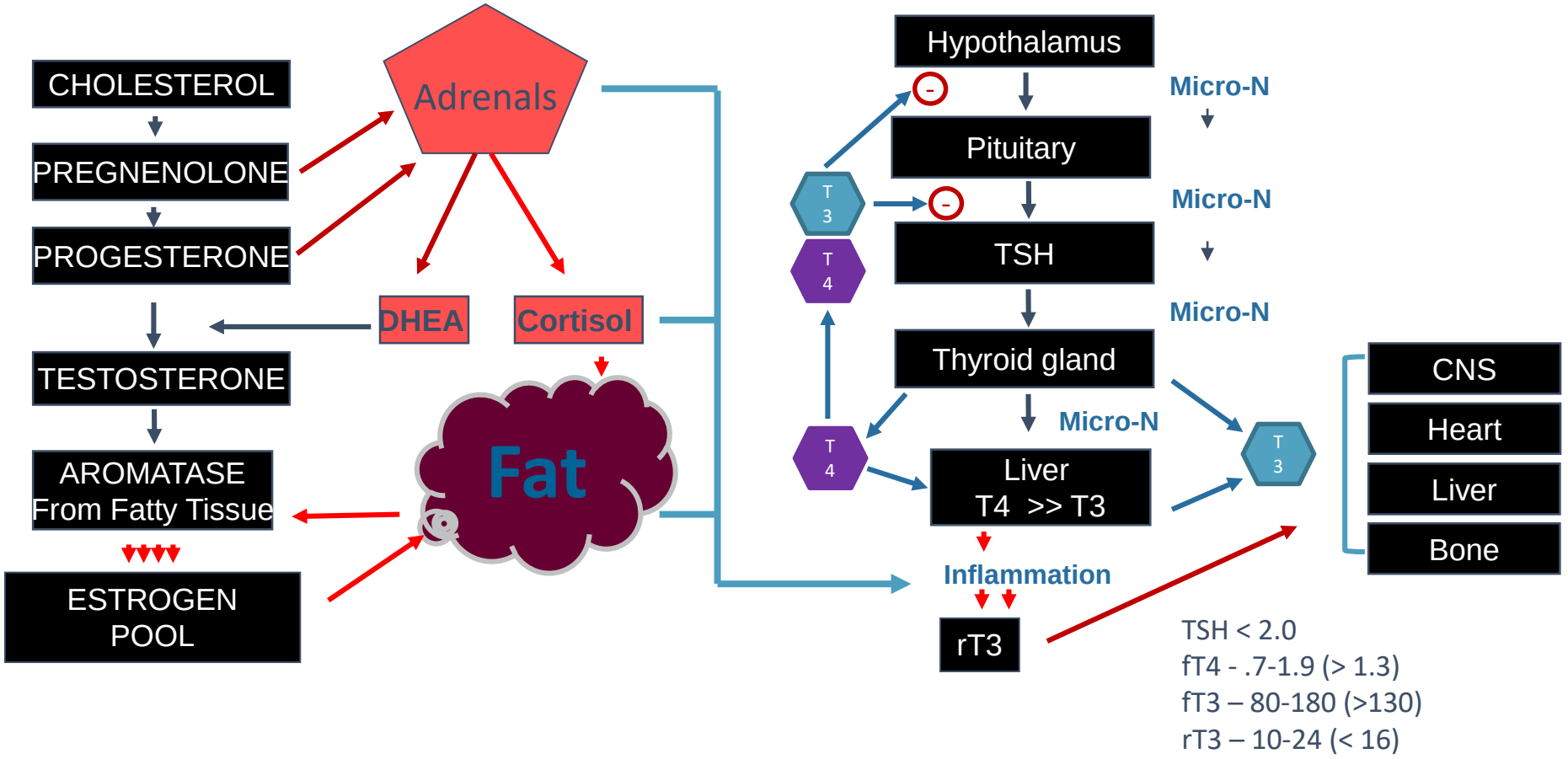
Pituitary dysfunction after concussion

- % of pituitary dysfunction varies with type and severity of concussion
- GH is most common hormone lost
- Then ACTH, FSH and LH then TSH
- Genetic predisposition and autoimmunity play a role



Tanriverdi F, et al. Pituitary dysfunction after traumatic brain injury: a clinical and pathophysiological approach. *Endocr Rev.* 2015;36:305–342.

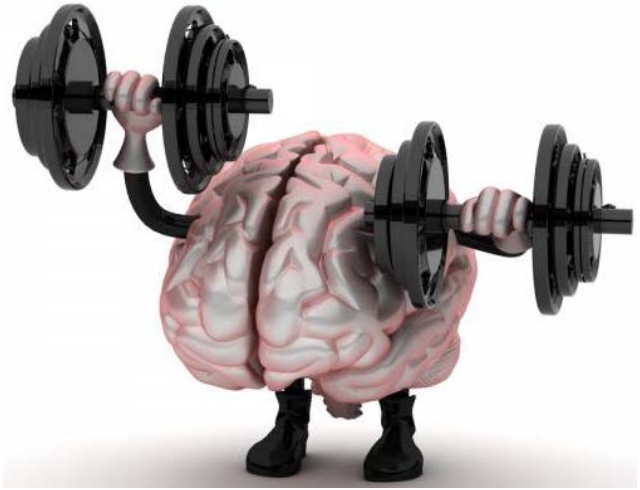
HPA & HPT AXIS



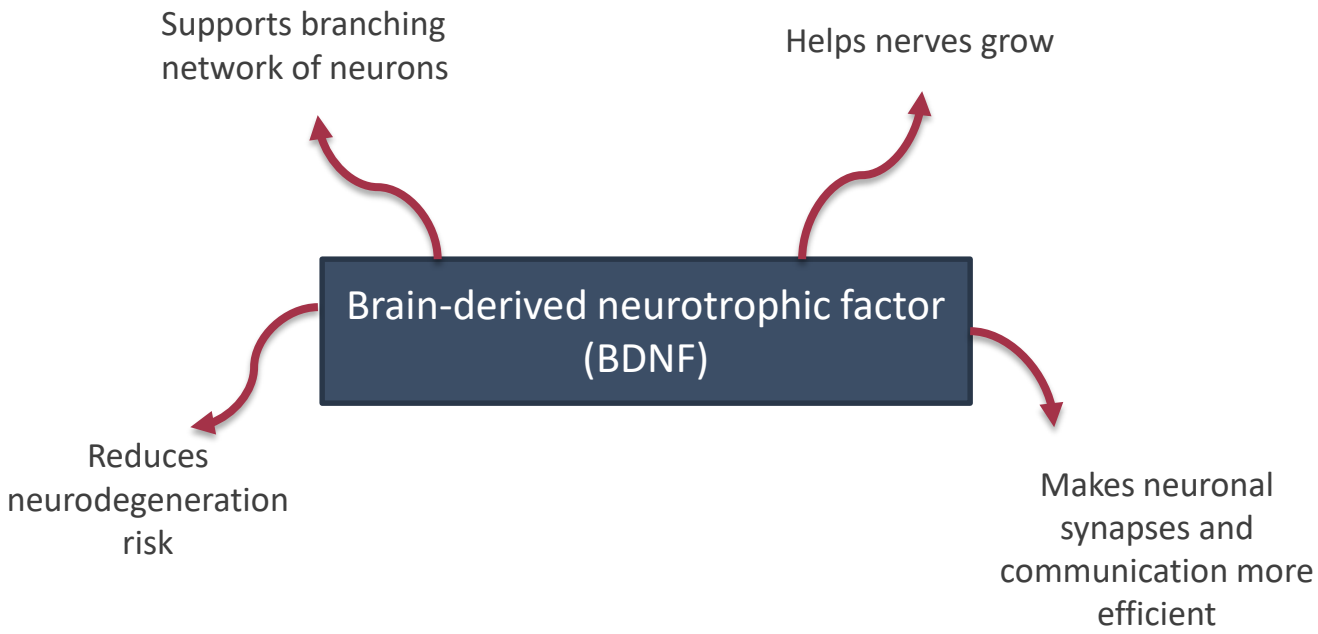
TSH < 2.0
fT4 - .7-1.9 (> 1.3)
fT3 – 80-180 (>130)
rT3 – 10-24 (< 16)

Benefits of exercise on the brain

- Cognitive functions and flexibility
- Neurotrophic effects
- Improves executive functions
- Improves stress tolerance
- Increases IQ
- Increases focus
- Short and long term memory
- Helps you think faster
- Inhibition and interference control



Dishman RK, et al. Neurobiology of exercise. Obesity (Silver Spring). 2012;14(3):345-356.



Exercise positively impacts BDNF

Nokia MS, et al. *J Physiol.* 2016;594(7):1855-1873.

Over training syndrome (OTS)

- Athletes train hard to optimize performance
- In many training cycles, athletes experience this short-term overreaching as they increase intensity and/or volume but recover rapidly and improve or maintain performance

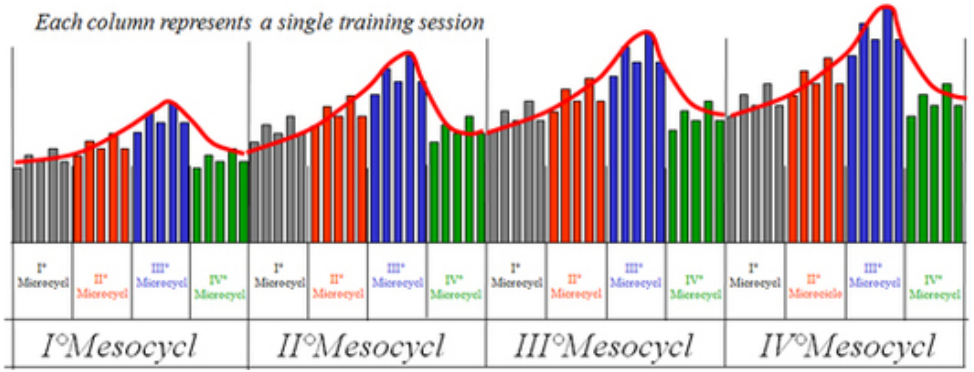


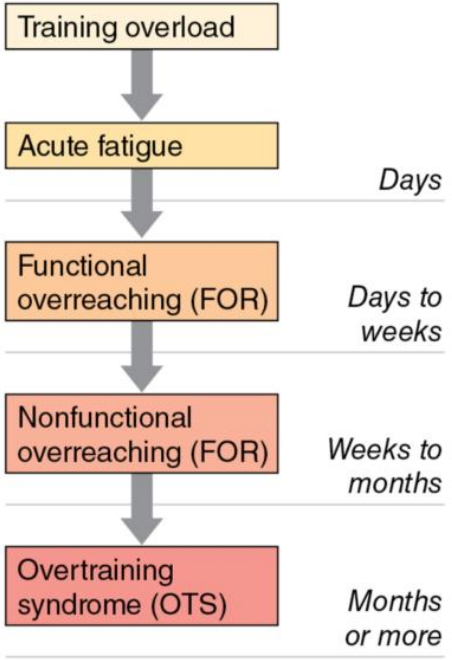
Image adapted from:sport-sys.com/what-is-periodization-training.html

Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Unfortunately, there is a fine line between improved performance and deterioration

- Cognitive Deterioration
  - Depression
  - Executive processing
  - Determination
  - Difficulty concentrating
  - Sensitive to environmental and emotional stress
  - Changes in personality
  - Focus
  - Decreased information capacity

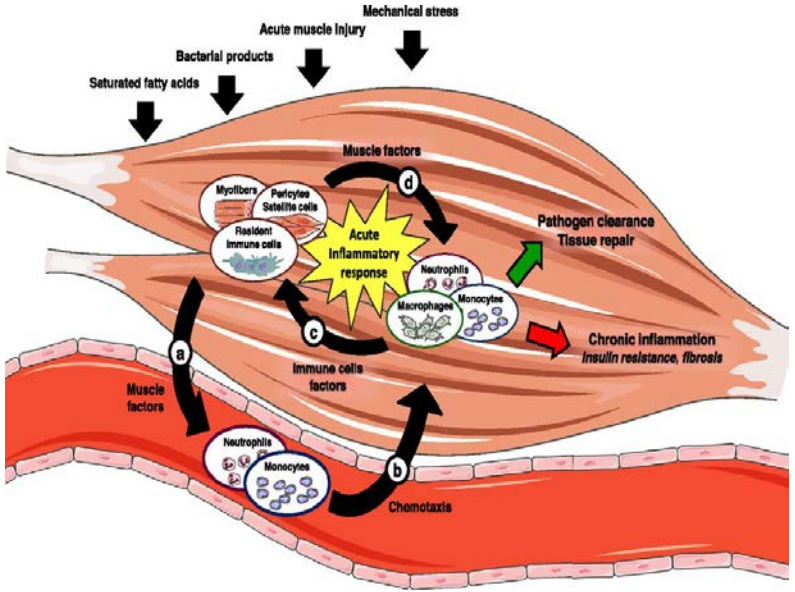
Over Training Syndrome (OTS)



Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Musculoskeletal injury and inflammatory response

- Cell walls damaged and releases inflammatory signals
- Responsible for the recruitment of immune cells
- This leads to the acute inflammatory response necessary for pathogen clearance and tissue repair

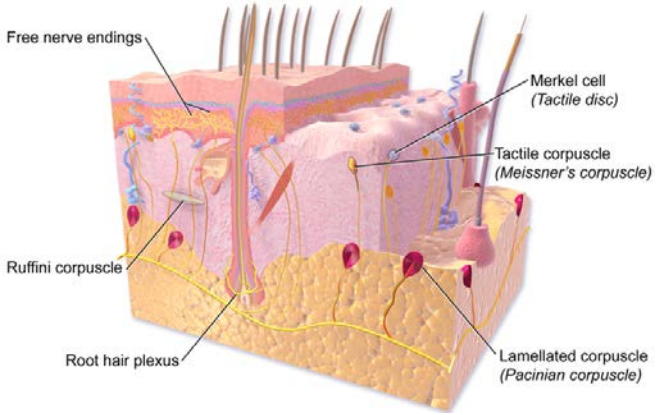


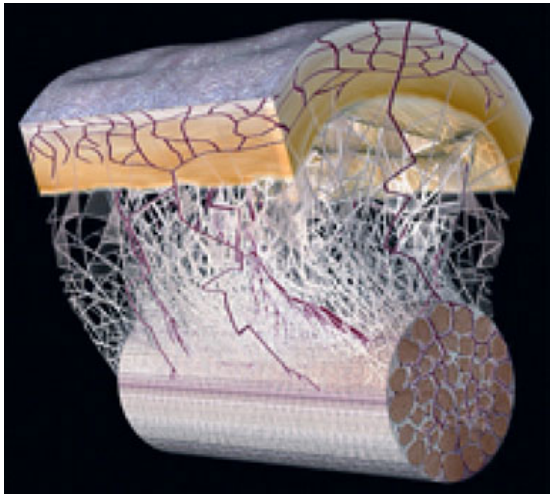
Fascial anatomy

- **GROUND SUBSTANCE**
  - Thixotropy
    - Ability to go from a gel to a liquid state
    - Like Jell-O, when cool, it's jelly and when its warm, it's a thick liquid
  - Mechanical stretch, body heat and bio-electric energy all contribute to keeping ground substance a liquid
    - Liquid state allows
      - Movement and stretch
      - Exchange of nutrients and cellular wastes removal
  - Hardened tissue lacks glide and damages tissue
    - Initiating an inflammatory response

Mechanoreceptor

- The fascial network possesses approximately 10x the sensory receptors as compared to its muscular counterpart (Van der Wall 2009)
- Muscle spindles
  - Fast adapting, low threshold
  - Intentional muscle movement
  - Golgi tendon organs
    - Senses tension and pressure
  - Ruffini corpuscles - capsule
    - Heavy pressure, joint movement, skin stretching
  - Panciniforms - synovium
    - Deep pressure, vibration and stretch





Musculoskeletal injury and inflammatory response

- Acute pain and inflammation can be protective
  - Aware of injury
  - A rapid warning relay to minimize physical harm.
  - Initiates protection, repair and recovery
- Chronic pain, however, serves no biologic function as it is not a symptom of a disease process but is a disease process itself
- Proinflammatory cytokines include
  - IL-1b, IL-6, IL-8, and TNF-α

Adaptive Microtrauma

↓

Local Acute Inflammation

↓

Local Chronic Inflammation

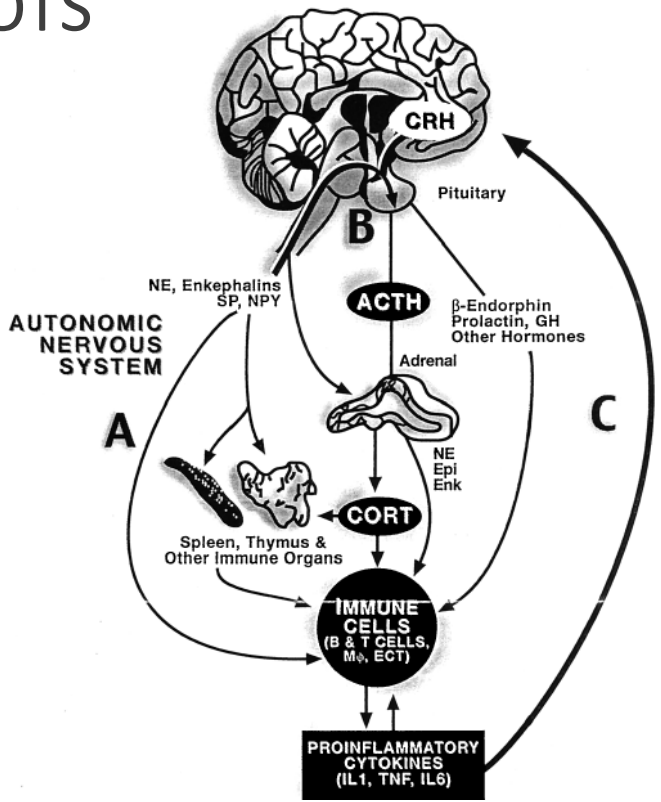
↓

Systemic Immune Inflammatory Response

Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Cognitive changes associated with OTS

- The brain and peripheral immune/inflammatory cells form a bidirectional communication network
- There is a strong relationship between systemic cytokines and psychological depression
- Immune activation and cytokines are involved in depression
- Depressed patients have significantly higher levels of IL-1 and IL-6 and TNF-α



Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Glutamine depletion and OTS

- Glutamine is the most abundant amino acid in human plasma and muscle and plays an important role in human metabolism
- Essential for lymphocyte proliferation and macrophage function
- Regular training depletes glutamine
  - decline in immune function
  - Increase in inflammatory mediators

Adaptive Microtrauma
↓
Local Acute Inflammation
↓
Local Chronic Inflammation
↓
Systemic Immune Inflammatory Response

Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Central nervous system fatigue and cognition

- Decrease BCAA's from exercise
  - Competes with tryptophan (TRY) uptake
- (TRY) brain concentration increases
  - Increases brain serotonin
    - Increases fatigue
    - Decreases determination
    - Difficulty concentrating
    - Depression

