Triad 3:
Cardio- Pulmonary, Neurovascular Relationships an Expanded Model of Cardiometabolic Risk

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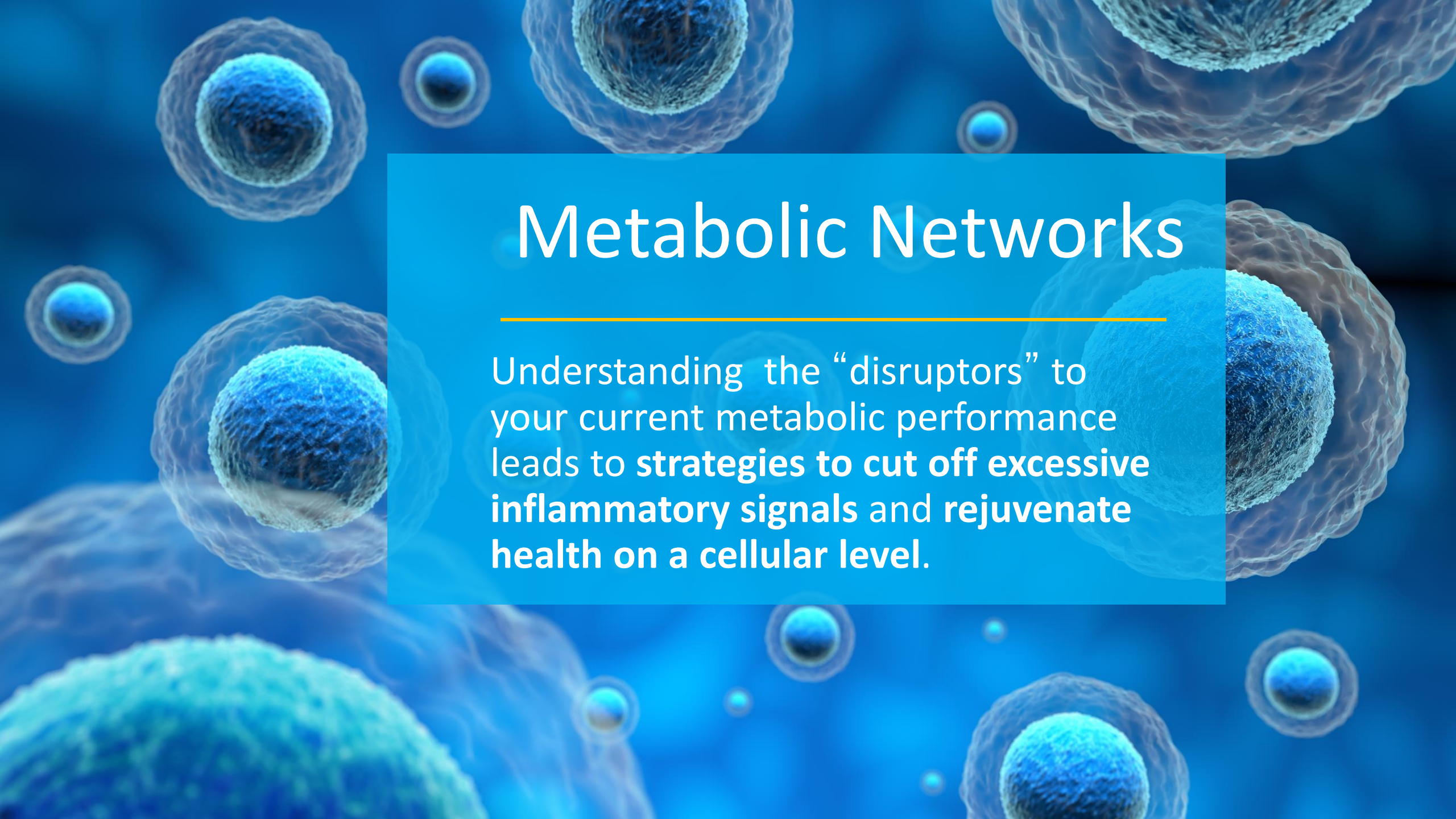
METABOLISM

The sum total of all the chemical reactions **driving how you feel today** and creating the chemistry **moving you toward future health**.

