A4M | MEDICINE REDEFINED

MODULE II

PEPTIDE THERAPY CERTIFICATION

Baseline Lab Testing to Establish Targeted Peptide Therapies

presented by :

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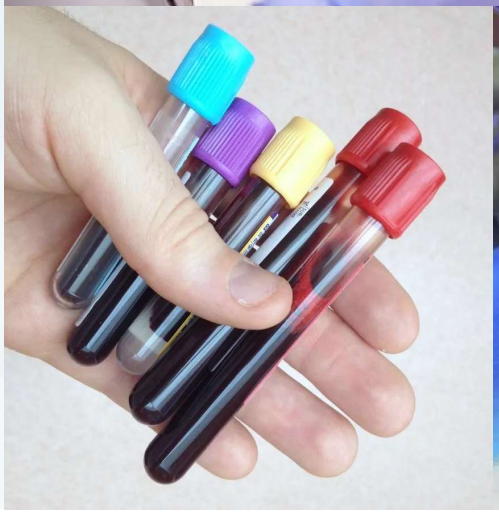

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Learning Outcomes

- **At the conclusion of this activity, the participant will be able to:**
  1. Understand what labs and biometrics will help you understand your patient's metabolic pathways.
  2. Discuss serum, salivary and urinary lab options.
  3. Be able to choose pertinent labs and create a more personalized metabolic panel to evaluate peptide therapeutics.

Laboratory Testing When Using Peptides

- Successful understanding a systems biology approach to balancing the body's individual chemistry
- Can be done through laboratory testing of parameters in blood and saliva
- Helps the practitioner assess the therapeutic efficacy of the peptide regimen

Remember: Factors That Affect Lab Results

- Sex
- Age
- Race
- Medical history including Rx, OTC and recreational drugs
- General health/metabolism
- Sleep and stress levels
- Not following testing requirements
- Look at the lab ranges – not all companies have the same values

Baseline Lab Testing

- Biometrics
  - BP, UpH, BMI, % body fat
- CBC and CMP
  - Includes MEB %
- Fasting glucose
- Hemoglobin A1c
- Insulin
- Thyroid panel – TPO/ThyAb, free T3, free T4, TSH, rT3
- Homocysteine
- Hs-CRP
- Estradiol, estrone, progesterone
- Stress/Cortisol 8am serum
- DHEA
- total Vitamin D Test
- Testosterone – free and total
- Vitamin B12
- RBC Magnesium
- Iron/Ferritin/RDW/% sat/TIBC
- PSA
- NMR lipoprofile

Add Ons

- Salivary and/or urinary cortisol
- Adiponectin
- Leptin
- oxLDL
- LDL-P
- GlycA
- Lpa
- ApoB
- 8-OHdG
- MSH
- MMA
- VEGF
- MMP-9
- LPS
- Zonulin
- Histamine

Core Testing – CMP and CBC

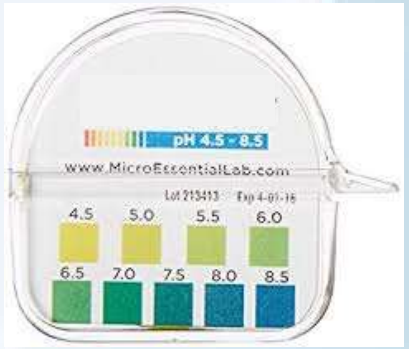
- Helps determine basic foundation of health
- Includes:
  - CBC (complete blood count)
  - CMP (comprehensive metabolic panel)
- Looks at
  - blood parameters
  - organs of detoxification
  - blood sugar and insulin regulation
  - electrolyte balance

CMP and CBC Tests

- Albumin
- Albumin/Globulin ratio
- ALP
- AST
- ALT
- Basophil %
- Bilirubin, total
- BUN
- Calcium
- Chloride
- CO2
- Creatinine
- Eosinophils %
- Globulin
- Ferritin
- Hematocrit
- Hemoglobin
- Iron
- MCH
- MCV
- MCHC
- **MPV**
- Monocytes %
- Potassium
- Protein
- RBC
- RDW
- Sodium
- WBC

Urinary and Saliva pH

- pH critical in determining biochemical balance
- Optimal pH salivary = 7 - 7.2
  (trending low 6.1-6.9, trending hi 7.3-7.8)
- Optimal pH urinary = 6.5-7 (trending low 6-6.49, trending hi 7.1-7.2)
- The more acidic (lower pH) = more inflammation
- More lactic acid produced at lower pH
- Mitochondria less efficient
- Joints and tissues stressed



Baliga S, et al. Salivary pH: a diagnostic biomarker. J Indian Soc Periodont. 2013;17(4):461-65.

pH

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

MEBs - %

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

Yang Z, et al. Comparisons of neutrophil-, monocyte-, eosinophil-, and basophil- lymphocyte ratios among various systemic autoimmune rheumatic diseases. APMIS. 125(10):863-71.

Intense Exercise Effects on CBC -WBC

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

Simpson RJ, et al. Exercise and regulation of immune functions. Prog Mol Biol Transl Sci. 2015;135:355-80.

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

MEB ranges (QUEST)

Monocytes %

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

Basophils %

- < 1 optimal
- Trending hi = 0.9-1

Eosinophils %

- <5 optimal
- Trending hi = 3.5-5

Total Vitamin D Test

- Vitamin D range = 30-100 ng/ml
- Optimal vitamin D level = 50-90 ng/ml
- Trending low = 30-49.9
  - 5,000 IU (125mcg) daily
  - Recheck in 90 days
- Alert Low = <30 ng/ml
  - 10,000 IU daily
  - Recheck in 90 days
- Alert high = >100
  - Can lead to toxicity including calcium deposits in soft tissue
- Add vitamin K2?

Iron

- Carries oxygen to cells
- Critical especially when exercising
- Necessary in thyroid hormone production also
- Low iron = poor oxidation of tissues = poor performance

Abbaspour N, et al. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164-74.

Iron

- Low iron caused by:
  - Bleeding, including the stomach, intestines or rectum
  - Heavy or excessive exercise
  - Thyroid hormone imbalances
  - Genetic problems
  - Environmental toxins
  - Low thyroid intake
  - Drug induced nutrient depletion
    - Antibiotics – Fluoroquinolones, tetracyclines, aminoglycosides
    - Indomethacin
    - Bile acid sequestrants
    - Levothyroid (Synthroid)
    - PPIs / H2 blockers

Abbaspour N, et al. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164-74.

Iron Ranges QUEST

- MALE
  - Range 50-180 mcg/dL
  - 100-160 optimal
  - Trending low = 50-100
  - Alert low <50

- FEMALE
  - 49 and younger
    - Range 40-190
    - Trending low – 40-80
    - Alert Low - <40

FEMALE

- 50 and older
  - Range 45 – 160
  - Optimal = 75-130
  - Trending low = 45-75
  - Alert low = <45

Ferritin

- Protein component of a red blood cell
- Correlates with amount of iron stored in body
- Pancreas connection
- Higher levels can indicate
  - high iron
  - insulin resistance
  - Chronic inflammation
  - Alcohol abuse
  - CKD – chronic kidney disease

Wang W, et al. Serum ferritin: past, present, future. Biochem Biophys Acta. 2010;1800(8):760-69.

Ferritin

- Lower levels can indicate
  - Low iron
  - Thyroid imbalances
  - Immune problems
  - Can cause fatigue, palpitations, numbness, cognitive decline

Wang W, et al. Serum ferritin: past, present, future. Biochem Biophys Acta. 2010;1800(8):760-69.

Ferritin QUEST

- Ranges gender and age specific
- Av. Male range = 38-380 ng/ml
  - Optimal = 100-300
  - Trending hi = 300.1-380
  - Trending low = 38-99.9
- Av. Female range = 16-232
  - Optimal = 90-180
  - Trending hi = 180.1-232
  - Trending low = 16-89.9

Cortisol Level: Triggers

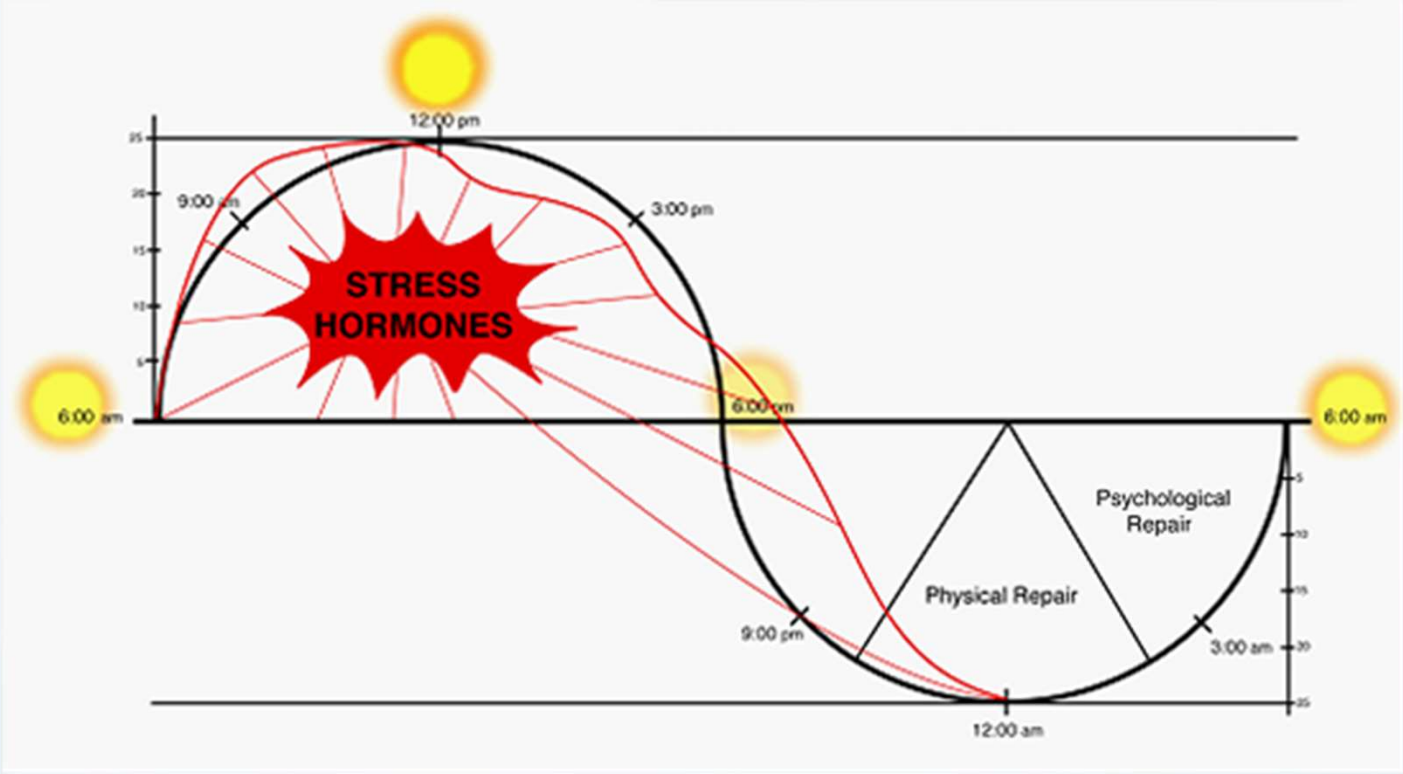
- Wired and Tired
- Tired and flat
- Poor sleep
- Poor performance
- Weight gain around the abdomen
- Mind racing
- Immune problems
  - Allergies and Asthma
  - Inflamed Joints
  - Poor exercise recovery

Cay M, et al. Effect of increase in cortisol level due to stress in healthy young individuals on dynamic and static balance scores. North Clin Istanb. 2018;5(4):295-301.

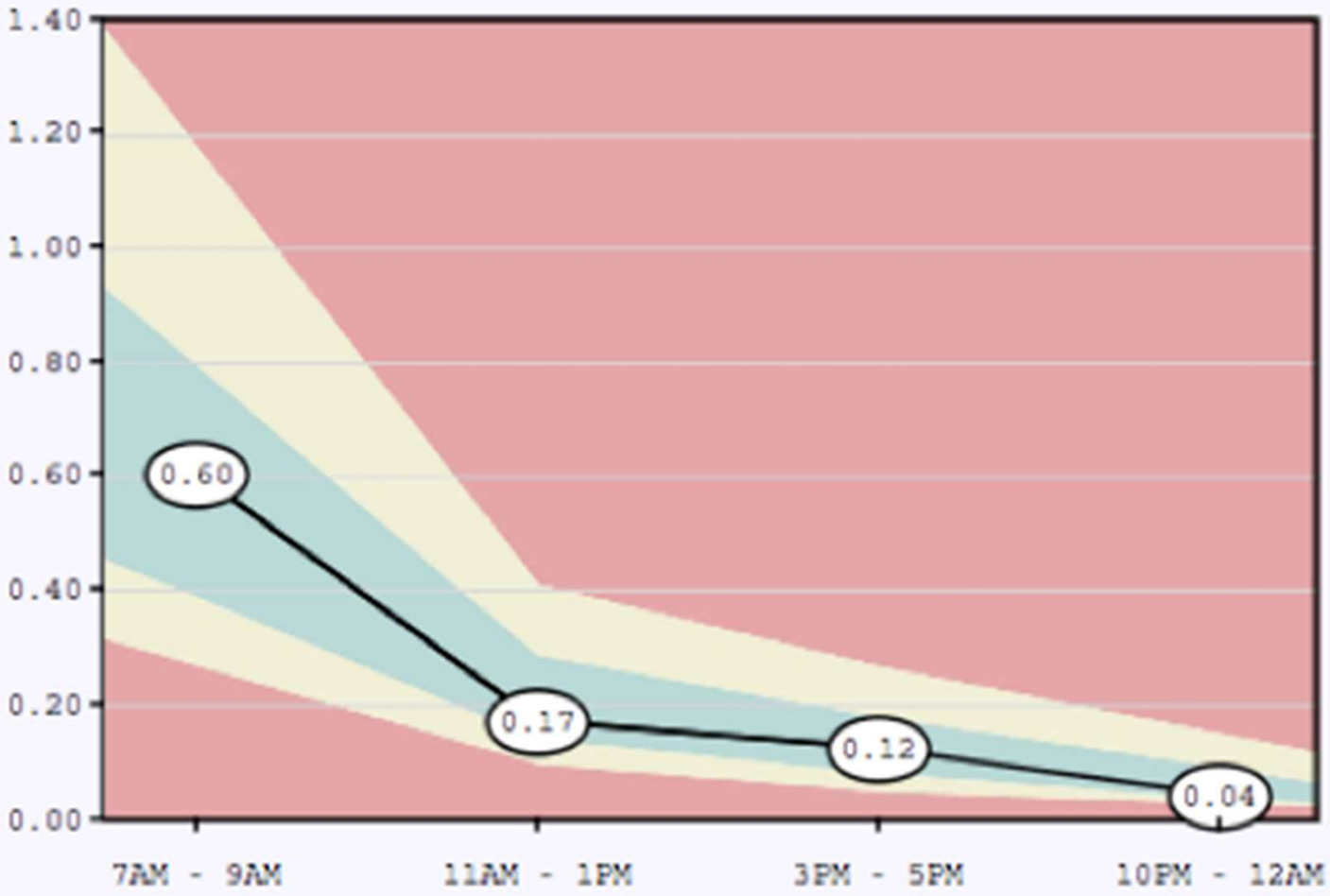
Serum Cortisol Ranges QUEST

- 8 am serum 4 – 22 mcg/dL
  - Alert LOW = <4
  - Trending Low = 4-11.9
  - OPTIMAL = 12-17
  - Trending high = 17.1-22
  - Alert High = >22
- Trending or alert Low
    - Adrenal concentrate is no anxiety and fatigue
    - or Adrenal cortex if anxiety present
    - or Licorice if BP stable
- Trending or alert High
    - Adaptogens
    - Adaptogens + Magnolia/Phellodendron/Theanin if cravings and feeling stressed, anxious