Adaptive Microtrauma

↓

Local Acute Inflammation

↓

Local Chronic Inflammation

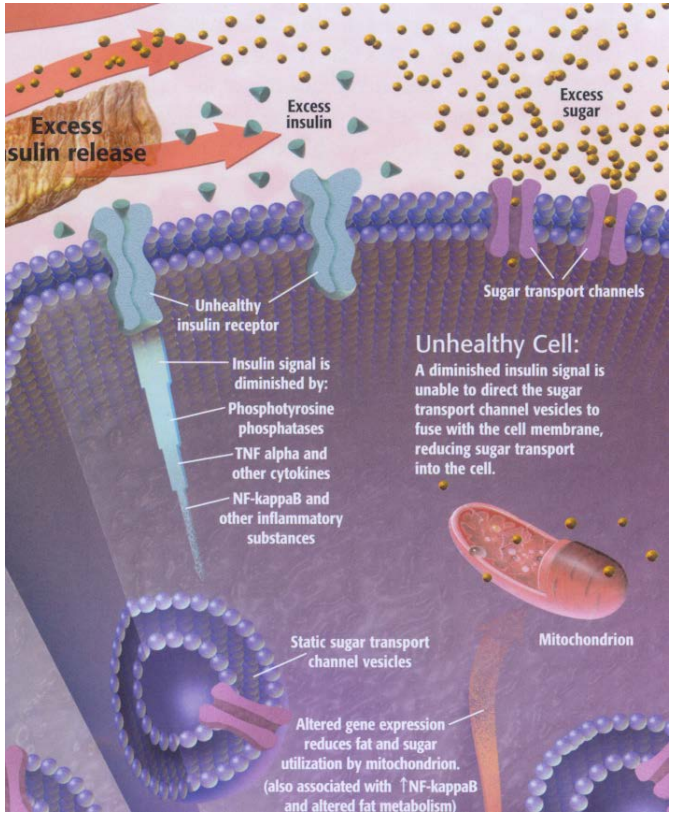
↓

Systemic Immune Inflammatory Response

Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Insulin resistance and OTS

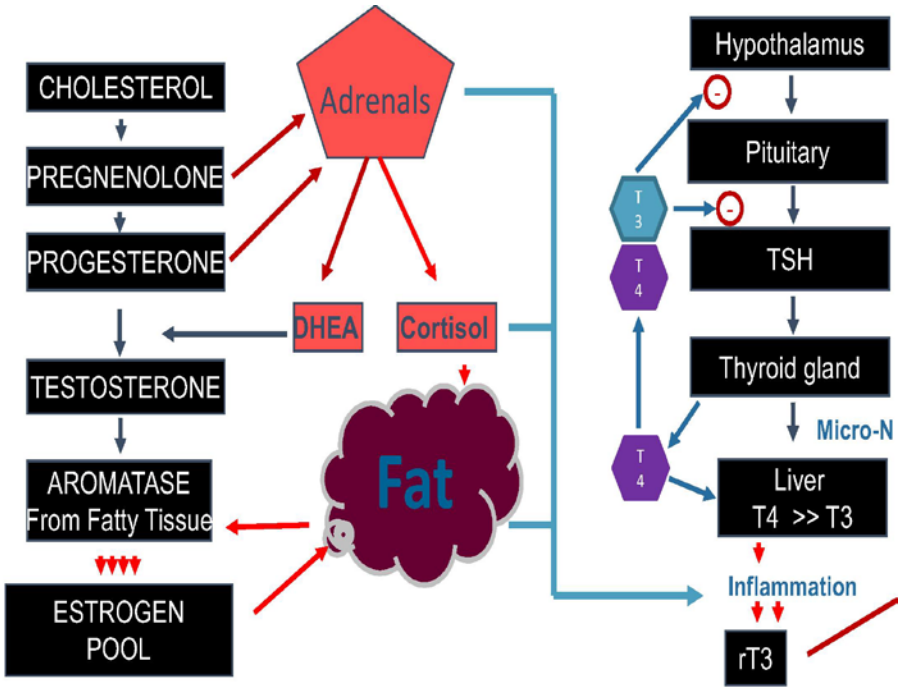
- Muscle tissue trauma interferes with transport of glucose and glycogen synthesis
- TNF-α decreases muscle concentration of GLUT-4 protein
  - Decreased glucose into tissue
  - Decreased glycogen concentration
  - Contributes insulin resistance
- Insulin resistance has frequently been reported as part of the metabolic response to systemic infection



Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

HPA, HPT Axis and OTS

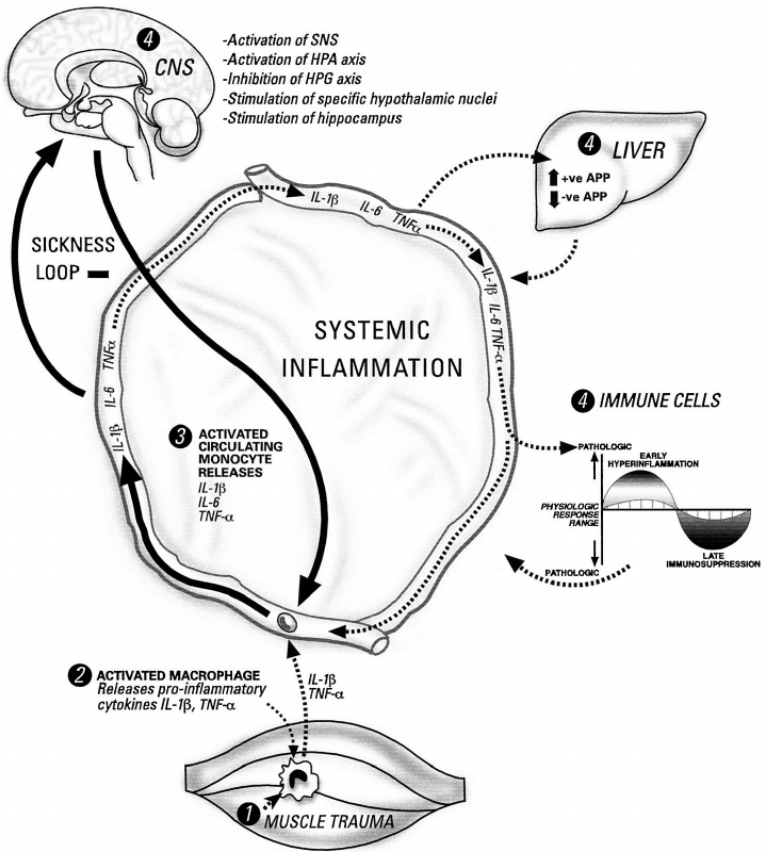
- Intense prolonged activity leads to increased cortisol
  - Decrease free testosterone
- Proinflammatory cytokines activate HPA axis
- Prolonged hyperstimulation is immunosuppressive



Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

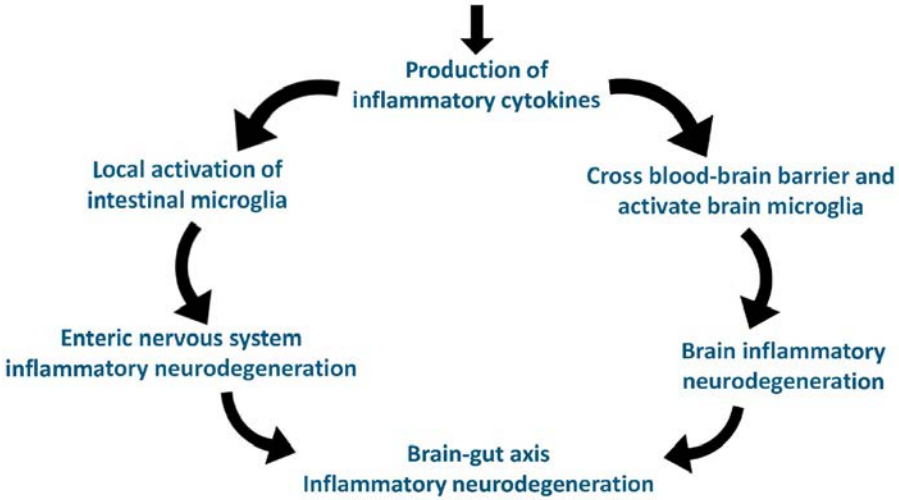
Overtraining syndrome

- Systemic inflammatory condition
- Catabolic
- Immunosuppressive
- Nutrient depleting
- Insulin Resistant
- Neurodegenerative
    - Similar to other cognitive disorders



Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc*. 2000;32(2):317–331.

Gut Brain Connection

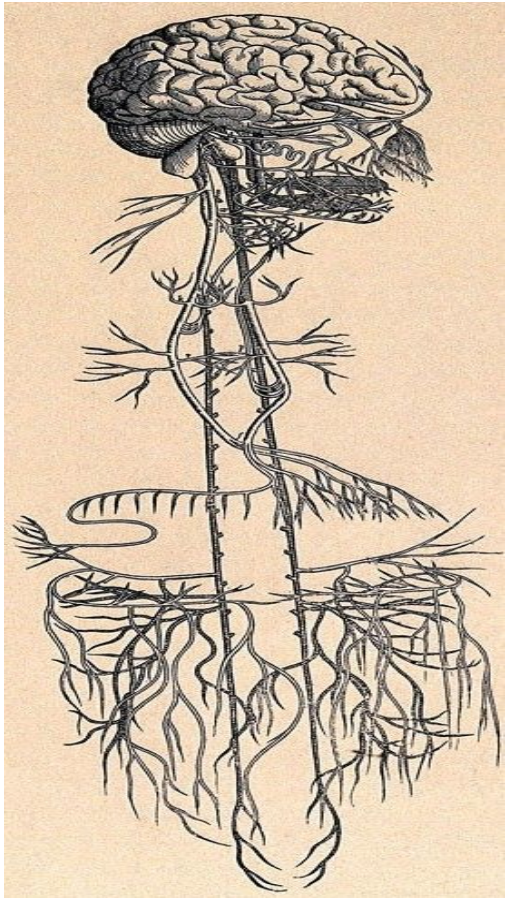


- IBS / IBD
- Ulcerative colitis
- Diverticulitis
- Celiac disease
- Crohn's disease
- GERD

- Infections
- SIBO
- Stomach ulcers
- *Candida*
- Viral
- Parasitic

Microbiome-Gut-Brain (MGB) axis: The vagus nerve

- Vagus nerve (10th cranial)
  - Longest nerve in the human body wanders from the brainstem to the lowest viscera of your intestines
- Vagus nerve is the driving force of the P∑NS
  - “Rest-and-Digest” or “Tend-and-Befriend” responses
  - ∑NS drives the “fight-or-flight” response

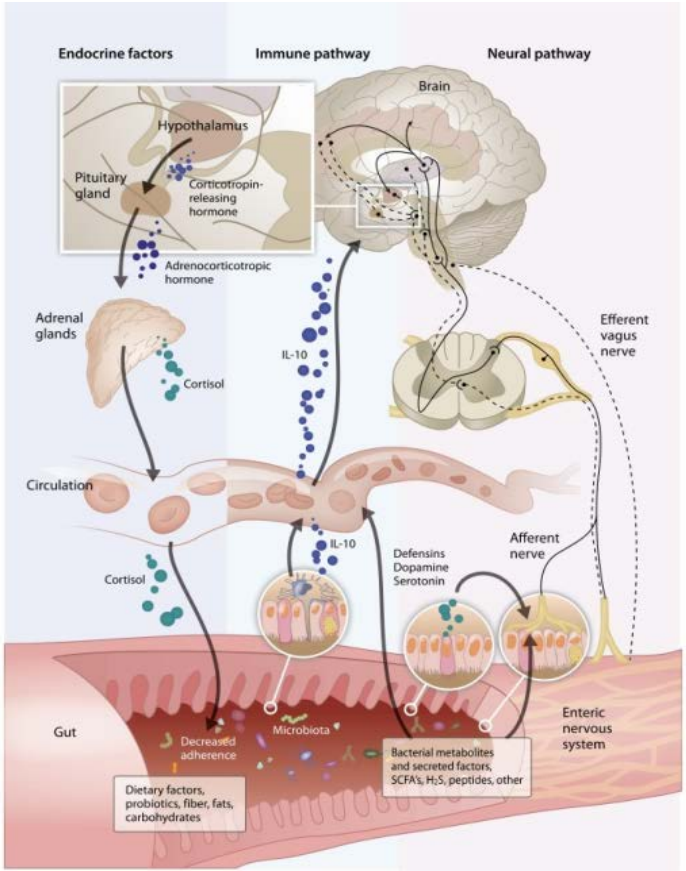


Source: Wellcome Library Public Domain

Lima-Ojeda JM, et al. “I am I and my bacterial circumstances”: linking gut microbiome, neurodevelopment, and depression. *Front Psychiatry*. 2017;8:153.