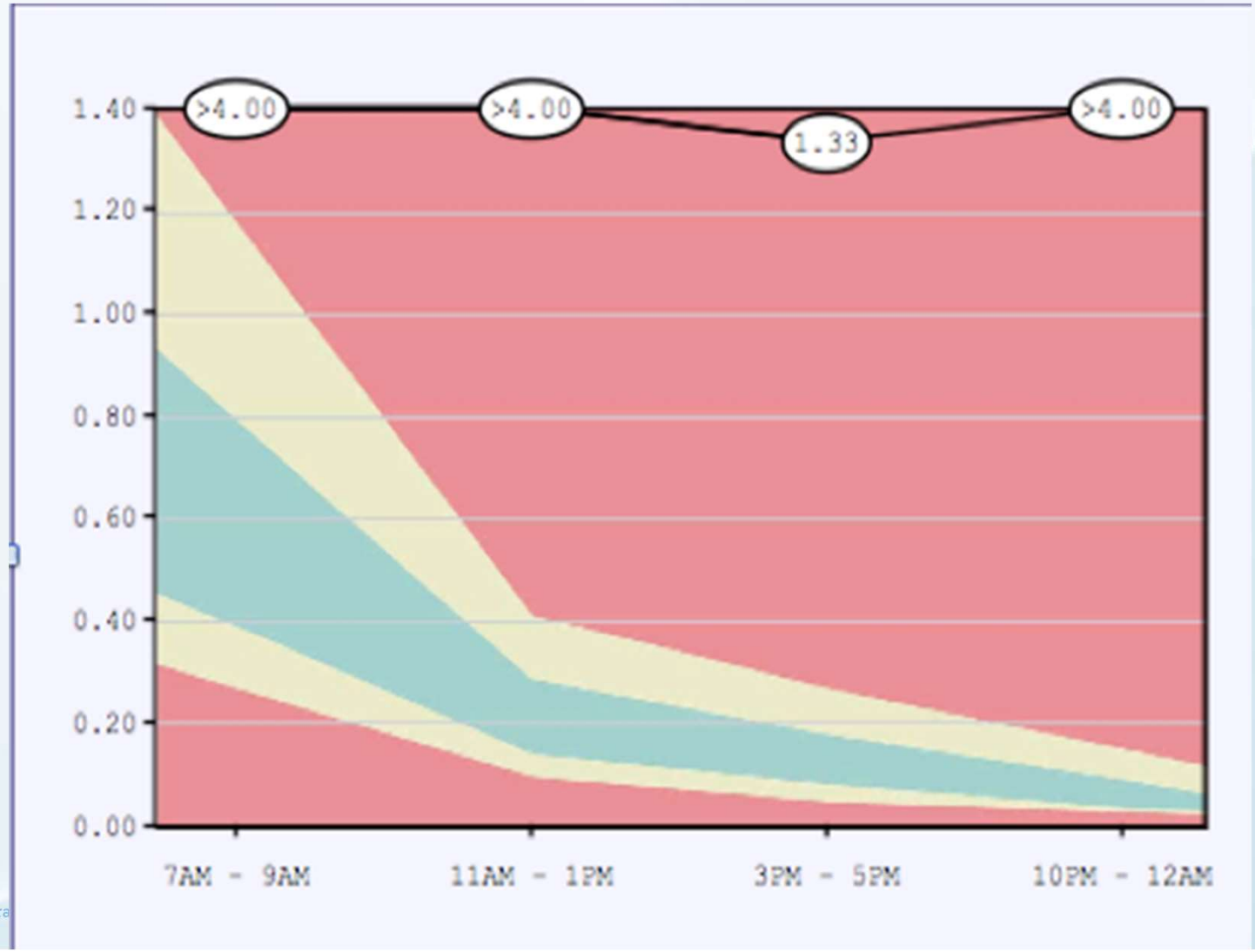
Normal Diurnal Hormone Release



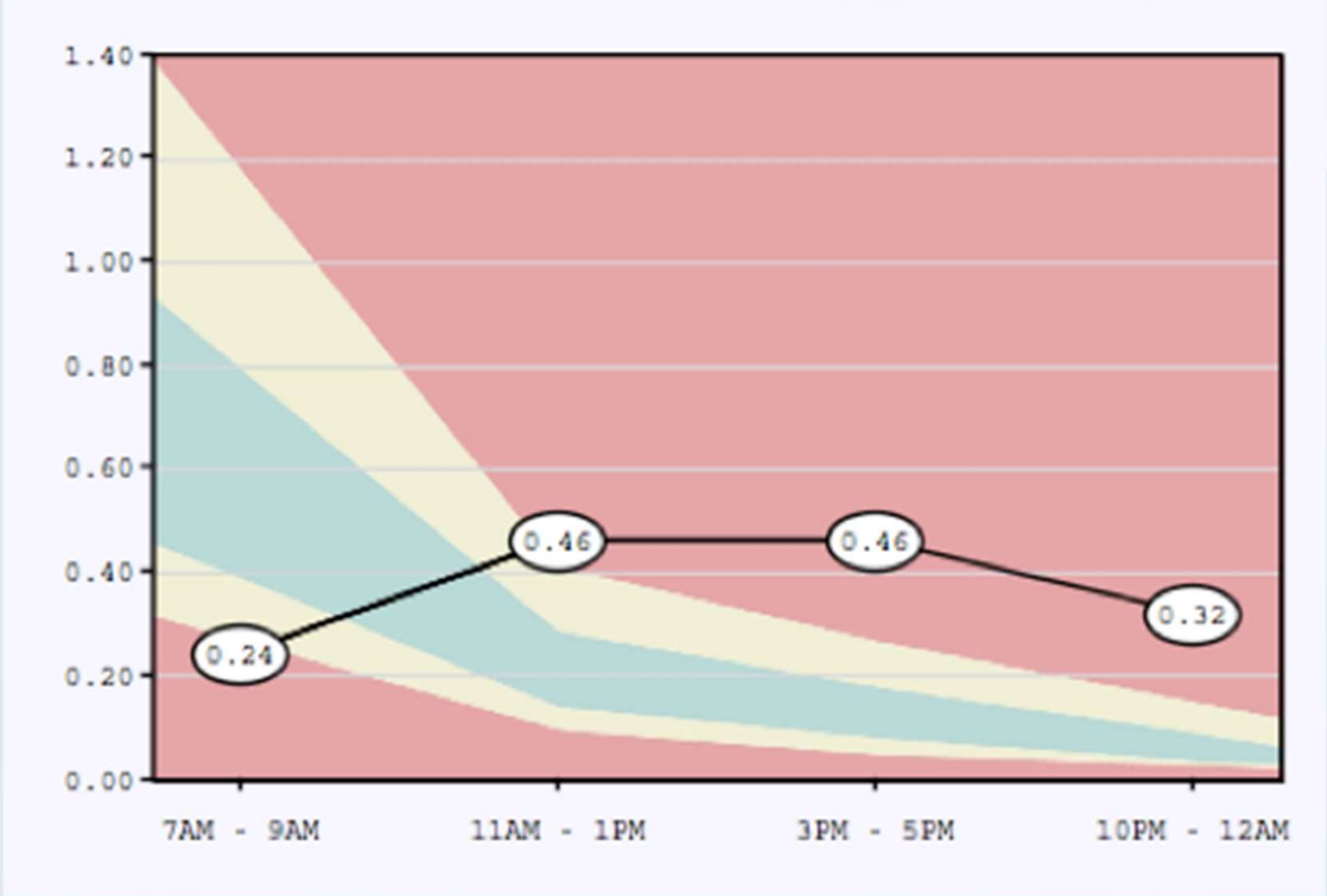
Cortisol – Normal Pattern



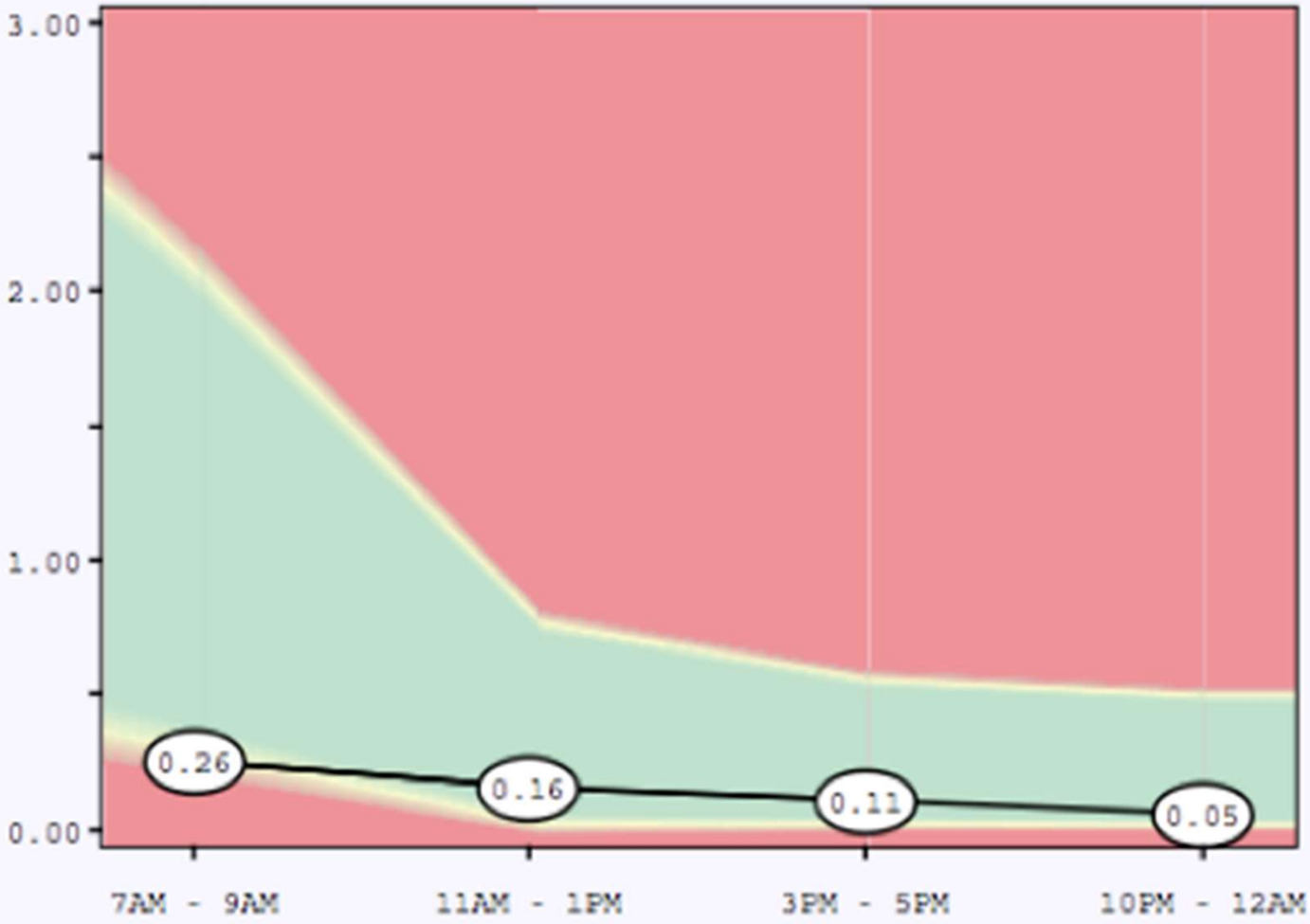
Cortisol - STRESS



Cortisol - STRESS



Cortisol - STRESS



DHEA and Weight Gain

- Cortisol imbalances lead to DHEA imbalances
- As cortisol rises, DHEA decreases
- Low levels of DHEA associated with increased weight gain, hormonal imbalances
- DHEA involved in regulation of glucose-6-phosphate
- High levels associated with:
  - adrenal tumors
  - PCOS – polycystic ovary syndrome

Khanfer R, Lord JM, Phillips AC. Neutrophil function and cortisol:DHEAS ratio in bereaved older adults. Brain Behav Immun. 2011l;25(6):1182-6.

DHEA LAB VALUES QUEST

- Serum DHEA-S – sulfated DHEA

- Serum
  - Ranges depend on age brackets (7 ranges between 18 and 71 years)
  - Average Male serum = 55-400mg/dL with OPTIMAL = 200-375
  - Average Female serum = 24-265 with OPTIMAL = 100-225

- **Manage low and trending low DHEA + high cortisol with DHEA supplementation**
  - Males alert low + trending hi or high cortisol = 50mg qd
  - Males trending low + trending hi or high cortisol = 25mg qd
  - Female alert low + trending hi or hi cortisol = 25mg/day
  - Female trending low+ trending hi or hi cortisol = 15mg/day

Thyroid Panel

- Used to evaluate thyroid health

- Includes
  - Free T4 (thyroxine)
  - Free T3 (triiodothyronine)
  - TSH (thyroid stimulating hormone)
  - TPO (thyroid peroxidase antibody)
  - ThyAB (thyroid antibodies) –optional
  - rT3 (reverse T3) - optional

- Important in weight loss – thermogenesis
- Diminished cognition

Thyroid Panel

- Serum blood draw, preferably in the morning
- Does not need to be fasting
- Low levels of thyroid hormones lead to weight gain
- T3 declines lead to mitochondrial deficiency
- Reduces insulin receptors

Maxxoccoli G, Carughi S, Sperandeo M, et al. Neuro-endocrine correlations of hypothalamic-pituitary-thyroid axis in healthy humans. J Biol Regul Homeost Agents. 20111;25(2):249-57.

Energy and Metabolism: Triggers

- Cold intolerance
- Diminished cognition and mood
- Food sensitivities
- Low energy throughout the day

Galgani J, Ravussin E. Energy and metabolism, fuel selection and body weight regulation. Int J Obes (Lond.). 2008;32(Suppl 7):S109-S119.

Thyroid Panel Ranges QUEST

- Free T3 ranges (2.3-4.2 pg/ml)
  - Alert low = <2.3
  - Trending low = 2.3-2.99
  - OPTIMAL = 3-3.9
  - Trending high = 4.0-4.2
  - Alert high = >4.2

  - Free T4 Ranges (0.8-1.8 pg/ml)
    - Alert low = <0.8
    - Trending low = 0.8-1.1
    - OPTIMAL = 1.11-1.69
    - Trending high = 1.7-1.8
    - Alert high = >1.8

Thyroid Panel Ranges QUEST

- TSH (0.4-4.5 mIU/L)
  - Alert low = <0.4
  - Trending Low = 0.4-1
  - OPTIMAL = 1.01-2
  - Trending high = 2.01-4.5
  - Alert high = >4.5
- TPO (<9 IU/ml)
  - OPTIMAL = 0-8.9
  - Trending high = 9-25
  - Alert high = >25

Thyroid: Nutrient Support

- Selenomethionine + iodine - 200mcg + 1mg daily iodine
- Ashwagandha root – *Withania somnifera* - Indian ginseng; 450mg BID std. 2.5-5% withanolides
- Fucus / Bladderwrack – high iodine content
- Coleus forskholii – 500mg BID std. 20% forskolin
- L-tyrosine – 500mg BID
- HPA support - adaptogens

GLUCOSE

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

Sharma K, et al. Stress-induced diabetes: a review. Cureus. 2022;14(9):e29142.

GLUCOSE TARGET RANGES

- Fasting blood glucose
  - Alert Low = <65
  - Trending Low = 65-72
  - OPTIMAL = 73-89
  - Trending high = 90-99
  - Alert High = >99
- Postprandial
  - 1 hr Target = < 125
  - 2 hr target = < 95

2008 Insulin Resistance Study

- 46,578 members of Kaiser Permanente Northwest
- FPG levels < 100 mg/dL (Jan '97-Dec 2000)
- No previous diagnosis of diabetes or impaired fasting glucose
- Subjects assigned to 1 of 4 categories (<85, 85-89, 90-94, or 95-99 mg/dL)
- Followed until developed diabetes, died, left the health plan, or until April 30, 2007
- Cox regression analysis--estimated risk of incident diabetes, adjusted for age, sex, body mass index, blood pressure, lipids, smoking, cardiovascular disease, and hypertension

Nichols GA, Hiller TA, Brown JB. Normal Fasting Plasma Glucose and Risk of Type 2 Diabetes Diagnosis. Am J Med. 2008;121(6). 519-524.

Study Results and Conclusions

- Every glucose rise of 1 point above 84, was correlated with a 6% increased risk of developing Type 2 diabetes

- **Insulin resistance, which leads to Type 2 diabetes, is developing at least a decade before detection by traditional lab markers**

Nichols GA, Hiller TA, Brown JB. Normal Fasting Plasma Glucose and Risk of Type 2 Diabetes Diagnosis. Am J Med. 2008;121(6). 519-524.

Fasting Plasma Glucose and T2D Risk – Updated Study

- 2022 long-term retrospective study
- N= 37,148 Japanese individuals w/ normal plasma glucose
- In 10 years, 1,028 patients developed T2D
- Cox regression analyses reveal:
  - **Risk for onset of T2D increased by 9.0 % per 1mg/dL increase in fasting plasma glucose from 90 – 99 mg/dL**

Munekawa C, et al. J Diabetes Invest. 2022;13:453-59.

Bottom Line

- RISK of T2D increases by 9 % for every point above 90mg/dL fasting plasma glucose

Munekawa C, et al. J Diabetes Invest. 2022;13:453-59.

Insulin Levels QUEST

- Range = 2 – 19.6 microIU / ml
- Optimal = 3-6
- Trending hi = 6-19.6
- Hi = > 19.6

Hemoglobin A1c (HbA1c)

- HbA1c or glycated hemoglobin
- Measurement of how well blood glucose is controlled over time
- Measures glycation
- Trending high or high generally indicate insulin resistance
- Can lead to damage to cardiovascular, kidney and nervous system
- RANGES (<5.7%)
  - OPTIMAL = < 5.3
  - Trending high = 5.3-5.7
  - Alert high = >5.7

Magnesium

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

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

Magnesium and Metabolic Performance

- Integral in ATP production
- Oxygen Uptake
- Central Nervous System Function
- Electrolyte balance Na/K pump function
- Glucose Metabolism
- Muscle Function
- Heart rate and function
- Bone density

Char V, Nizamliogly M, Mogulkoc R, et al. Effects of magnesium supplementation on blood parameters at rest and after exercise. Biol Trace Elem Res. 2007;115(3):205-12.
Chen HY, Cheng FC, Pan HC, et al. Magnesium enhances exercise performance via increasing glucose availability in the blood, muscle and brain during exercise. PLoS One. 2014;9(1):e85486.

Magnesium

- Metabolic imbalances associated with trending low or alert low magnesium levels :
  - Muscle/aches and pains, and spasms
  - Increased serum CRP-hs
  - Lowered testosterone
  - Headaches
  - Elevated blood pressure
  - Anxiousness or nervousness
  - Insomnia
  - Increased risk for metabolic syndrome, insulin resistance and type 2 diabetes,
  - Weight gain, obesity
  - Heaviness in legs, muscles feel weak
  - Fatigue

Char V, Nizamliogly M, Mogulkoc R, et al. Effects of magnesium supplementation on blood parameters at rest and after exercise. Biol Trace Elem Res. 2007;115(3):205-12.
Chen HY, Cheng FC, Pan HC, et al. Magnesium enhances exercise performance via increasing glucose availability in the blood, muscle and brain during exercise. PLoS One. 2014;9(1):e85486.

Magnesium Dosage

- Manage with 200-300 mg BID (elemental) magnesium chelate
  - 7.5-10mg/kg elemental – mainly for building storage or athletes
- If sleep a problem, dose Mag 300-600 mg at bedtime
- Mag. Threonate – 2,000 mg 1-2 times daily , contains 144mg magnesium

RBC Magnesium QUEST

- Magnesium serum levels do not give accurate cellular levels
- Need to perform RBC magnesium level - amount of intracellular Mag
- Range RBC Mag: (4-6.4 mg/dL)
  - Alert Low = <4
  - Trending Low = 4.1-5.5
  - OPTIMAL = 5.6-6.2
  - Trending high = 6.3-6.4
  - Alert High = >6.4

Serum Magnesium QUEST

- Serum 1.5-2.5 mg/dL

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

Vitamin B12

- Important in nervous system
  - Improves cognitive function
  - Neuroprotective
- Methylation – helps regulate homocysteine levels
- Ranges QUEST = 200-1100 pg/ml
  - Trending low = 200-400
  - Optimal = 400-950
  - Trending high = 950-1100
  - High > 1100

Ryan-Harshman M. Vitamin B12 and health. Can Fam Physician. 2008;54(4):536-41.

Testosterone

- Testosterone important in multiple aspects of aging and healthspan for men and women
- ANABOLIC
- Testosterone drops an average of 1 to 1.5% per year after age 30
- Low levels also linked to non-alcoholic fatty liver disease (NALFD)

Mody A, et al. Relevance of low testosterone to non-alcoholic fatty liver disease. Cardiovasc Endocrinol. 2015;4(3):83-89.

Testosterone

- Low levels of testosterone:
  - Fatigue
  - Mood changes; cognitive decline
  - Decreased libido
  - Loss of muscle tone, strength
  - Skeletal problems
  - Increased erectile dysfunction in men
  - Sleep disorders
  - Kidney issues
  - Weight gain, especially around mid-section
  - Increase incidence of insulin resistance/type 2 diabetes
  - Increased incidence of coronary artery disease and other cardiovascular problems
  - Increased Metabolic Syndrome
  - Increased risk of cancers

Traish AM, Miner MM, Morgentaler A, et al. Testosterone deficiency. Am J Med. 2011;124(7):578-87. PMID: 21683825.

Testosterone in Men

Low Testosterone = INCREASED RISK OF ALL-CAUSE MORTALITY in MEN

Traish AM, Miner MM, Morgentaler A, et al. Testosterone deficiency. Am J Med. 2011;124(7):578-87. PMID: 21683825.

Testosterone Insufficiency

- 2008 Endocrine Society report studied 2000 German men aged 20 to 79 and found that those with low testosterone had a 2.5 times greater risk of dying over the next 10 years - all cause mortality
- Schneider HJ, et al. Prevalence of low male testosterone levels in primary care in Germany: cross-sectional results from the DETECT study. Clin Endocrinol (Oxf). 2009;70(3):446-54.

- 2006 study reported in JAMA showed that of 858 men over the age of 40, followed for 4 to 8 years, those with low testosterone had 88% increased risk of death in that time frame.

Shores MM, et al. Low serum testosterone and mortality in male veterans. Arch Intern Med. 2006;166(15):1660-5.

Testosterone Insufficiency

- 2014 meta-analysis of 20 observational studies
- CONCLUSION:
  - Men with low total testosterone, low SHBG OR low free testosterone significantly more likely to have MetS

Brand JS, et al. Testosterone, sex-hormone binding globulin and the metabolic syndrome in men: an indvidual participant data meta-analysis of observational studies. PLoS One. 2014;9(7):e100409.

Low T and Kidney Disease

- **Low "T" is common and impairs quality of life (QoL) in patients with CKD**
  - 2020 study of 25 male patients (ages 55-84) with stages III–IV CKD
  - GFR = 15-59
  - Serum T levels were <350 ng/dl (TD)
  - **Treated with testosterone gel for 3 months**
  - RESULTS: significant improvement in :
    - T levels, Hb, Hct, grip strength
    - Improved quality of life in moderate – severe CKD
    - May delay progression of CKD

Yeo JK, et al. Effects of testosterone treatment on quality of life in patients with Chronic kidney disease. Am J Mens Health. 2020;14(3):1557988320917258.

Testosterone Labs

- Test total testosterone and free testosterone
- SHBG (serum hormone binding globulin) can bind up serum testosterone
- Total levels can look fine, but free (ACTIVE) levels may be low with excess SHBG
- Testosterone/DHEA ratio

Testosterone Ranges QUEST

- Testosterone serum, total MEN (250-1100)
  - Alert low = <250 ng/dL
  - Trending low = 250-650
  - OPTIMAL = 650-900
  - Trending high = 900-1100
  - Alert high = >1100

- Testosterone serum, total FEMALES (2-45)
  - Alert low = <2 ng/dL
  - Trending low = 2-15
  - OPTIMAL = 15.1-28.9
  - Trending high = 29-45
  - Alert high = >45

Testosterone Ranges QUEST

- Testosterone serum, free MEN (46-224 ng/dL)
  - Alert low = <46
  - Trending low = 46-70
  - OPTIMAL = 70-168
  - Trending high = 168.1-224
  - Alert high = >224

- Testosterone serum, free FEMALES (0.2-5 ng/dL)
  - Alert low = <0.2
  - Trending low = 0.2-2.25
  - OPTIMAL = 2026-4.5
  - Trending high = 4.6-5
  - Alert high = >5

hsCRP – C-reactive protein

- CRP major marker in chronic inflammation
- hs = high sensitivity
- Correlated with
  - Sleep problems
  - Insulin resistance/blood sugar imbalances
  - Cardiovascular events
  - Metabolic syndrome
  - Weight gain

Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018;9:754.

hs-CRP QUEST

- Range = <3
- 1 or less is optimal
- Trending high = 1.1 – 3
- > 3 is high

NMR LIPID profile + Add-ons

- Total Cholesterol
- LDL-C
- LDL-P
- HDL
- Triglycerides
- Ox-LDL
- LpPLA2
- Lpa
- ApoB