Microbiome-Gut-Brain (MGB) axis: The vagus nerve

- MGB axis regulates gastric/intestinal function and energy homeostasis
- MGB axis modulates immune and endocrine systems, HPA axis, neurotransmitter pathways, and growth factors
- Inflammation within this network may be the basis of neurodevelopment vs neurodegeneration



Lima-Ojeda JM, et al. “I am I and my bacterial circumstances”: linking gut microbiome, neurodevelopment, and depression. *Front Psychiatry*. 2017;8:153.

Infection:
Fungus - *Candida*

- Neurotransmitters
  - Inhibits tryptophan to 5-HTP
    - Uncontrolled cravings
    - Not willpower -- chemistry
  - Inhibits tyrosine to dopamine
    - Low conversion to Epi/Nor-EPI
    - Cannot fight off infection
- Oxalate crystals
  - Further increase inflammation
  - Form stones
  - Common in ADD, ADHD, autism spectrum

Ketogenic diet’s impact on:

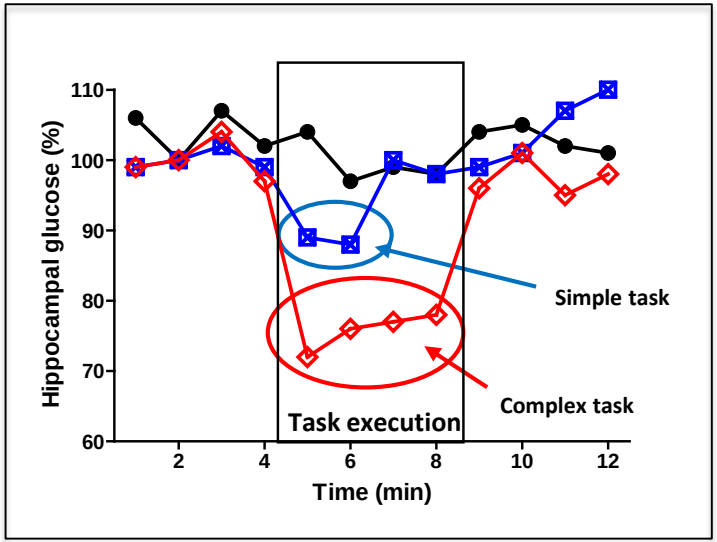
Fuel for the brain

Alzheimer's disease

Cognition and aging

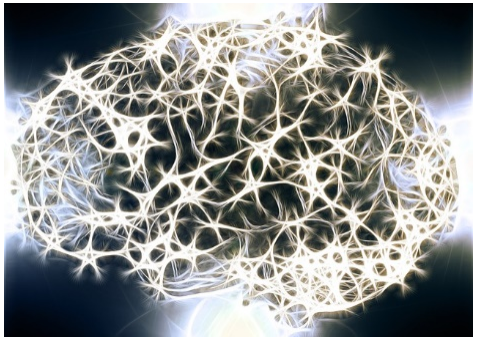
The human brain is extraordinarily expensive

- The human brain comprises 2% of body mass, while requiring approximately 25% daily energy demands (500 kcal)[1]
- **Despite its significant energy requirements, the brain has limited capacity to store glucose**
- The hippocampus is a brain area associated with the execution and retention of learning and memory processes



Adapted from: McNay EC et al. *Proceedings of the National Academy of Sciences* 2000; 97(6): 2881-2885

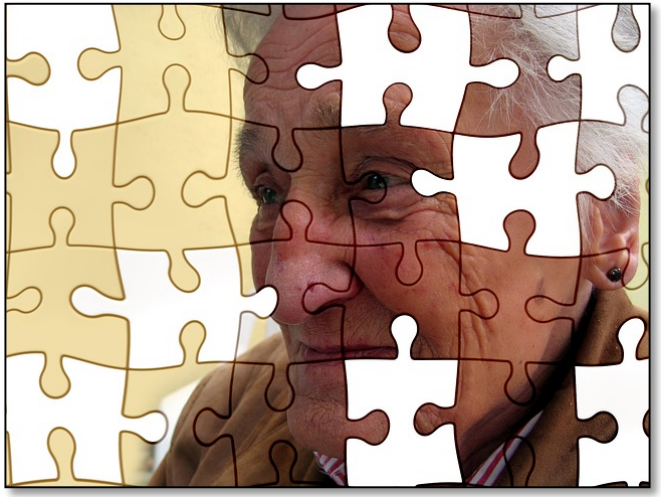
- During the execution of cognitively demanding tasks, a decrease in hippocampal glucose levels is observed[2]
- More complex tasks deplete hippocampal glucose levels further
- ***Cognitive performance is limited by fuel availability in the hippocampus***



1. Mink JW et al. *American Journal of Physiology* 1981; 241(3): R203-12

Impaired brain glucose utilization and cognitive decline

The healthy young brain relies solely on glucose to obtain energy for its functional and structural needs[1]



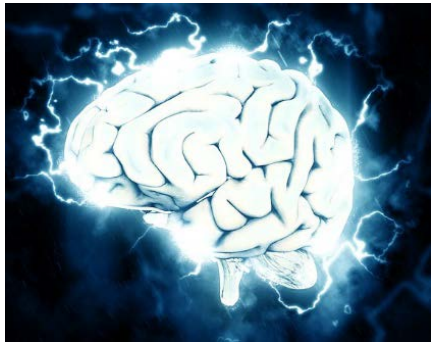
During healthy aging, brain **glucose uptake is 10-15% lower and can be up to 35% lower in certain brain areas in neurological disorders** such as Alzheimer's Disease (AD)[1-5]

This hypometabolism has led researchers to coin the term 'Type 3 Diabetes' when referring to AD

Brain uptake of ketones appears to remain normal in the brains of patients with Alzheimer's Disease[5]

1. Hoyer S. *Annals of the New York Academy of Science* 1991; 640:53-8
2. Nugent S et al. *Neurobiology of Aging* 2014; 35:1386-95
3. Mosconi L et al. *Neurobiology of Aging* 2008; 29:676-692
4. Castellano C et al. *Journal of Alzheimer's* Disease 2015; 43(4):1343-53
5. Cunnane S et al. *Frontiers in Molecular Neuroscience* 2016; 9:53

Can the brain use ketone bodies?

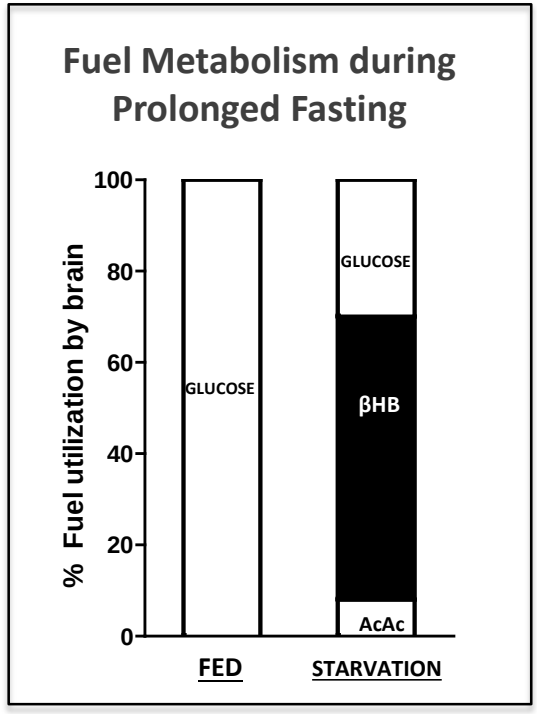


- Common misconception: brain can only use glucose
- Ketone bodies are the only alternative source of energy for the brain (as it cannot utilize FFAs)
- Both rodent and human studies have shown **increased uptake of ketone bodies by the brain**[1,2] following:
  - ✓ Peripheral infusion of ketones
  - ✓ Prolonged fasting
  - ✓ Ketogenic diet

1. Pifferi F et al. *Epilepsia* 2008; 14(2):51-58
2. Cunnane S et al. *Frontiers in Molecular Neuroscience* 2016; 9:53

Can the brain use ketone bodies?

- When obese subjects underwent prolonged fasting (water access only for 4 to 6 weeks), researchers were able to investigate cerebral energy metabolism during nutrient (glucose) deprivation[3]
- They observed that **up to 70% of brain's energy demands were provided by ketone bodies** available in circulation (blood) and taken up by the brain[3]

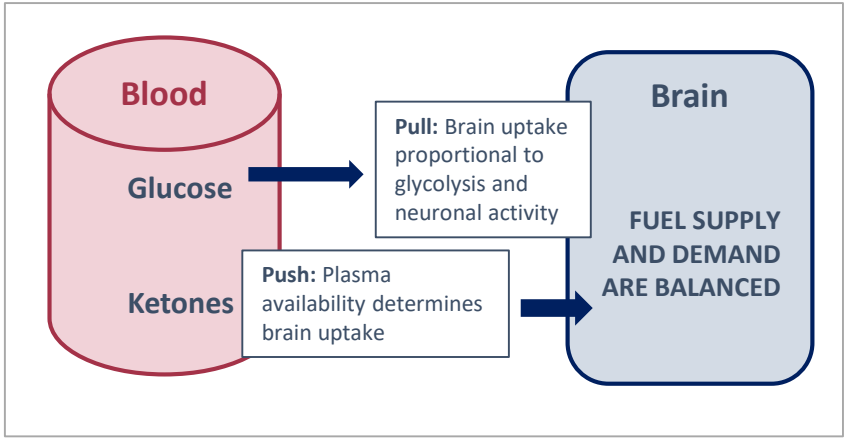


Adapted from: Owen OE et al. *The Journal of Clinical Investigation* 1967; 46(10):1589-95

Can the brain use ketone bodies?