Basic Lipids

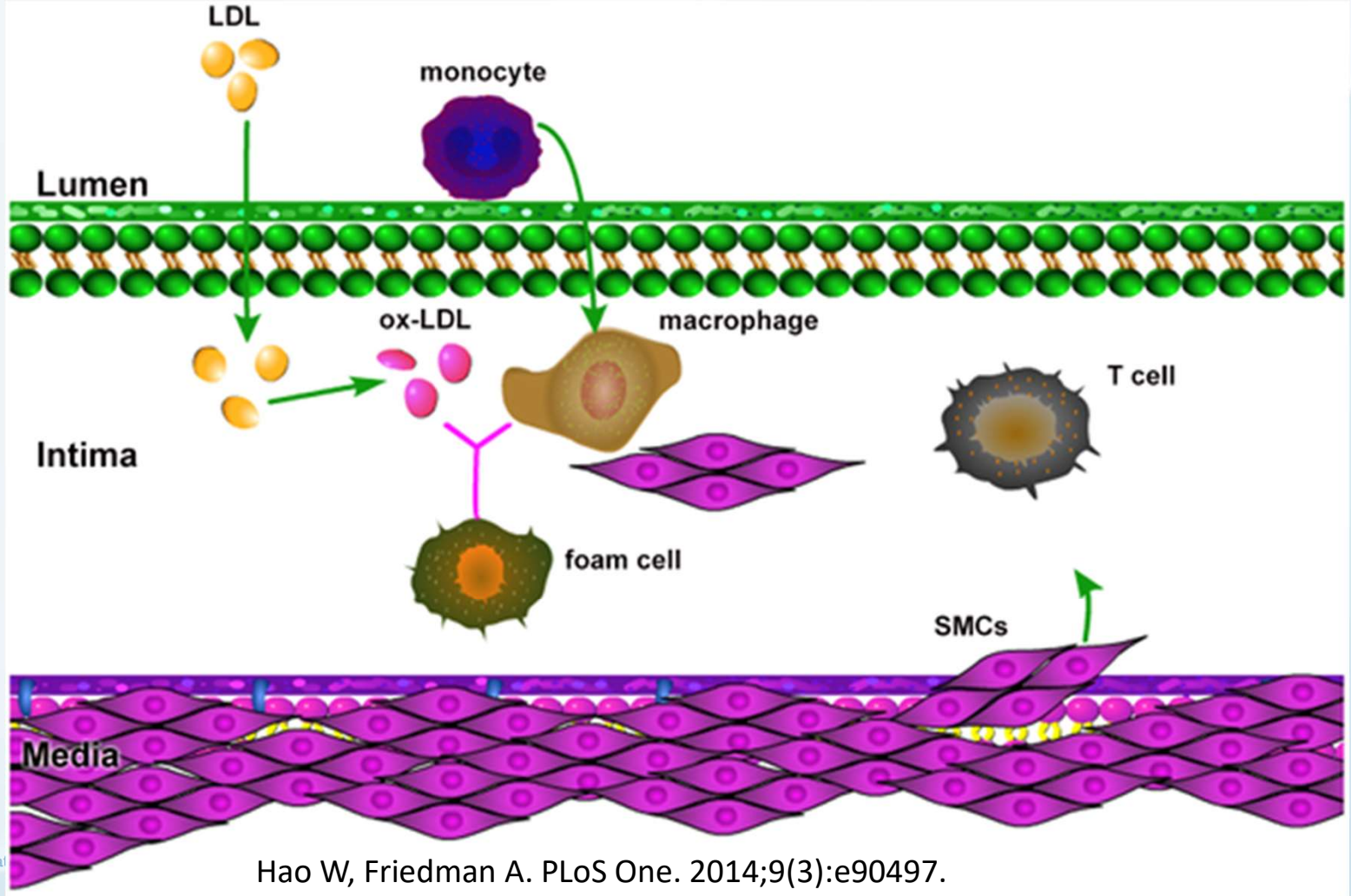
- Total Cholesterol
  - Range = 125-200mg/dl
  - Optimal = 125-180
- LDL-C
  - <100 mg/dl
  - OPTIMAL = 75-95
  - Trending hi = 95.1 – 100
- HDL
  - Gender specific
  - Males >40
  - Females >50
- Triglycerides
  - <150 optimal
  - Want to be <100
  - 100-150 trending hi
  - Alcohol use night before test can increase levels

oxLDL

- Oxidized LDL
- Proinflammatory component of lipids
- Transforms macrophages into foam cells
  - Major constituent of arterial plaque
- Increased levels correlate with increased risk of coronary artery disease (CAD) and IR/T2D
- Oxidation of LDL triggered by IR and chronic stress
- First sign of metaflammation
- MetS risk increased 4x w/ increase LDLox
- Levels increase as CAD severity increases
- Range < 60 U/L

Holvoet P, et al. Association between circulating oxidezed low-density lipoprotein and incidence of the metabolic syndrome. JAMA. 2008;299:2287-93.

oxLDL



Hao W, Friedman A. PLoS One. 2014;9(3):e90497.

LDL-P

- LDL-Particle number
- nmol/L
- Lower the value, less risk for cardiovascular disease
- Stronger correlation to CVD than LDL-C

Cromwell WC, et al. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study – Implications for LDL Management. J Clin Lipidol. 2007;1(6):583-92.

Lipid Add-Ons

- Lpa
  - < 75 nmol/L optimal
  - > 125 is HIGH
- LpPla2
  - ≤ 123 nmol/min/ml optimal

GlycA

- Novel marker for systemic inflammation
- Also CV disease risk
- Low intra-individual variability
- Metainflammatory and autoimmune patients
- Reflects both increased glycan complexity and circulating acute phase protein levels during local and systemic inflammation
- Levels associated wth IL-6, TNF-alpha, fibrinogen, hsCRP, serum amyloid A, $LpPLA_2$
- Levels also associated with increased production of anti-microbial peptides (AMPs), circulating leukocytes and neutrophil activity

Connelly MA, et al. GlyA, a novel biomarker of systemic inflammation and cardiovascular disease risk. J Transl Med. 2017;15:219.

GlycA

- GlycA increased in chronic inflammation and febrile conditions
- GlycA also correlates with markers of MetS:
  - Body mass index (BMI)
  - Insulin resistance, Type II
  - BP
  - Ratio of leptin to adiponectin
- This makes GlycA a reliable CV risk marker **BEYOND** hsCRP
- Also marker for progression of CV risk to T2DM
- LabCorp range = < 400 umol/L ; . 400 = high risk

Connelly MA, et al. GlyA, a novel biomarker of systemic inflammation and cardiovascular disease risk. J Transl Med. 2017;15:219.

Zonulin Testing

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

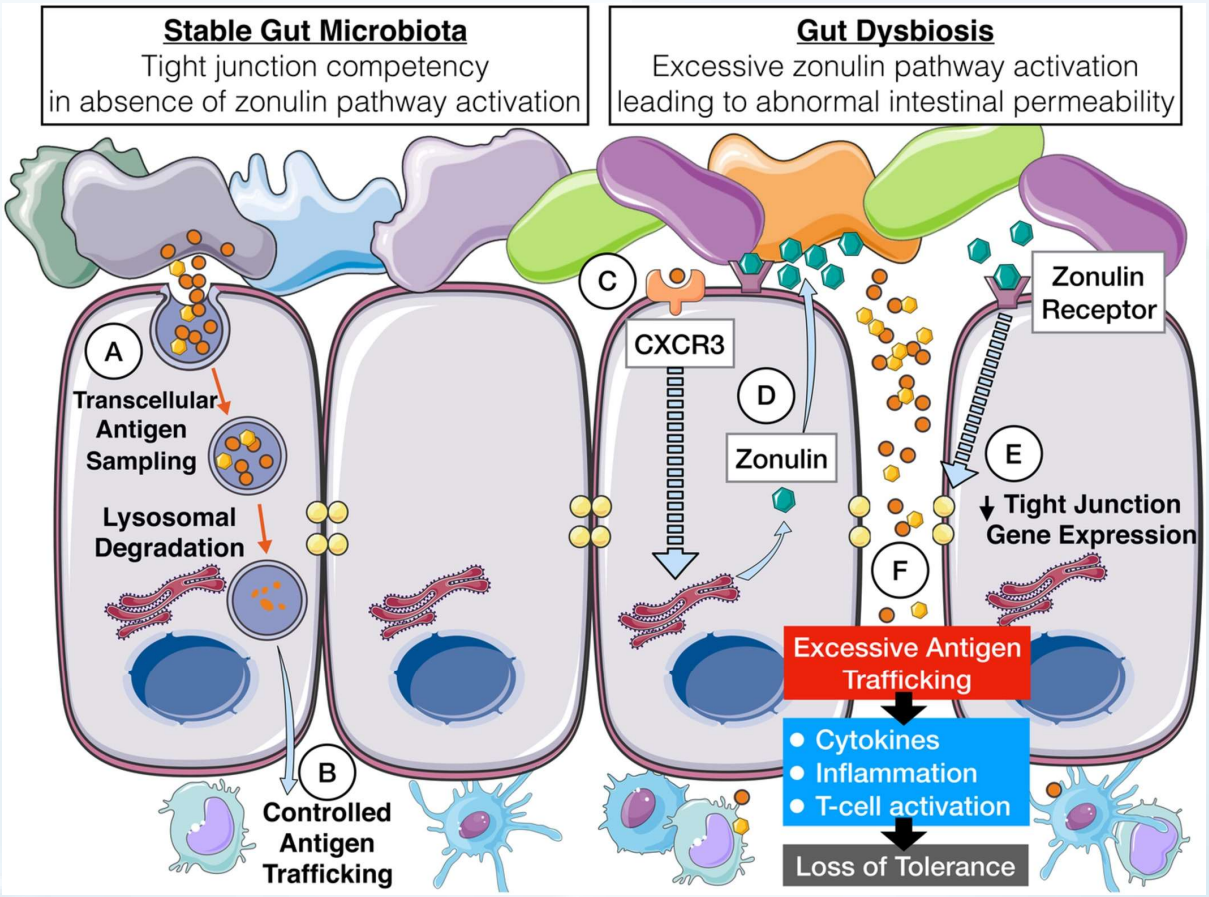
Heickman LKW, et al. Zonulin as a potential putative biomarker of risk for shared type 1 diabetes and celiac disease autoimmunity. Diab Metab Res Rev. 2020;36(5):e3309.

Zonulin

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

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

Zonulin and GUT Dysbiosis



Heickman W, et al. Diabet Metab Res Rev. 2020;36(5):e3309.

Metainflammatory Conditions Associated w/ Increased Zonulin

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

Disease	Model	References
Aging	Human	37, 38
Ankylosis spondylitis	Human	39
Attention deficit hyperactivity disorder	Human	40
Autism	Human	41, 42
Celiac disease	Human	15– 20, 23– 27, 43– 48
Chronic fatigue syndrome/myalgic encephalomyelitis	Human	49
Colitis – inflammatory bowel diseases	Human	50, 51
Colitis	Mouse	52
Environmental enteric dysfunction	Human	53
Gestational diabetes	Human	54, 55
Glioma	Human	56
Glioma	Cell	57
Insulin resistance	Human	58
Irritable bowel syndrome	Human	59, 60
Hyperlipidemia	Human	61
HIV	Human	62– 66
Major depressive disorders	Human	67, 68

Fasano A. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F10000 Res. 2020;9:10.12688/f1000research.20510.1

Zonulin and LPS levels in Centenarians

- Increased intestinal permeability is an important event leading to endotoxemia
- Gut microbiota lipopolysaccharide accelerates inflammaging
- Endotoxemia-induced inflammation has been associated with insulin resistance and atherosclerosis, ultimately increasing the risk of coronary heart disease
- 2018 study reported Disease-free centenarians had significantly lower levels of serum zonulin and lipopolysaccharide than young patients with acute myocardial infarction
  - They also had significantly lower concentrations of serum lipopolysaccharide than young healthy controls

Kim K, et al.. BMC Microbiol. 2016;16:9

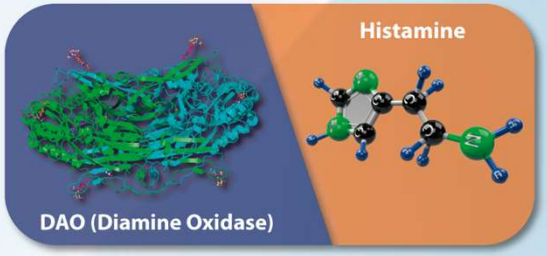
Zonulin

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

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

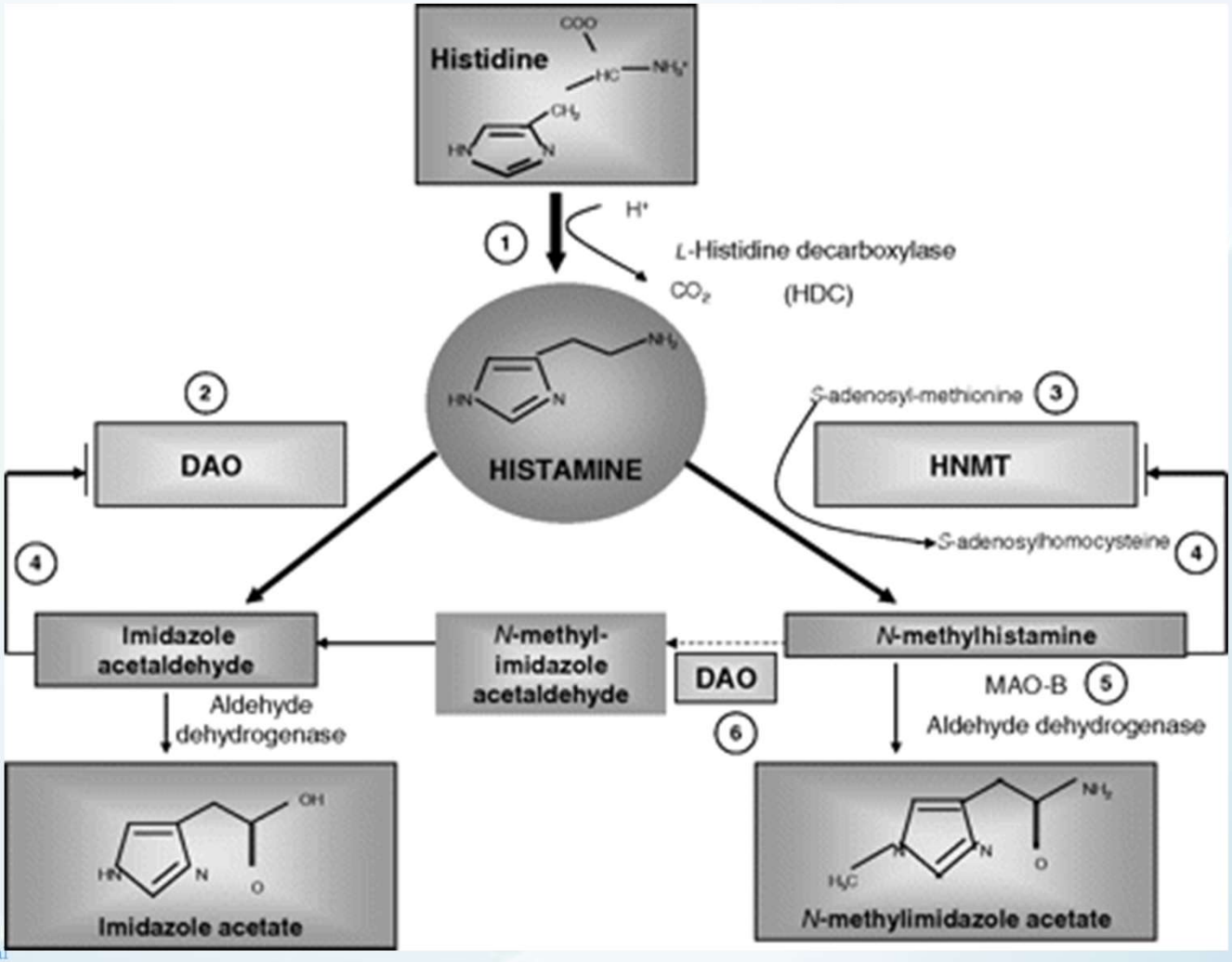
Histamine Testing

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



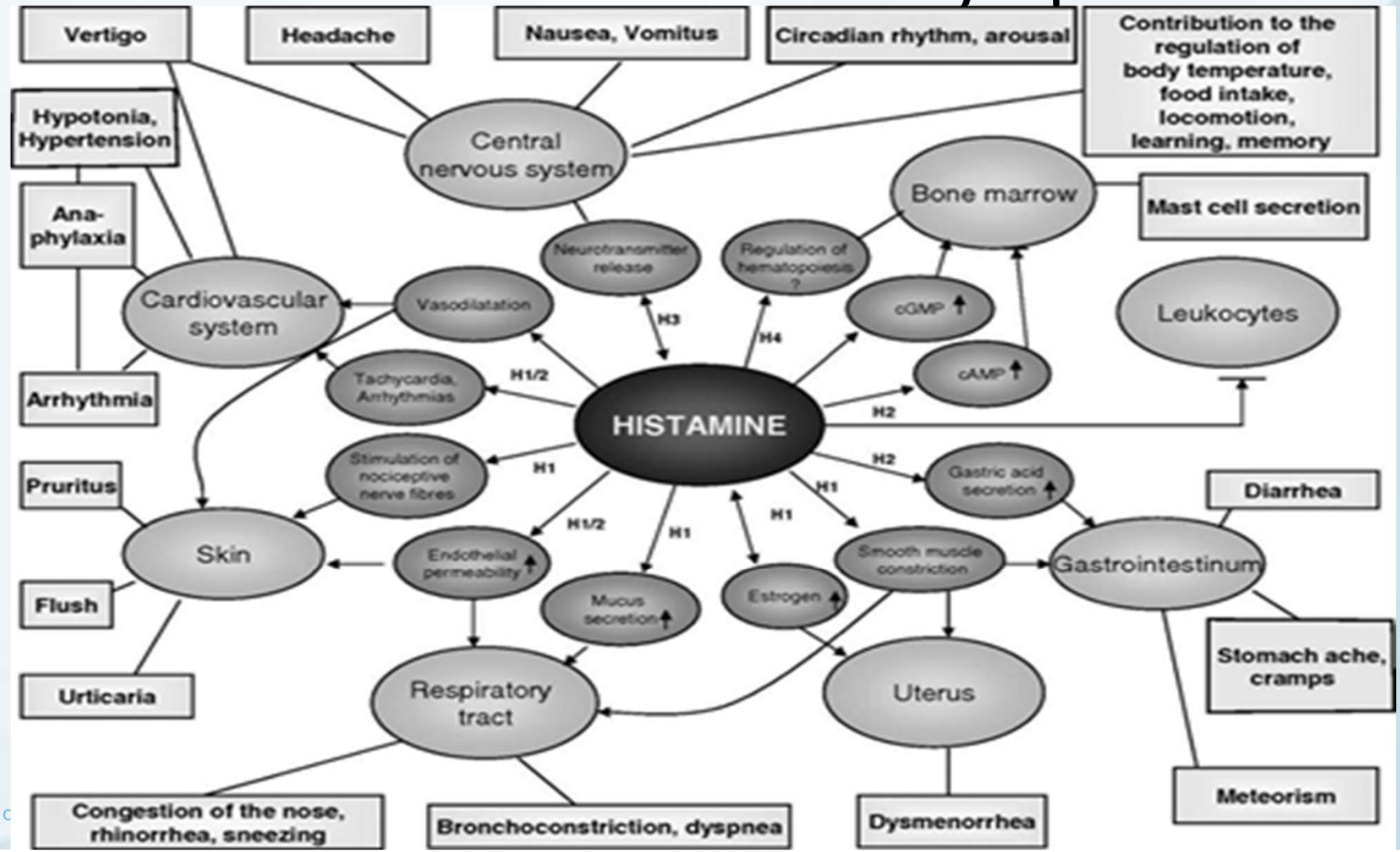
Maintz L, etla. Histamine and histamine intolerance. Am J Clin Nutr. 2007;85(5):1185-96.

Histamine Metabolism Review



Maintz L, Novak N. Am J Clin Nutr. 2007;85(5):1185-96.

Histamine Intolerance Symptoms



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

Histamine Testing

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

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

Histamine Testing

- Urinary histamine range – varies w/ sex, age

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

Foods Reported to Block DAO Enzyme

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

Support for Histamine Symptoms

- DAO Supplements
  - DAO from porcine kidney protein concentrate
  - 300mcg (10,000 HDU*) BID – 20,000 HDU daily

    *HDU = histamine digesting unit

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

Support for Histamine Symptoms

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

Galectin-3 (Gal-3)

- Gal-3 = pleiotropic protein
- Binds to B-galactoside residues in glycoproteins
- Mainly expressed and released in damaged brain by reactive microglia
- Interacts w/ immune receptors including TREM2 and TLR4

Siew JJ, et al. Neurosci. 2024;134(2):e165523.

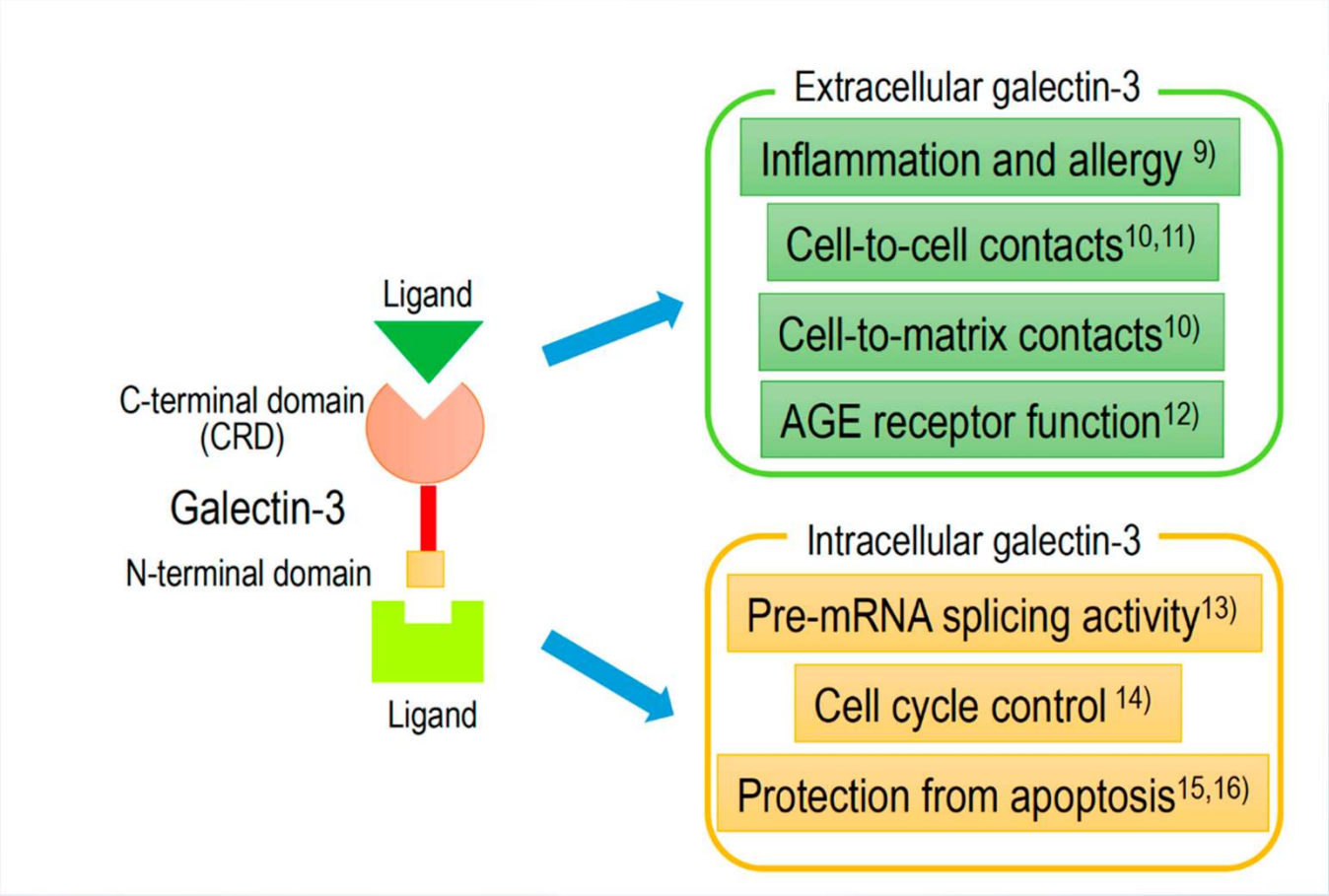
Galectin-3 (Gal-3)

- Plays role in :
  - Cell growth
  - Inflammation/neuroinflammation
  - Fibrosis – including heart failure progression
  - Involved in microglial activation in CNS
  - Expression of both M1 &M2 macrophages

- Levels may be increased in cancer and other conditions associated w/ organ fibrosis including cardiac failure, TBI, stroke, AD/dementias

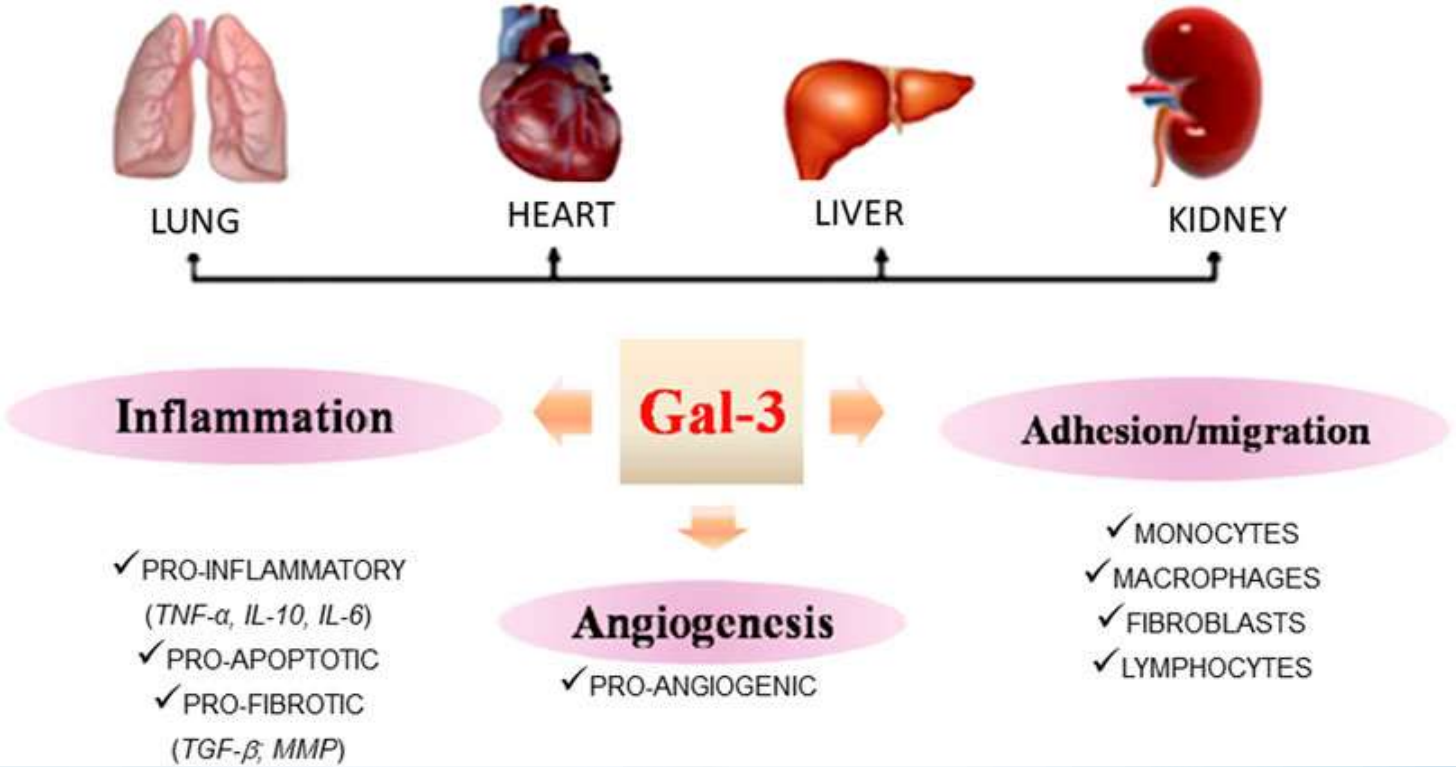
Siew JJ, et al. Neurosci. 2024;134(2):e165523.

Gal-3 Intra- and Extracellular Functions



Hara A, et al. Biomolecules. 2020;10:389.

Tissue Expression of Gal-3 and Role in Inflammation



Seropian IM, et al. Front Physiol. 2023;14:1304735.

Gal-3 - Diabetes

- Gal-3 levels elevated in chronic inflammatory diseases including obesity, diabetes and its complications
- Gal-3 levels significantly higher in T2D patients
- **Elevated Gal-3 levels associated w/ increased HbA1c, FPG and hsCRP**
- Study findings suggests Gal-3 may play a role in the progression of prediabetes stage to diabetes stage

Li Y, et al. Rev Endocr Metabol Disorders. 2022;23:569-77.