- Higher circulating levels of ketone bodies result in **higher brain uptake and utilization of ketones** for its energy demands[1]
- **Preserved uptake and utilization of ketone bodies** in the brains of mild cognitively impaired (MCI) patients, whereas glucose uptake and utilization decreases 20-30%[1-5]

'Push and Pull' mechanism comparing brain uptake of ketones vs glucose



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1. Hoyer S. *Annals of the New York Academy of Science* 1991; 640:53-8
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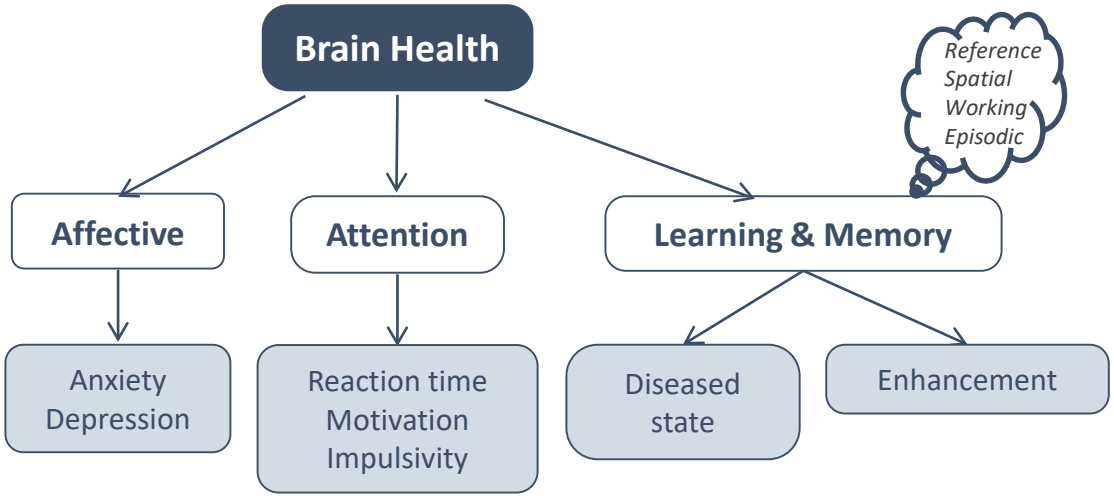
3. Mosconi L et al. *Neurobiology of Aging* 2008; 29:676-692
4. Castellano C et al. *Journal of Alzheimer's Disease* 2015; 43(4):1343-53
5. Cunnane S et al. *Frontiers in Molecular Neuroscience* 2016; 9:53

Brain health comprises more than memory

- Emerging science suggests that *optimizing cerebral energy metabolism with ketone bodies* may benefit a wide array of neurological conditions[1]
- Research groups have recently started investigating the *potential therapeutic benefits* of ketogenic diets on neurodevelopmental and affective disorders[1,2]
- ***Subjective reports and anecdotal evidence* suggest a beneficial effect of ketogenic diets on mood[3], anxiety and attention and further research is needed to validate these claims**



1. Stafstrom CE et al. *Frontiers in Pharmacology* 2012; 3:59
2. Murphy P et al. *Biological Psychiatry* 2004; 56:981-83
3. El-Mallakh RS & Paskitti ME *Medical Hypothesis* 2001; 57(6):724-26

Emerging science – novel research areas

Healthy aging	Cognition	Stress	Microbiome
• Longevity • Reduce age associated morbidity	• Augmentation • Prevention of decline • Biohacking	• Resilience • Prevention	• Gut-brain axis • Increased diversity

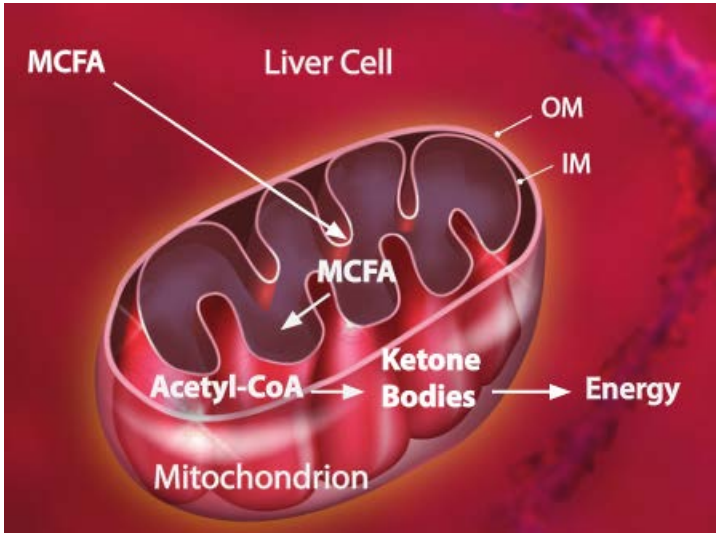
Supplements to support ketosis

1. Turmeric: results in blood glucose stabilization and lowered triglyceride levels *Neerati P, et al. Phytother Res. 2014;28:1796–1800.*
2. Chromium: increases production and release of glucose transport molecule called glut-4 enzymes in liver and muscle tissue Qiao W, et al. *Biol Trace Elem Res*. 2009;131(2):133-142.
3. Acetyl-L-carnitine: critical for fat metabolism and energy production in the cellular mitochondria
4. ALA: unique and powerful antioxidant that has both water and fat soluble properties
5. Omega-3 “SMASH” fish:
    a) Ensures omega-3 to omega-6 ratio
    b) Natural anti-inflammatory
    c) Contributes to keto diet high-fat intake requirement

Choosing the right fat for ketogenic programs

How do medium chain triglycerides (MCT) increase ketone bodies?

- MCTs contain 6 to 12 carbon atoms:
  - Caproic acid (C6:0), aprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0)
- MCT's freely cross the inner membrane
  - Faster metabolism to ketones for an efficient fuel
  - Transiently increases endogenous ketones
  - Doesn't store as fat

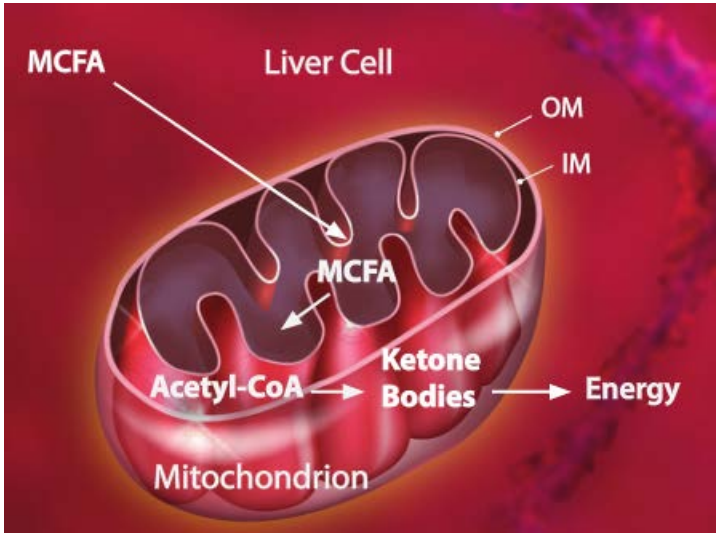


MCFA= medium chain fatty acids, OM = outer membrane, IM = inner membrane

Bach AC, Babyan VK, *Am J Clin Nutr,* 1982 Nov;36(5):950-62

Exogenous ketone (βHB) salt

- Exogenous ketone supplementation **induces acute ketosis**
  - beta-hydroxybutyrate (BHB) is bound to a salt (sodium, calcium) to improve absorption
  - 11.7 g. of BHB Acutely induces ketosis within 15 mins for at least 1 hour
- Anecdotally, reduces “Keto Flu” symptoms and can facilitate adherence to ketogenic diet
- In animal models, acute and chronic oral βHB salts:
  - Increased plasma ketone levels
  - Correlated positively with HDL-C and negatively with blood glucose levels, adipocyte volume and serum lipolysis products[1-2]
  - **In rodents, sustained ketosis for longer periods than βHB administration alone[1]**



MCFA= medium chain fatty acids, OM = outer membrane, IM = inner membrane

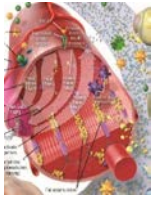
1. Kesl et al. *Nutrition & Metabolism* 2016;13:9,
2. Caminhotto RO et al. *Nutrition & Metabolism* 2017; 14:31

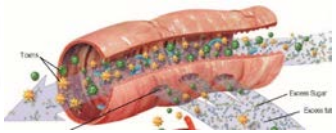
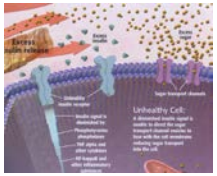
Ketogenic meal replacement

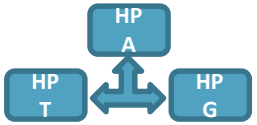
- 14 g of fat
- 20 g of protein
- 5 g of carbohydrates
- 3 g of MCT
- 24 essential vitamins and minerals
- 220 calories per serving

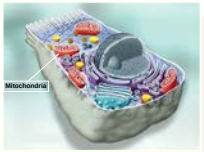
The 4 R Program

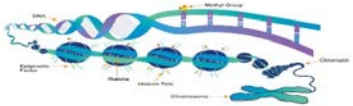
- Remove
- Replace
- Reinoculate
- Repair



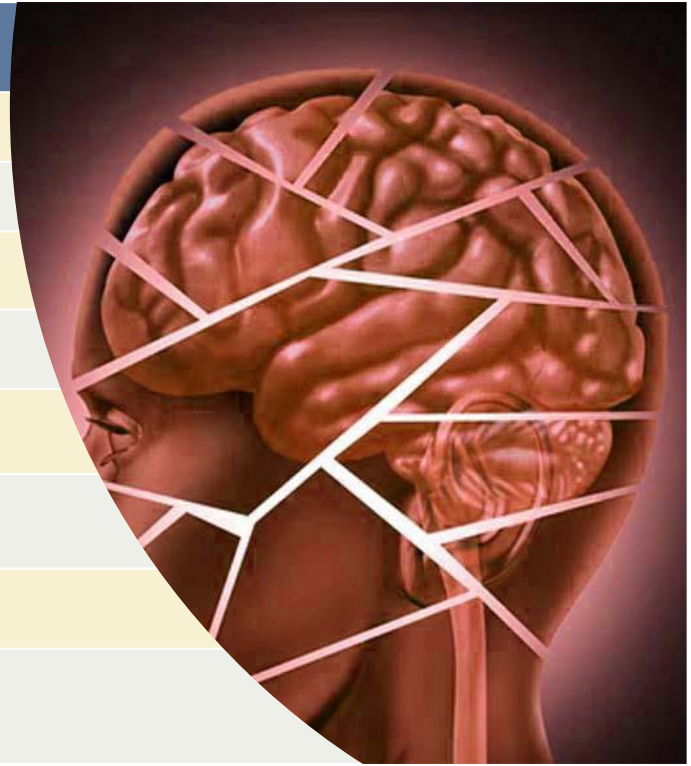




4R Keto/Detoxification program

Ingredients
Keto diet, keto meal replacement, MCT oil (10 g/day)
Concentrated aromatic oils, berberine
Strain-specific probiotics per patient’s condition
Enzymes: HCL, pancreatic, lipotropic, address biofilm
Specialized pro-resolving mediators (1,500 mg/day)
Xanthohumol, curcumin, boswellia, ginger extracts
IMO and HMO prebiotics, glutamine
A low-allergy blend of soluble and insoluble fiber