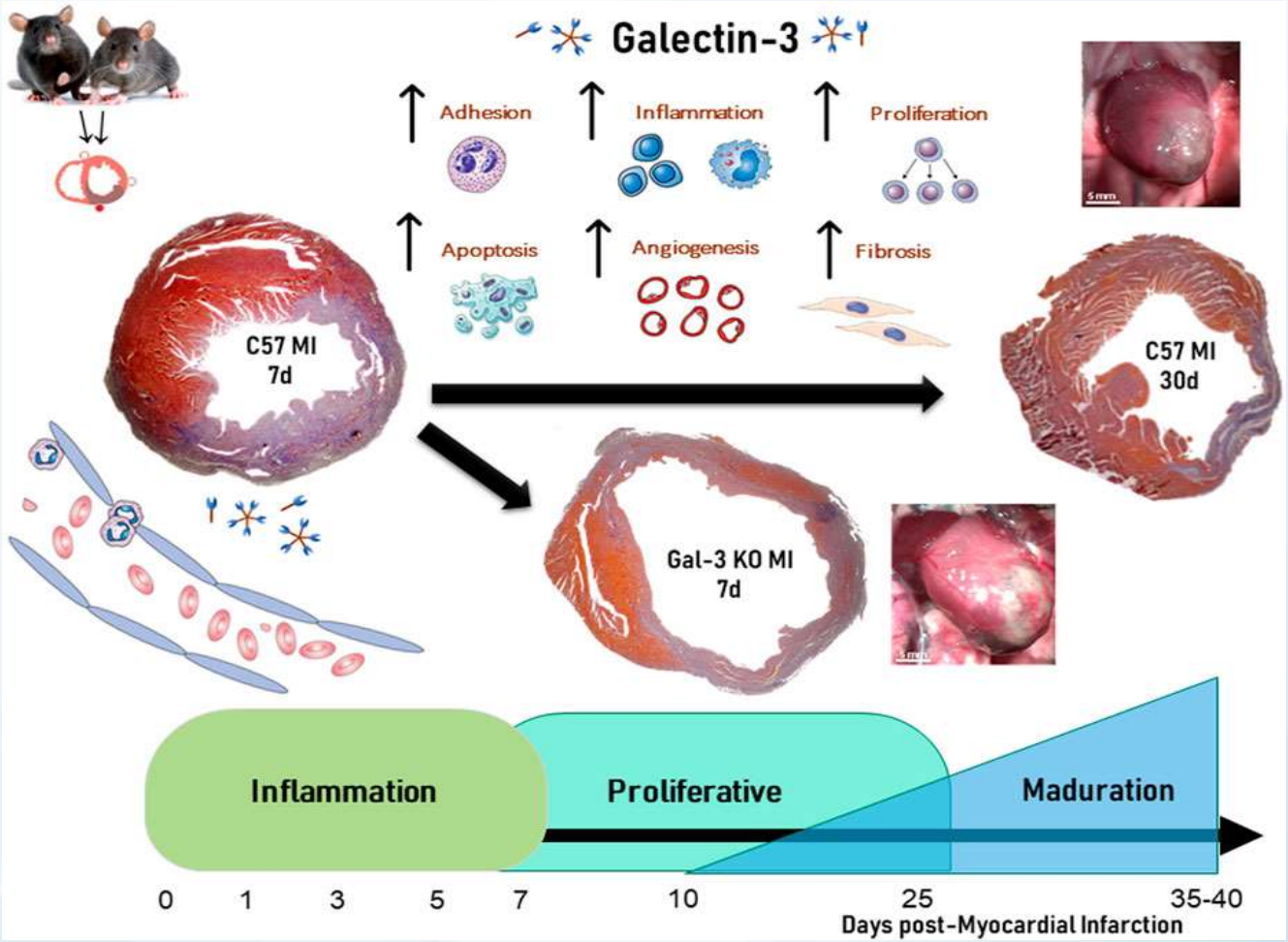
Gal-3 CVDs

- Gal-3 central player in development and progression of atherosclerosis and heart failure

- Promotes CV issues through multiple mechanisms:
  - Infiltration of inflammatory cells
  - Proliferation of fibroblasts
  - Cardiomyocyte hypertrophy
  - Collagen synthesis

Seropian IM, et al. Front Physiol. 2023;14:1304735.

Gal-3 CVDs



Key Concept

A doubling in galectin-3 level over the course of 6 months, irrespective of baseline value, identifies a high-risk patient in whom additional care management efforts and advanced therapies are warranted

McCullough PA, et al. Rev Cardiovasc Med. 2011;12(4):200-10.

Gal-3 Lab Testing

- Gal-3 lab range : < 22.2 ng/ml (LabCorp)
  - Levels ≤ 17.8 ng/ml – low risk of adverse outcomes
  - 17.8 – 22.2 ng/ml – moderate risk of adverse outcomes
  - > 22.2 ng/ml = high risk of adverse outcomes
- Studies report levels of Gal-3 > 25.9 predict patients likely to rapidly progress to heart failure

Siew JJ, et al. Neurosci. 2024;134(2):e165523.

Gal-3 COVID-19

- Severe COVID-19 associated w/ systemic hyperinflammation leading to acute respiratory distress syndrome, multi-organ failure and death
- 2022 study n= 156 patients Sars-CoV-2 positive
- Increased Gal-3 levels reported to correlate well between severe and non-severe outcomes
- Also correlated w/ other markers of COVID-19 severity (hs-CRP, NLR, D-dimer, neutrophil count)
- Authors concluded Gal-3 levels independent predictor of severe outcome

Cervantes-Alvarez E, et al. Sci Reports. 2022;12:1856.

Gal-3 Neurological Issues

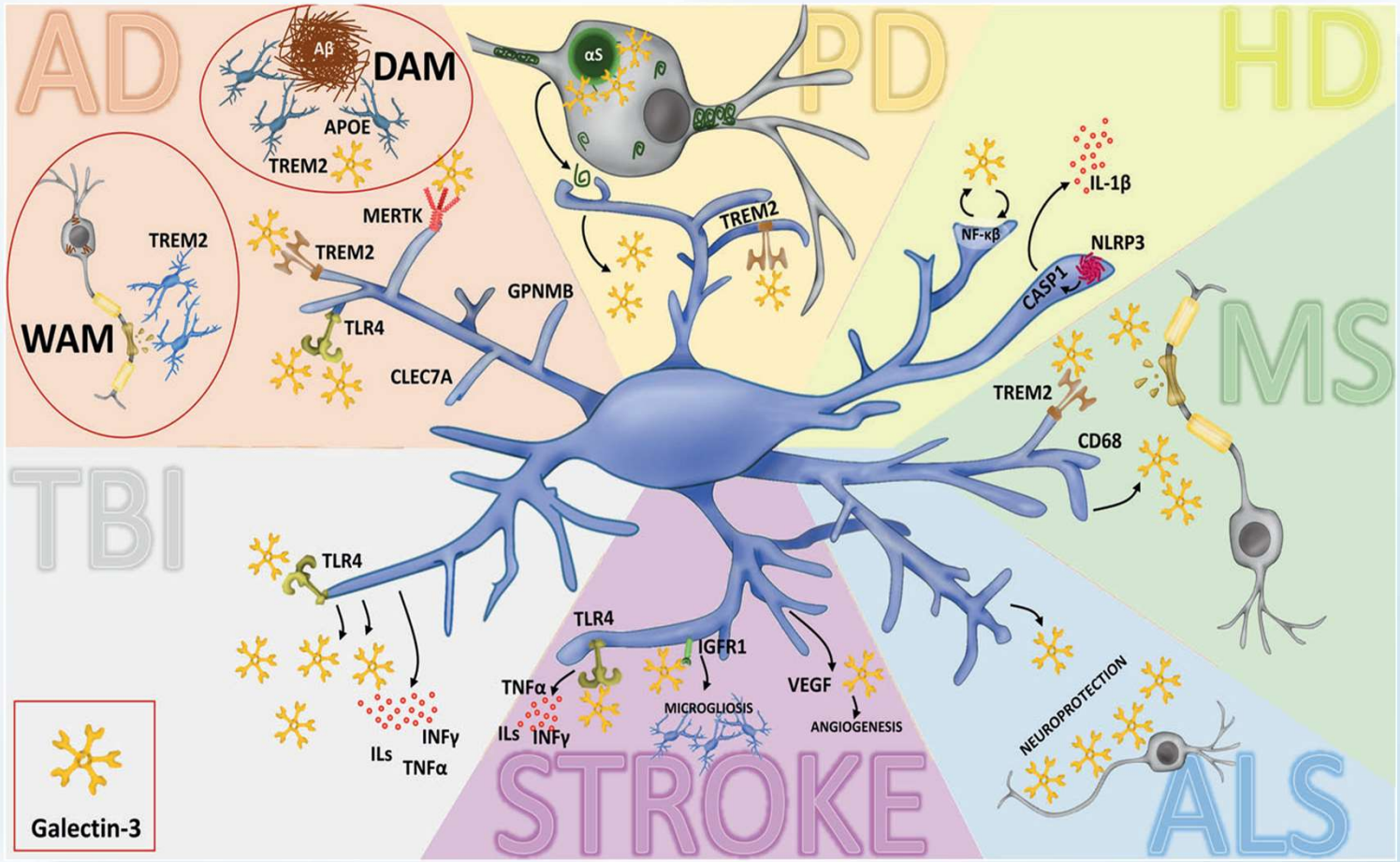
- Dysregulated microglial activation and altered homeostatic functions contribute to neurodegeneration

- Microglia express multiple types of lectins (e.g., galectins)
  - Serve as critical checkpoints for microglial activation
  - Contribute to neurodegeneration

- **Gal-3 is a proinflammatory autocrine mediator that can**:
  - Bind toll-like receptor 4 (TLR4)
  - And the receptor expressed on myeloid cells-2 (TREM2)
  - **KEY PLAYER in microglial mediated neuroinflammation**

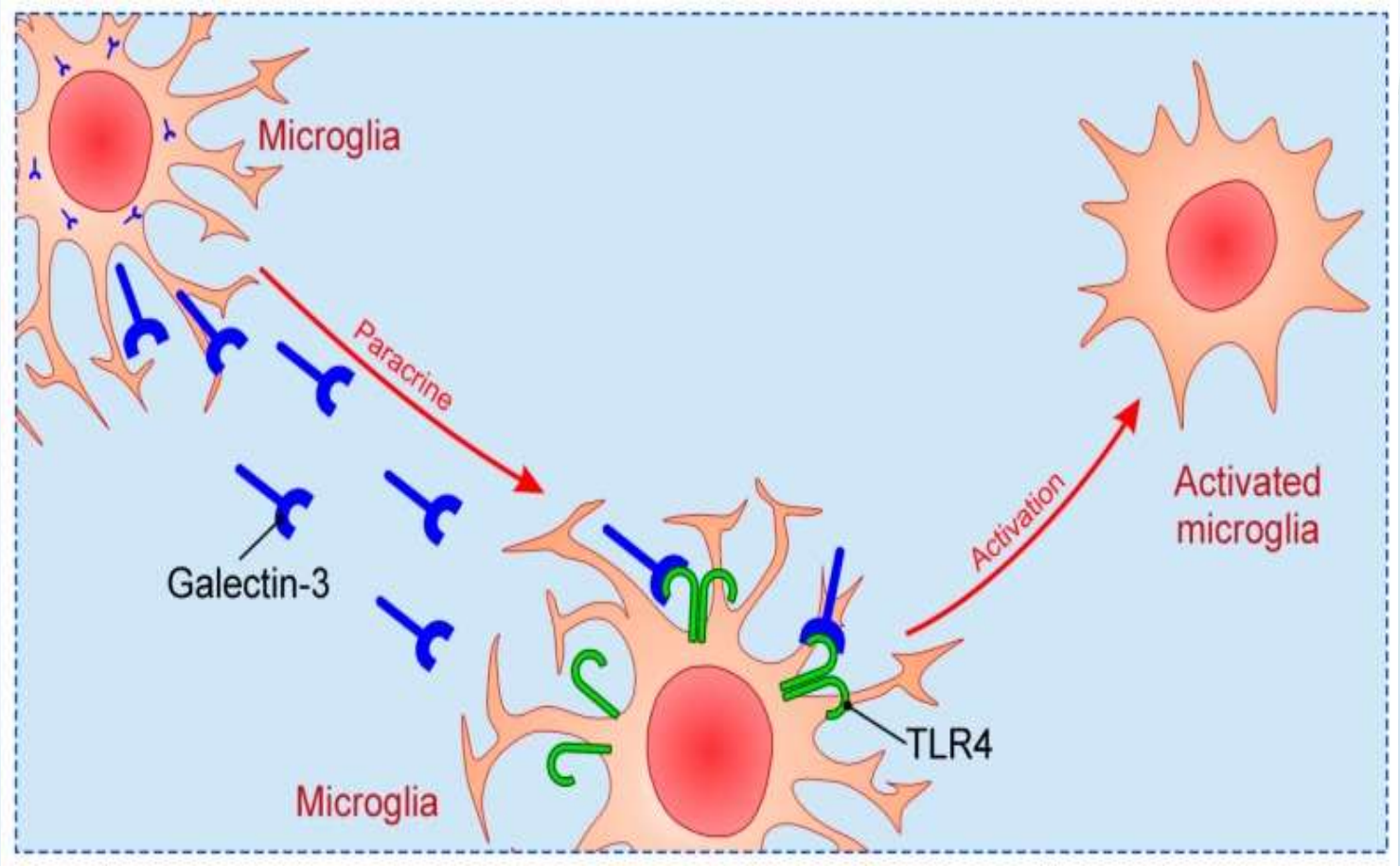
Weigand AJ, et al Brain Commun. 2020;**2**(1):fcz046.

Gal-3 in Microglia-Associated Neurodegenerative Diseases



Garcia-Revilla J, et al. Cell Death Dis. 2022;13:628.

Gal-3 Influence on Neuroinflammation



Srejovic I, et al. Glactin-3: Roles in Neurodevelopment, neuroinflammation, and behavior. Biomolec. 2020;10(5):798.