The 4R Program - 1. Remove

- Concentrated aromatic oils: thyme, oregano, sage, lemon balm
  - For upper respiratory issues/sinusitis/GI health
- Berberine, oregon grape, coptis root, Chinese herbs, ginger, licorice, skullcap
  - Inflammation, intestinal support, dyslipidemia, dysglycemia, dysbiosis
- MCT oil containing caprylic acid: a natural anti-fungal
- *Strain-specific probiotics:*
- *S. boulardii, L.rhamnosus* HN001, *Bifidobacterium lactis* HN019
  - Anti-Viral-Bacterial-Yeast. Prevents pathogen adhesion. Protects sinus and GI mucosal cells
- NCFM, *Bifidobacterium lactis* Bi-07, Bi-04, *Lactobacillus plantarum* Lp-115, *salivarius* Ls-33, *Streptococcus thermophilus* St-21, *S. boulardii*
  - Immune health, digestive support, Anti-Viral-Bacterial-Yeast

The 4R Program - 2. Replace

I. Low-gastric acidity:
   - Betaine HCI combined with pepsin

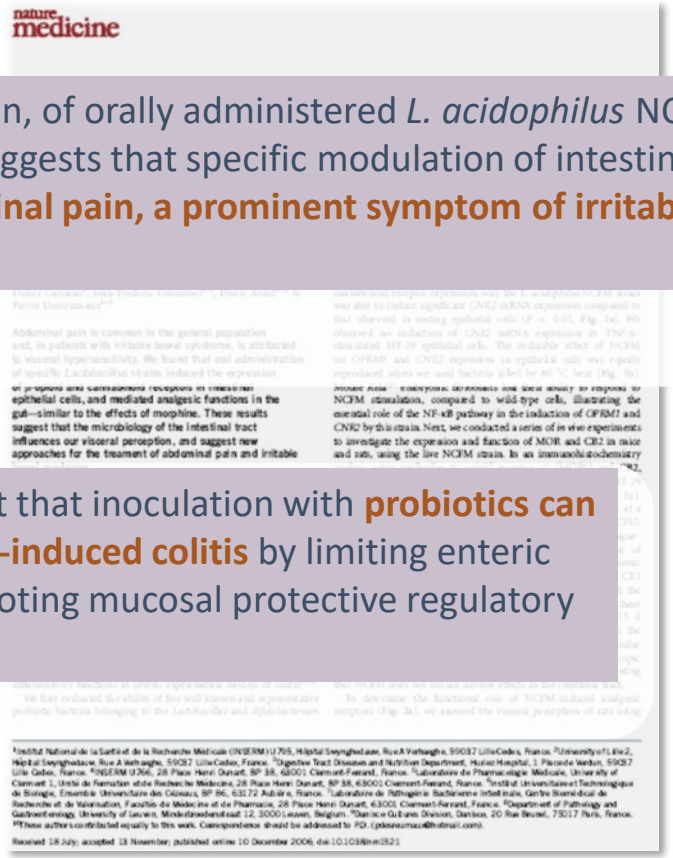
II. Pancreatic enzyme insufficiency:
   - Protease, amylase and lipase with specific enzymes to break down pathogen bio-film

III. Lipotropic nutrients:
   - To aid in liver and gallbladder function

3. Reinoculate—Probiotics

- *L. acidophilus* NCFM and *B. lactis* Bi-07 (60 billion live organisms) – designed to relieve recurring bowel distress and related functional discomforts, such as occasional bowel urgency
  - Helps relieve abdominal discomfort, bloating, cramping, bowel irritation, and occasional urgent bowel movements

Probiotics (*L. acidophilus* NCFM and *B. lactis* BI-07) have been studied clinically in numerous models of bowel distress



The similar efficacy, in treating pain, of orally administered *L. acidophilus* NCFM and a standard dosage of morphine suggests that specific modulation of intestinal flora may be a … **treatment for abdominal pain, a prominent symptom of irritable bowel syndrome"**

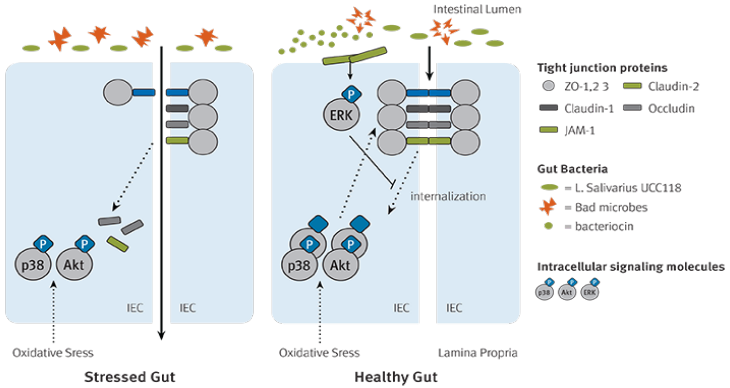
Nat Med. 2007 Jan;13(1):35-7.

"These observations suggest that inoculation with **probiotics can effectively prevent bacteria-induced colitis** by limiting enteric bacteria infection and promoting mucosal protective regulatory immune responses."

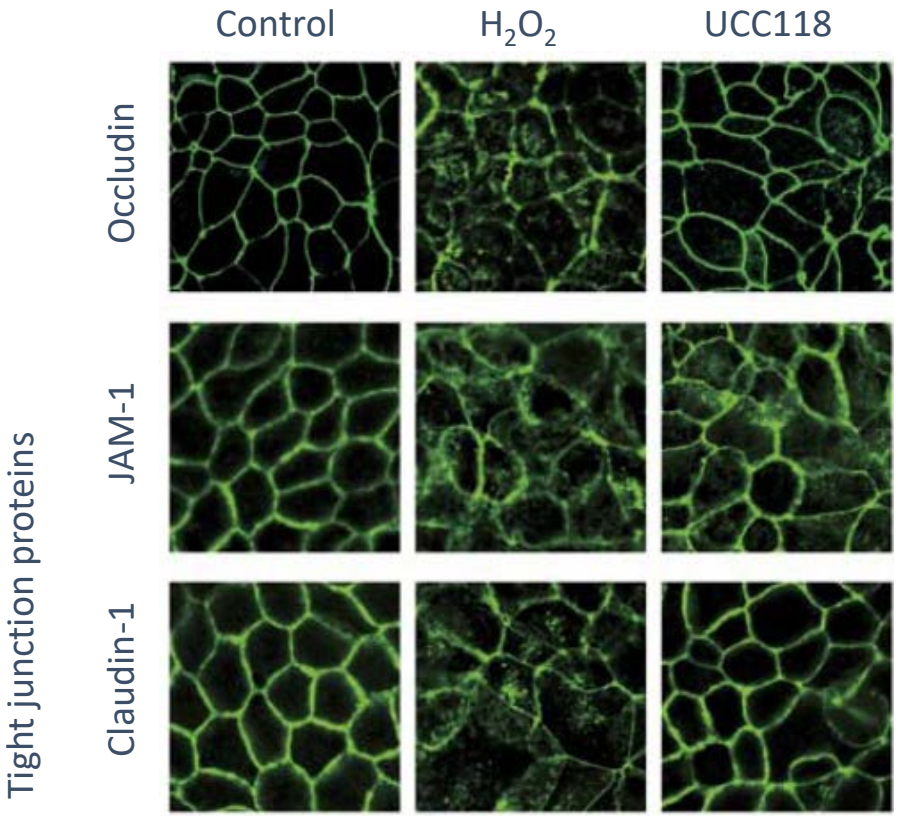
Pediatr Res. 2005 Dec;58(6):1185-91.

4. Regenerate/Repair

- *L. salivarius UCC118*
  - *Repair* Tight Junction Proteins
- Xanthohumol
  - Clinically proven anti-inflammatory
- Bioavailable curcumin
- Isomalto-oligosaccharides (IMOs)
  - Encourages SCFA's for mucosal and intestinal health
- Human milk oligosaccharides (HMO)
  - Prebiotic to nourish beneficial bacteria
- Alanine-glutamine dipeptide designed for enhanced absorption, stability, and solubility
  - Energy source for intestinal mucosal cell
- Specialized pro-resolving mediators (SPM)



Miyauchi et al. *Am J Physiol Gastrointest Liver Physiol*. 2012 Nov 1;303(9):G1029-41



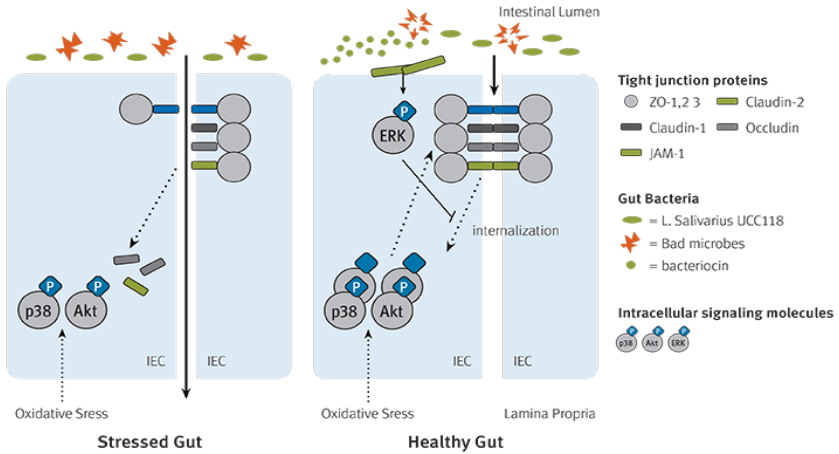
Pretreatment with UCC118 prevents disruption of intestinal epithelial cell tight junctions, in a validated in vitro model of human intestinal epithelial cell oxidative stress