Gal-3 Dementia/Alzheimer's

- Large-scale proteomic analysis of brains in individuals with AD recently identified Gal-3 in a microglial metabolism module enriched with genetic risk factors of AD

- Alzheimer's disease - characterized by:
  - Accumulation of amyloid-β plaques,
  - Aggregation of hyperphosphorylated tau (pTau) - tauopathy
  - **Microglia activation and inflammation**

- **Gal-3 plays crucial deleterious role in microglial activation in Alzheimer's disease (AD) and other forms of dementias**

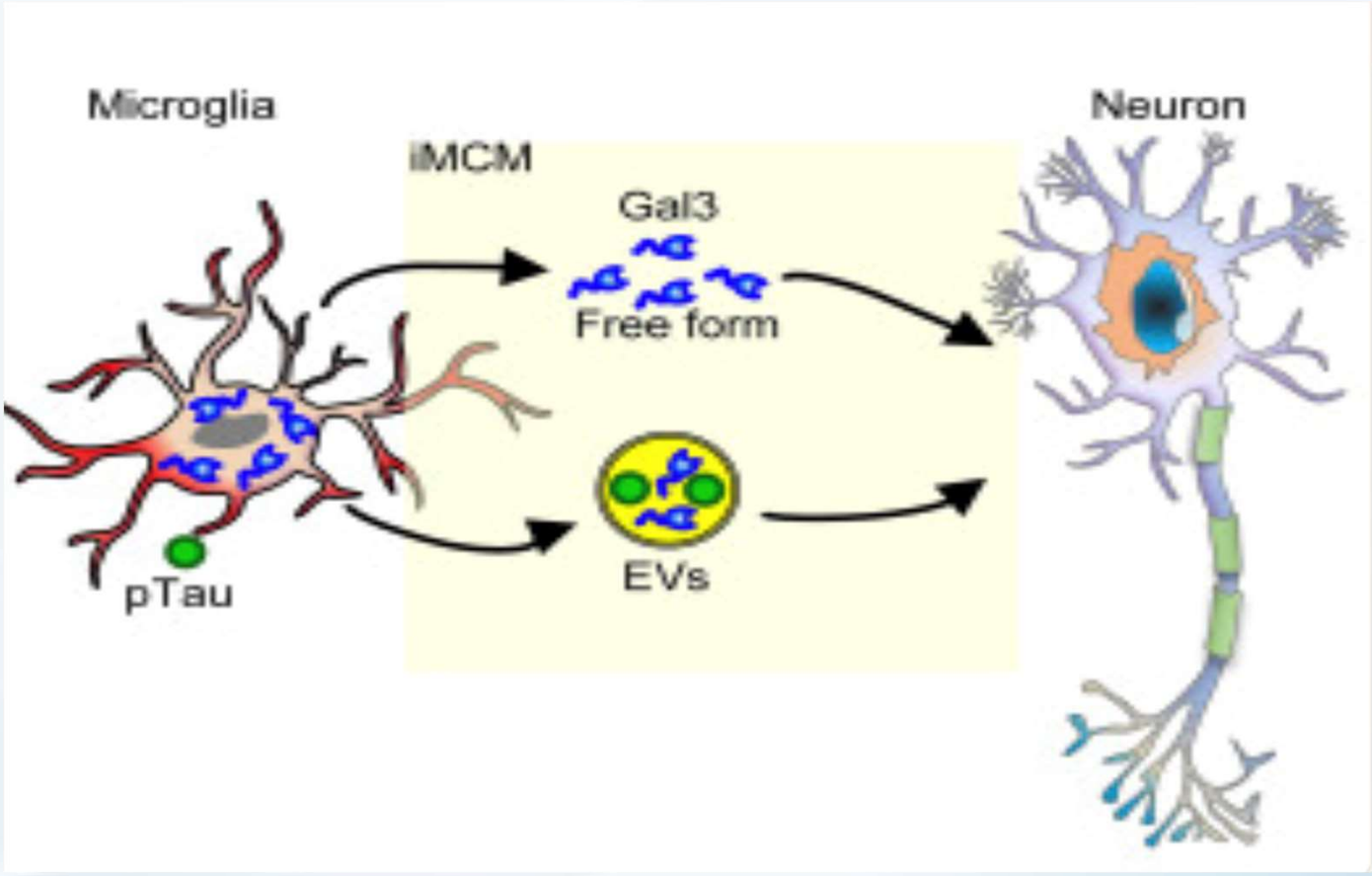
Siew JJ, et al. Neurosci. 2024;134(2):e165523.

Gal-3 Dementia/Alzheimer's

- Gal-3 upregulated in microglia with tauopathy
  - Both forms of GAL-3 upregulated - free and extracellular vesicular–associated (EV-associated) – see illustration next slide
- pTau reported to trigger release of Gal-3
- **Gal-3 suppression reported to reduce plaque burden and improve cognitive behaviors in lab animals**

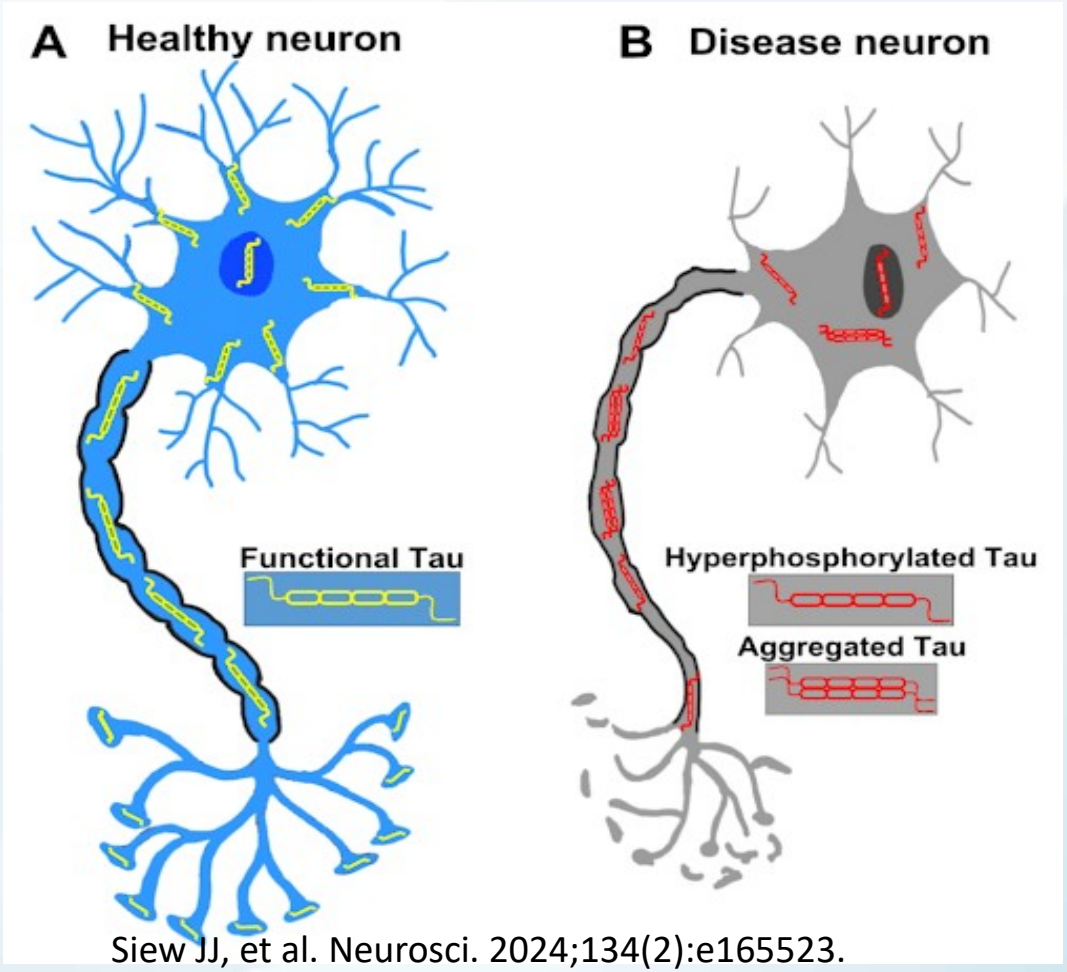
Siew JJ, et al. Neurosci. 2024;134(2):e165523.

Gal-3 in free form and in EVs exacerbates the effect of pathological tau



Siew JJ, et al. Neurosci. 2024;134(2):e165523.

Tauopathy and Effects on Neurons



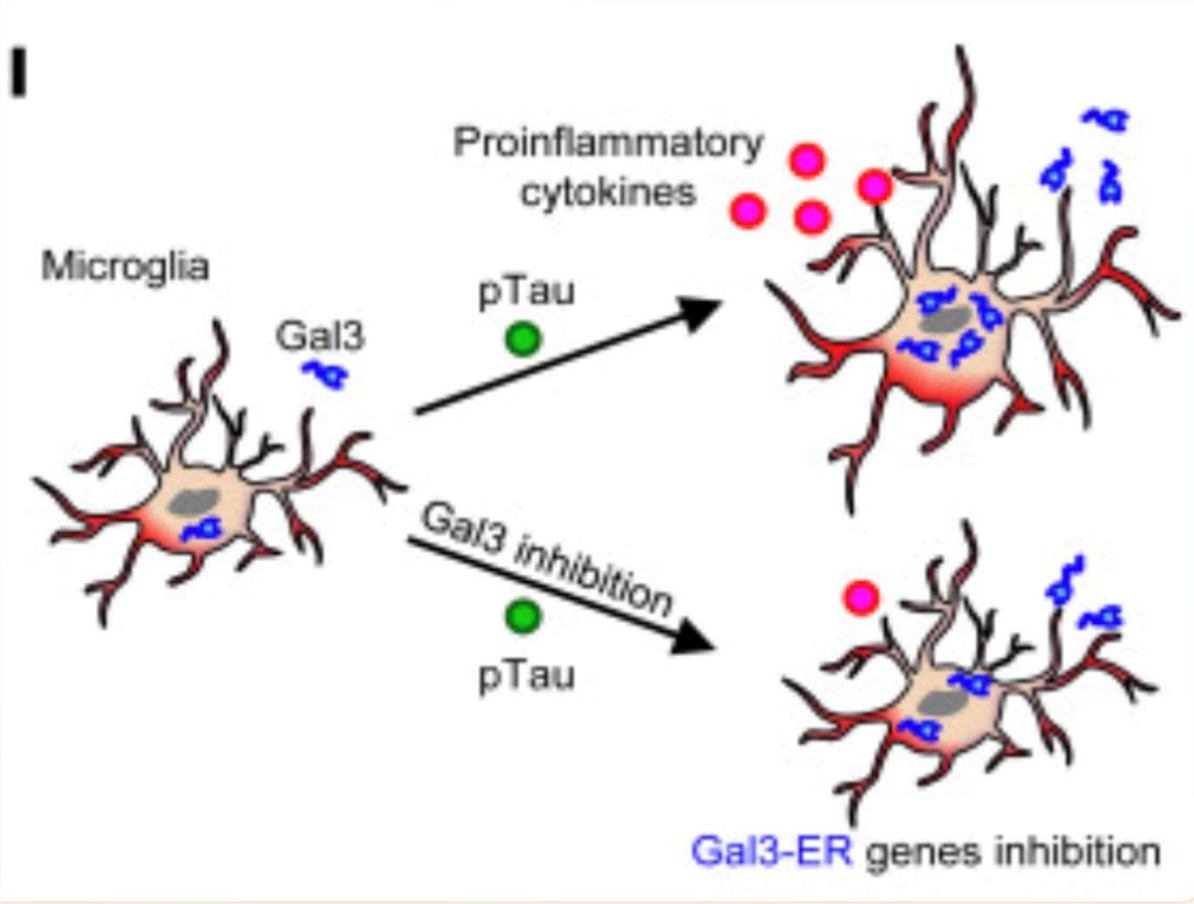
Siew JJ, et al. Neurosci. 2024;134(2):e165523.

Study

- A 2020 neuroimaging study of 513 participants demonstrated that individuals with both amyloid and tau pathologies exhibited a 27% prevalence of AD
- In contrast, those with only amyloid pathology had just 6% prevalence of AD
- Genetic and epigenetic studies of patients with AD reveal that many genes associated with AD risk are enriched in microglia and are related to immune responses

Weigand AJ, et al. Is tau in the absence of amyloid on the Alzheimer's continuum?: A study of discordant PET positivity. *Brain Commun.* 2020;**2**(1):fcz046.

Gal-3 Influence on Neuroinflammation



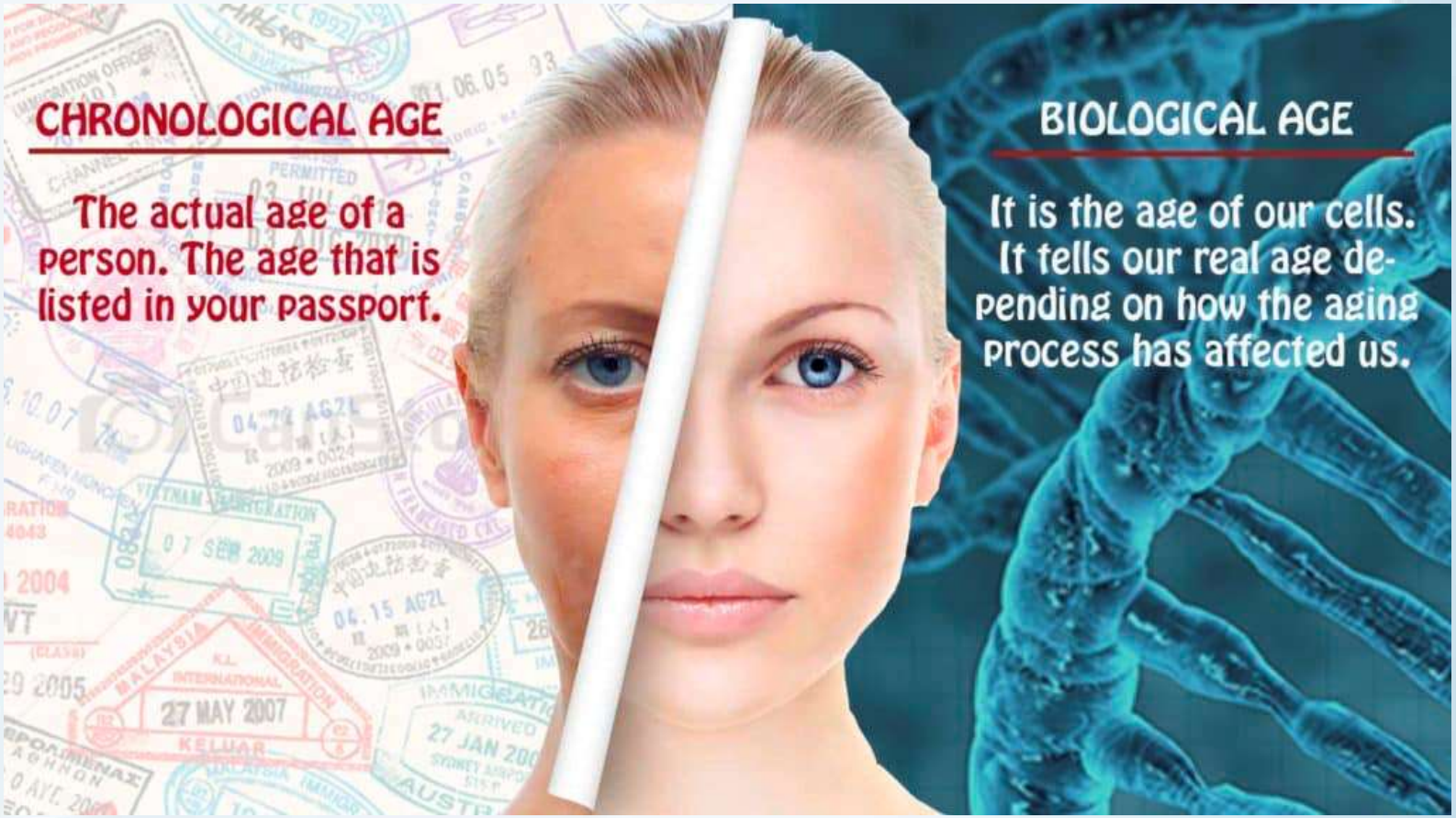
Siew JJ, et al. Neurosci. 2024;134(2):e165523.

GAL-3 Levels Correlate w/ Neurodegenerative Diseases

Authors, year	Disease	Sample	Galectin-3 levels	Main clinical findings
Ashraf and Baeesa, 2018	Alzheimer's disease	Serum CSF	↑	↓CF
Ashraf and Baeesa, 2018	Amyotrophic Lateral Sclerosis	Serum CSF	↑	No impact on CF
Yazar et al., 2021	Alzheimer's disease	Serum	↑	↓CF (↓MMSE) ↑Duration of disease
Wang et al., 2015	Alzheimer's disease	Serum	↑	↓CF (↓MMSE)
Cengiz et al., 2019	Parkinson's disease	Serum	↑	A good predictor for advanced-stage disease
Yazar et al., 2019	Idiopathic Parkinson's disease	Serum	↑	↑UPDRS scores ↑duration of disease
Siew et al., 2019	Huntington's disease	Serum	↑	↓CF (↓MMSE) ↑Disease burden Correlation with UHDRS scores
Yan J. et al., 2016	Amyotrophic Lateral Sclerosis	Serum	↑	↑Duration of disease
Zhou et al., 2010	Amyotrophic Lateral Sclerosis	CSF spinal cord tissue	↑	Disease biomarker
Wang et al., 2021	Acute ischemic stroke	Serum	↑	↓CF (↓MoCA)
Yan X.J. et al., 2016	Intracerebral hemorrhage	Serum	↑	↑Inflammation ↑Injury severity ↑Mortality
Liu et al., 2016	Subarachnoid hemorrhage	Serum	↑	↑Disease severity Poorer prognosis
Sävman et al., 2013	Birth asphyxia	CSF	↑	Severe clinical course poorer prognosis

CSF, cerebro-spinal fluid; CF, cognitive functions; MMSE, Mini Mental State Examination; UPDRS, Unified Parkinson's Disease Rating Scale; UHDRS, Unified Huntington's disease Rating Scale: MoCA. Montreal Cognitive Scale.

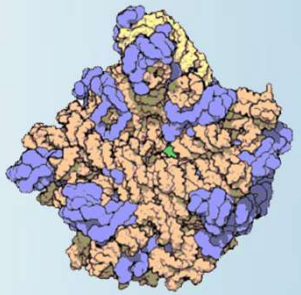
Biological vs. Chronological Age



How Do We Effectively Measure Aging?

8 Hallmarks of Biological Aging

- Genomic instability
- Telomere attrition
- Epigenetic alterations
- Loss of proteostasis
- Dysregulated nutrient sensing
- Mitochondrial dysfunction
- Cellular senescence
- Stem cell exhaustion
- Altered intercellular communication



Lopez-Otin C, et al. The Hallmarks of Aging. Cell. 20134;153(6):1194-1217.

How Can Biological Age be Determined?

- Biological age is determined by looking at the state of the **immune system** and the level of **inflammation/inflammaging**
- How? IgG glycans are targets
- Type of IgG glycans change the function of IgG from pro-inflammatory to anti-inflammatory and vice versa
- Both pro- and anti-inflammatory needed for a healthy immunity
- Trick is to balance inflammation – squelch metaflammation before it wreaks havoc on systems biology and affects biological aging

Kristic J, et al. Glycans are a novel biomarker of chronological and biological ages.*J. Gerontol. A Biol. Sci. Med. Sci.* 2014; **69**: 779-789

How Can Biological Age be Determined?

- Enzymatic glycosylation represents one of the most frequently occurring posttranslational modifications of proteins in the body
- Protein-linked sugar chains play a variety of highly specific roles
  - “Fine tune” interactions between cells and molecules
- Abberant glycosylation found in a variety of pathological conditions:
  - Next page….

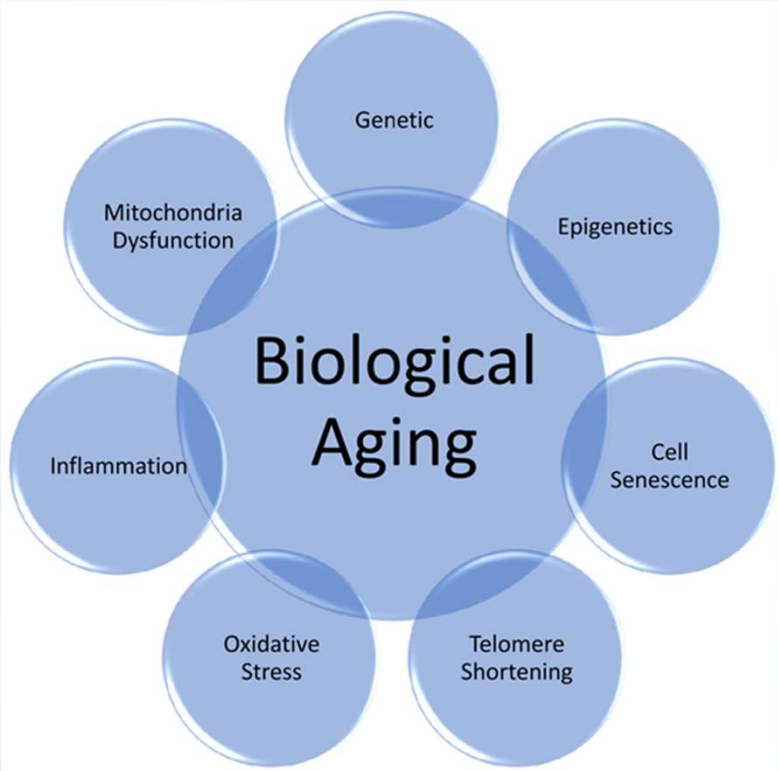
Kristic J, et al. Glycans are a novel biomarker of chronological and biological ages.*J. Gerontol. A Biol. Sci. Med. Sci.* 2014; **69**: 779-789

IgG Glycosylation – Links to Health Conditions in the Literature

- Increased aging
- Metaflammation
- Blood glucose/insulin regulation
- Cardiometabolic risks like dyslipidemia, HTN, atherosclerosis
- GUT microbiome disruption
- GUT issues – i.e. IBDs, *C. difficile* infections
- Weight gain
- Hormonal issues
- Kidney dysfunction
- Autoimmune conditions
- Altered immune function
- Allergic conditions
- Mental health issues
- Neurological issues – Parkinson's
- Poor performance and recovery
- COPD
- Pain issues
- Cancers

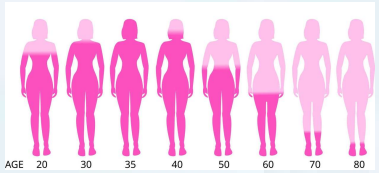
www.glycanage.com/publications/

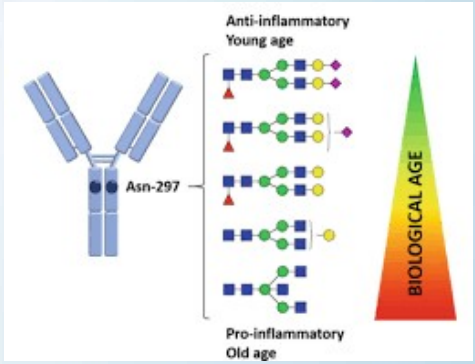
How Do We Effectively Test for Biological Aging ?



ENTER…Aging-Glycan Aging Testing

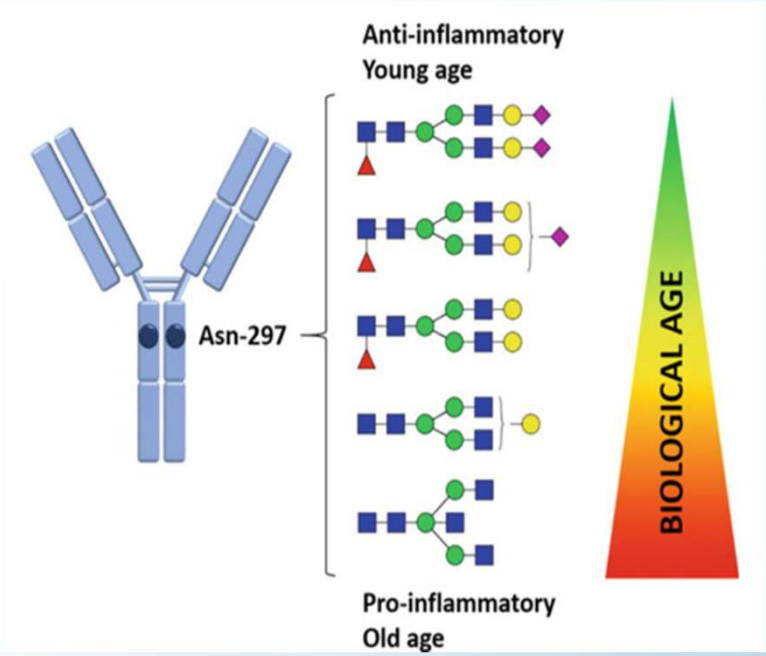
Can We Revert the Aging-Glycan Aging Clock of Biological Age and Stop Inflammaging?





A Comprehensive Biological Age Test – Aging-Glycan Aging

- Science-based testing
- Reveals age of the **immune system and degree of inflammation present**
- Biological age is NOT a fixed variable
- Useful in:
  - Anti-aging therapies
  - Lifestyle improvement
  - Improving resiliency
  - Measuring and improving fitness recovery

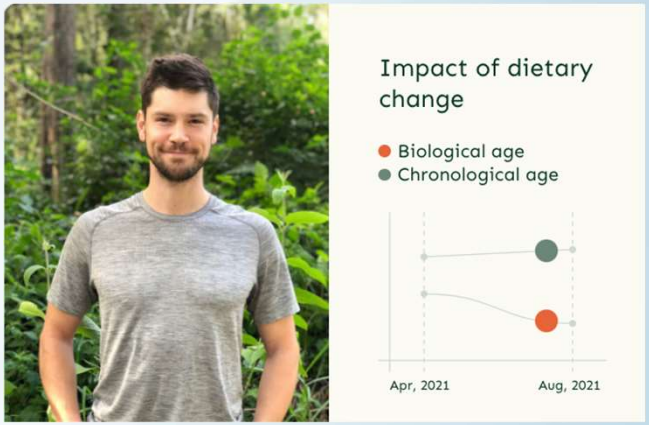


Aging-Glycan Aging Test Measures:

Ways to improve biological aging

- Impact of systems biology approach to aging
- Impact of dietary changes
- Impact of Exercise
  - Training and recovery impact
  - Impact of bodybuilding
- Impact of supplements – nutrients
- Impact of lifestyle changes
  - Stress control, job and home dynamics, sleeping habbits

Etc…



Can Biological Age be Improved?

- Case studies report biological age can be improved
- Getting someone's biological age to **equal or surpass** the chronological age means:
  - Systems biology in sync and functioning properly
  - Metainflammatory pathways improved
  - Metabolism creating adequate energy
  - Decrease Inflammaging
- How?
  - Next slide….

Can Biological Age be Improved?

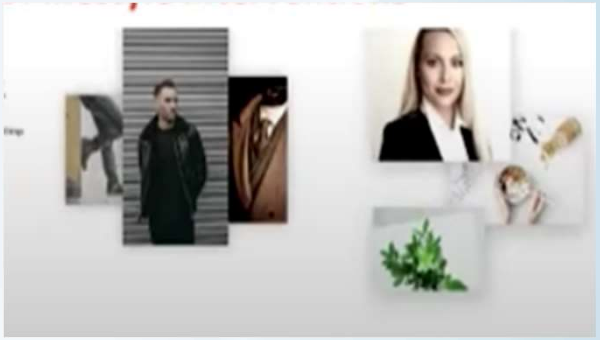
- How? A Systems Biology Approach
  - Stress control
  - Improved sleep patterns
  - Metabolic control – blood glucose/insulin, thyroid, weight
  - Hormonal balance
  - Working on the GUT
  - Detoxification improvement
  - Exercise regimen
  - DIETARY changes
  - Supplements to support systems biology

Follow-up Testing

- Appropriate lifestyle, supplements and nutritional changes should be implemented after Aging-Glycan aging testing
- **Generally**, it takes 3-6 months for changes to make differences in biological age

Aging-Glycan aging Testing Bottom Line

- Help motivate people to live healthier lives



- Characterizes current immune and inflammatory status

- Help support chronic diseases by adjusting systems biology issues

- **Lend insight into the current state of metaflammation and inflammaging**

- Evaluates effects of lifestyle interventions including diet, supplements and exercise

Thank You

Questions?
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