Miyauchi et al. *Am J Physiol Gastrointest Liver Physiol*. 2012 Nov 1;303(9):G1029-41

4. Regenerate/Repair

L. salivarius UCC118 on tight junction proteins

- *UCC118* prevents the internalization of tight junction proteins after oxidative stress
  - Not all strains of *Lactobacillus salivarius* have this capacity
  - *UCC118* protects tight junction functionality in intestinal epithelial cells

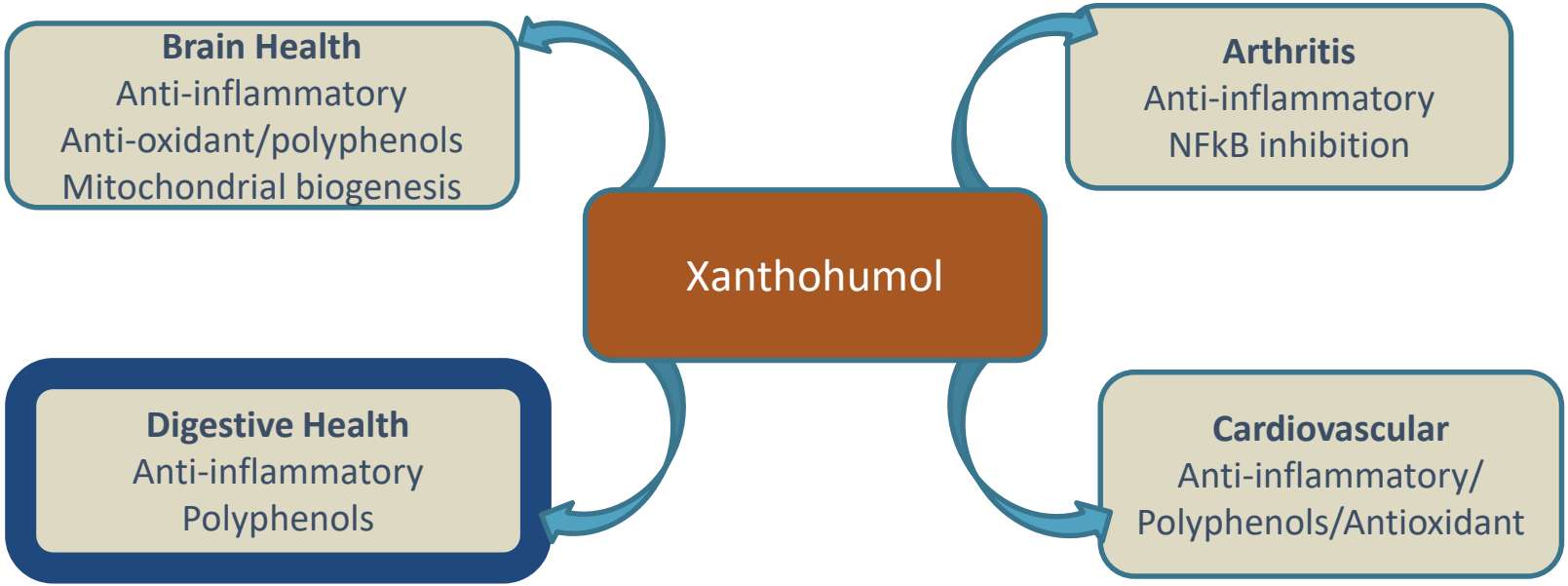


Miyauchi et al. *Am J Physiol Gastrointest Liver Physiol*. 2012 Nov 1;303(9):G1029-41

4. Regenerate/Repair

Xanthohumol strong clinical data

Excellent science: *>250 publications in preclinical science*



Bioavailable form of curcumin

- Shows potent anti-inflammatory activity—may help reduce inflammation-signaling molecules, such as NF-κB, TNF-α, COX-2, and PGE_2[1]
- Shows potent antioxidant activity – may help improve overall redox status through influencing antioxidants Nrf2, HO-1, and NQO1[2]
- Delivers significant concentrations of biologically active free curcuminoids—regarded as major limitation for efficacy of curcumin supplementation[2]
- Blend of stable curcuminoid and galactomannan compound (from fenugreek) designed for great bioavailability and more reliable clinical outcomes

1. Vecchi Brumatti L, Marcuzzi A, Tricarico PM, Zanin V, Giradelli M, Bianco AM. Curcumin and inflammatory bowel disease: potential and limits of innovative treatments. *Molecules*. 2014;19(12):21127-21153.
2. Rajasekaran SA. Therapeutic potential of curcumin in gastrointestinal diseases. World J Gastrointest Pathophysiol. 2011;2:1-14. 35. González-Reyes, S. Guzmán-Beltrán S, Medina-Campos ON, Pedraza-Chaverri J. Curcumin pretreatment induces Nrf2 and an antioxidant response and prevents hemin-induced toxicity in primary cultures of cerebellar granule neurons of rats. *Oxid Med Cell Longev*. 2013;2013:801418
3. Krishnakumar IM, Abhilash M, Gopakumar G, Dinesh K, Balu M, Ramadasan K. Improved blood–brain-barrier permeability and tissue distribution following the oral administration of a food-grade formulation of curcumin with fenugreek fibre. *Journal of Functional Foods*. 2015;14:215-225.

Isomalto-oligosaccharide (IMO) prebiotic fiber

The average American is only eating 10 - 15g of fiber

Adults should consume 25 – 35g of fiber

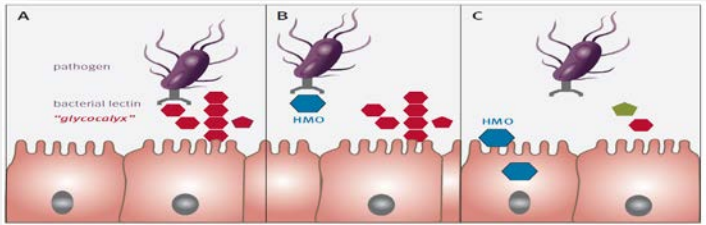
- IMO is soluble fiber, gentle prebiotic fiber source from tapioca
- Produces short-chain fatty acid (SCFA) like acetate, propionate and butyrate as end products of fermentation
- Inhibits the growth and activities of harmful microorganisms and contributes to stimulation of the growth of *Lactobacilli* and *Bifidobacteria*

Vernazza CL, et al. *J. Appl. Microbiol*. 2006;100;846-853.

Key targeted ingredients address dysbiosis

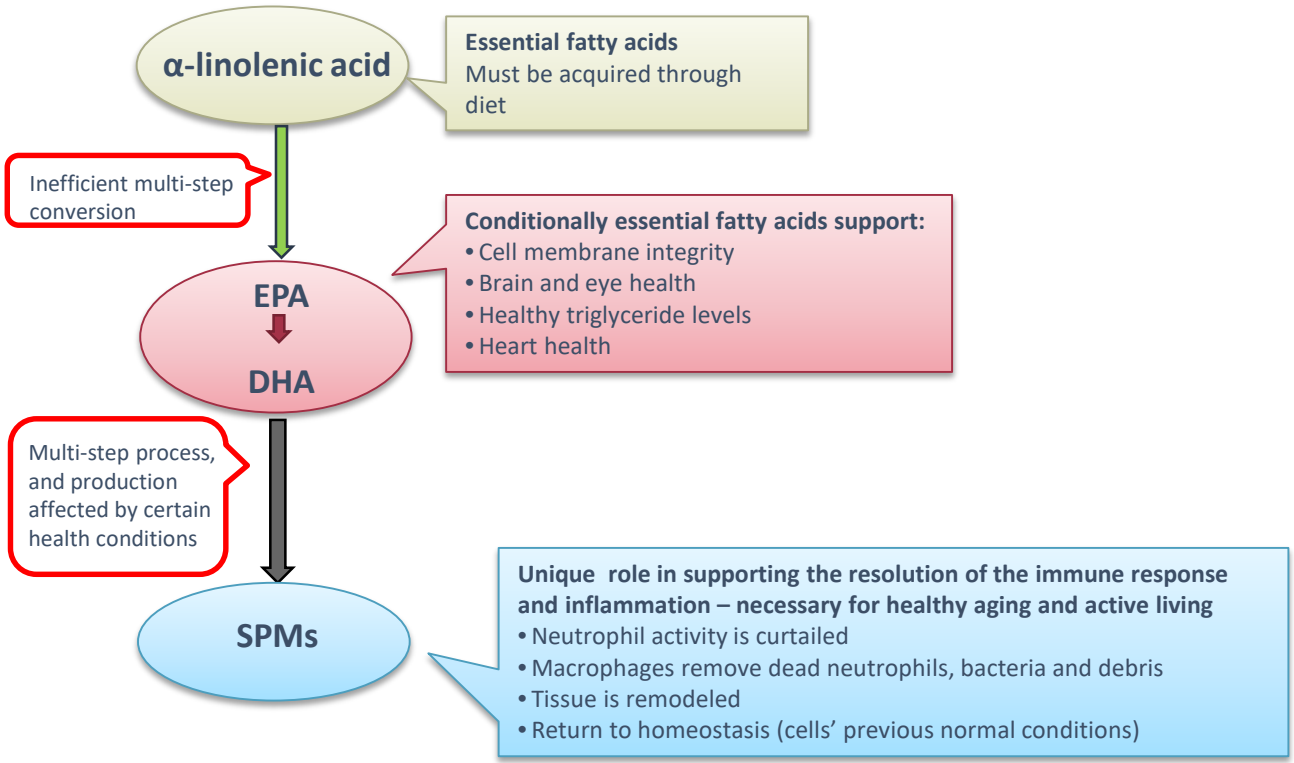
- ✓ HMOs occur naturally in human milk
- ✓ HMOs have prebiotic effect
- ✓ HMOs mimic structures found on surface of intestinal epithelia that bind unwanted bacteria serving as decoy receptors
- ✓ HMOs (2'-FL) selectively promote bacterial growth (*in vitro*) affecting butyrate production

2'-FL is the most abundant HMO



- ✓ IMO is soluble fiber, well-tolerated prebiotic fiber source from tapioca
- ✓ Produces short-chain fatty acid (SCFA) like acetate, propionate and butyrate as end products of fermentation
- ✓ Inhibits growth and activities of harmful micro-organisms and contributes to stimulation of the growth of *Bifidobacteria*

Specialized pro-resolving mediators (SPMs)

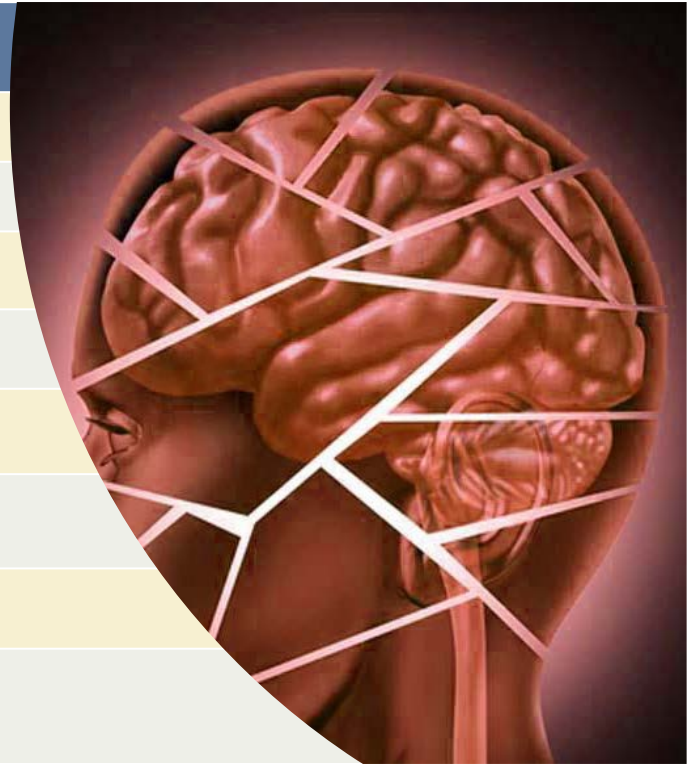


Inefficient conversion to meet needs in the face of inflammation, particularly in an already compromised host

Serhan CN. *Nature*. 2014;510:92-101.

4R Keto/Detoxification program

Ingredients
Keto diet, keto meal replacement, MCT oil (10 g/day)
Concentrated aromatic oils, berberine
Strain-specific probiotics per patient’s condition
Enzymes: HCL, pancreatic, lipotropic, address biofilm
Specialized pro-resolving mediators (1,500 mg/day)
Xanthohumol, curcumin, boswellia, ginger extracts
IMO and HMO prebiotics, glutamine
A low-allergy blend of soluble and insoluble fiber



Brain inflammation & proactive care

Formula Ingredients
Keto diet, keto meal replacement, MCT oil (10 g/day)
50:50 blend of *L. acidophilus* NCFM & *B. lactis* Bi-07
Digestive enzymes: lipotropic
Acetyl-L-Carnitine, N-Acetylcysteine,
Specialized pro-resolving mediators (1,500 mg/day)
Xanthohumol, curcumin, boswellia, ginger extracts
IMO and HMO prebiotics, glutamine
Magnesium, taurine, Ca, L-5-MTHF
Additional Supplements
Omega-3: 2-4 g DHA; D3: 5000 IU; CoQ10: 200 mg; ALA: 300 mg; Mag L-threonate: 2 g; Creatine: 20 g

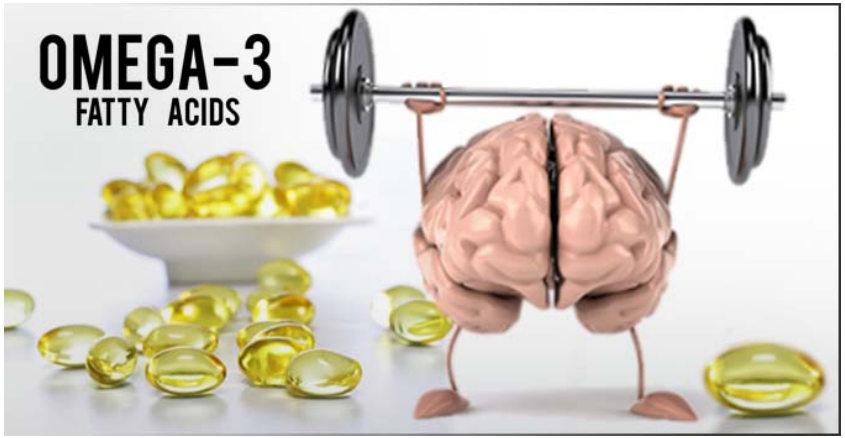
Creatine

- Maintain function of the mitochondria
- Improves blood flow in the brain improves both short and long term symptoms
- Short term:
  - Post TBI—concussion—creatine shown to reduce the duration of post-concussion amnesia

Rubenstein C. *Nutritional Perspectives: Journal of the Council on Nutrition of the American Chiropractic Association.* 2016;39(3):21-22

Omega-3 fatty acids (Ω3) improve brain function

- Aggressive intake of (Ω3's) benefit TBI, concussion, and post-concussion syndrome patients
- (Ω3's) exert positive effects on brain functions
  - White matter integrity
  - Grey matter volume in frontal temporal, parietal and limbic areas
  - Increased BDNF
  - Decreased peripheral fasting insulin



Michael DL. Concussions of brain injury—can omega-3 intake aid in brain health recovery. *J of the Am Col of Nutri*, 2016;35(5):469-75.
Witte AV, et al. *Cereb Cortex.* 2014;24(11):3059-3068.

DHA

- Ten published preclinical trials
- DHA supplementation reduces:
  - Axonal and neuronal damage
  - Inflammation
  - Apoptosis
  - Oxidative stress
  - Cognitive impairment
  - Neurotransmitter decline

ClinicalTrials.gov NCT01903525

Boswellia and curcumin

- Boswellia serrata (BS)
  - Reduces inflammation and improves cognitive outcomes
    - Moein P, et al. *Brain Inj.* 2013;27(12):1454-1460.
- Curcumin
  - Systemic anti-inflammatory that raises BDNF
  - Reduces acute activation of microglia/macrophages and neuronal apoptosis *Journal of Neuroinflammation*, 2014
  - Study presented in the *Experimental Neurology Journal* 2016 revealed that curcumin counteracted the outcome of traumatic brain on oxidative stress, synaptic simplicity and cognition

Alpha lipoic acid

- Lowers oxidative stress at the BBB
- Protects against free radical damage
- Improves insulin sensitivity and lowers blood sugar
- Chelates metals
- Improves endothelial function
- Lowers blood pressure
- Decreases dementia risk
- Improves the lipid profile
- Activates AMPK, Nrf2, and SIRT1
- Inhibits NF-KB

Elevate brain magnesium (L-threonate)

- Drives magnesium into the cerebrospinal fluid and then into neurons
  - Enhanced synaptic density and plasticity
  - Effects are unique to L-threonate
- Improvements in spatial memory and orientation
- Prevents loss of synapses and decline of memory
- Clears and prevents toxic beta amyloid plaques
- Suppresses the expression of the enzyme responsible for amyloid deposits by 80%

Sun Q, et al. Regulation of structural and functional synapse density by L-threonate through modulation of intraneuronal magnesium concentration. *Neuropharmacology*. 2016;108:426-39.
Li W, et al. *Mol Brain*. 2014;7:65.
Yu X, et al. *FASEB J*. 2015 Dec;29(12):5044-58.

Taurine:
Reduces brain inflammation and brain aging

- Protects brain cells against environmental toxins including lead and organic pesticides
- Prevents dysfunction of mitochondria within brain cells
- Protects brain cells against excitotoxicity
- Enhances GABA, which directly opposes excitotoxic effects
- Improves memory
- Reduces brain inflammatory processes
- Stimulates proliferation and new neuron formation to sustain learning and memory
- Protects brain cells against destruction following a stroke
- Attenuates damage caused by beta amyloid protein, a major contributing factor in Alzheimer's disease

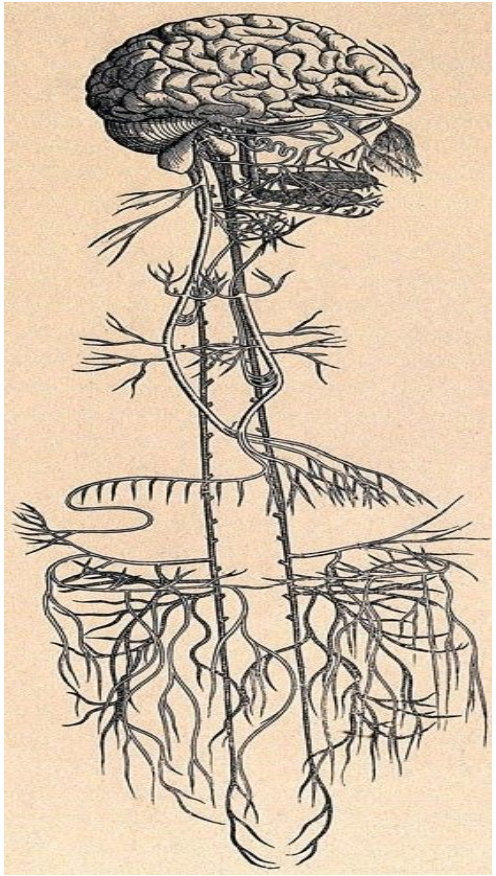
Louzada PR, et al. Taurine prevents the neurotoxicity of beta-amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders. FASEB J. 2004;18:511–518.

Acetyl L-carnitine

- Energizes the brain
- Increases levels of important neurotransmitter chemicals needed for memory, focus, and learning
- Repairs the damage done to brain cells caused by stress and poor nutrition
- Relieves depression
- Speeds stroke recovery
- Slows Alzheimer’s
- Helps damaged nerves and diabetic neuropathy

In conclusion

- The Microbiome-Gut-Brain Axis is an afferent/efferent communication super highway
- Inflammatory markers can cross the BBB
  - May lead to neurodegeneration and cognitive decline
- Ketogenic diet has shown to be anti-inflammatory
- 4R Functional Medicine protocol with a focus on key nutrients to restore neurological health



Source: Wellcome Library Public Domain

Questions?

Please join us for the evening reception:
5 - 6:30 pm
Bayview Ballroom
Drinks and hors d'oeuvres

Presentation by Dominic D'Agostino, PhD:
Plant-Centric Keto Diet

Thank you

Metagenics Institute

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Personalizing Cognitive Health for Optimal Outcomes

University of Miami's 7th Annual Integrative Medicine Conference

Pre-Conference Session

April 26, 2018

Miami, FL