METABOLISM

Directly under the influence of Global Metabolic Inflammatory Signaling =

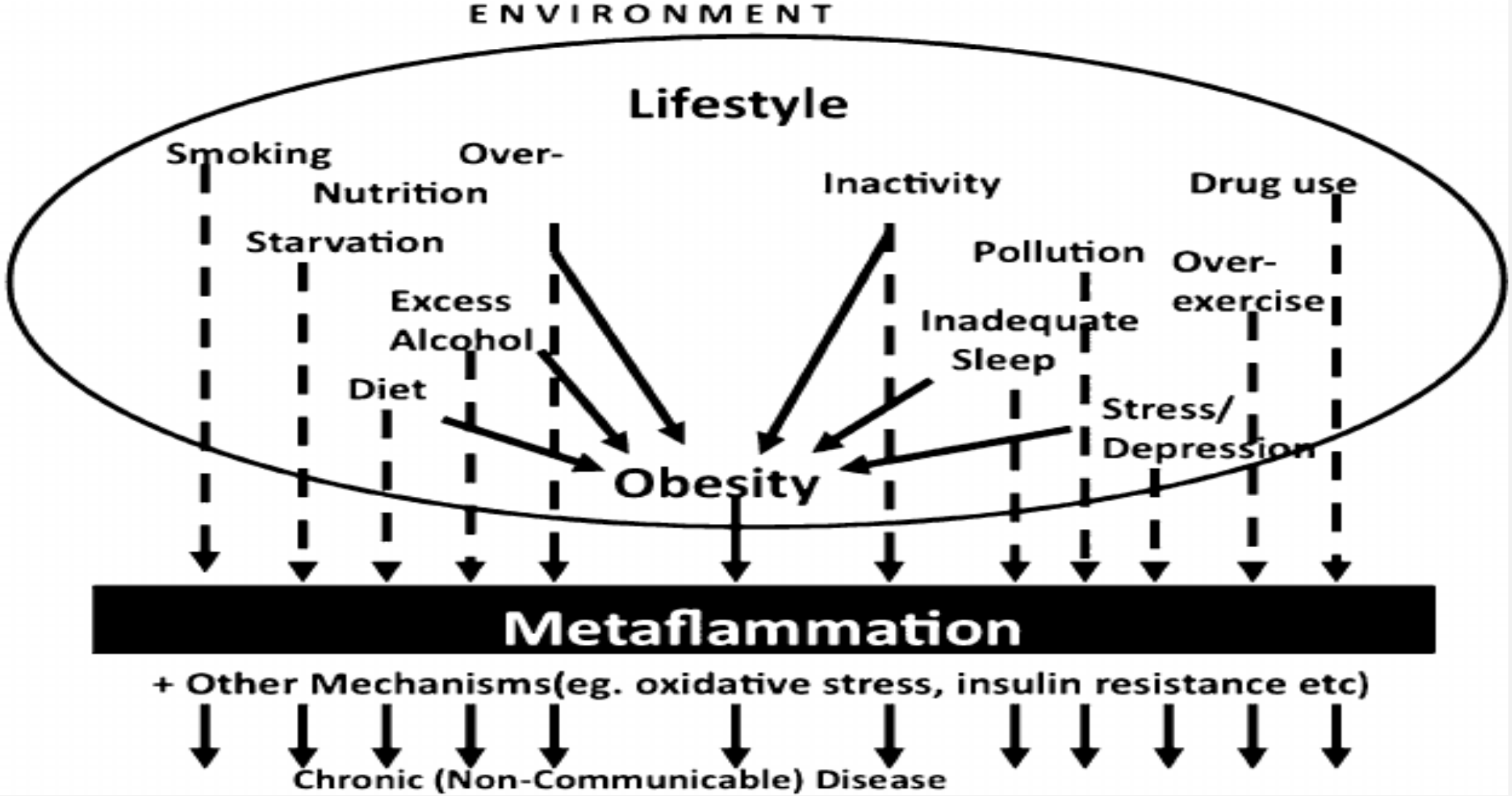
Metaflammation drives Metabolic Dysregulation



Metabolic Networks

Understanding the “disruptors” to your current metabolic performance leads to **strategies to cut off excessive inflammatory signals** and **rejuvenate health on a cellular level**.

Metaflammation Constructs

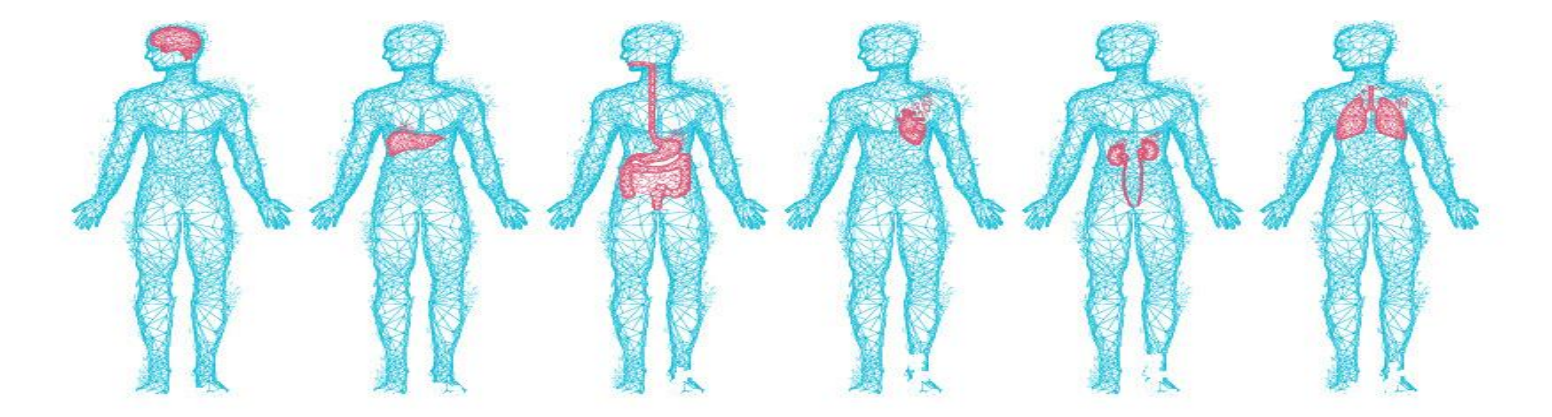


Egger G, et al. Obesity Reviews. 2008;10(2):237-49.

“Healthy Aging”

- Takes a systems biology approach to treatments
- Tones functional interconnectivity of organ systems

  KEY = controlling **Metaflammation**

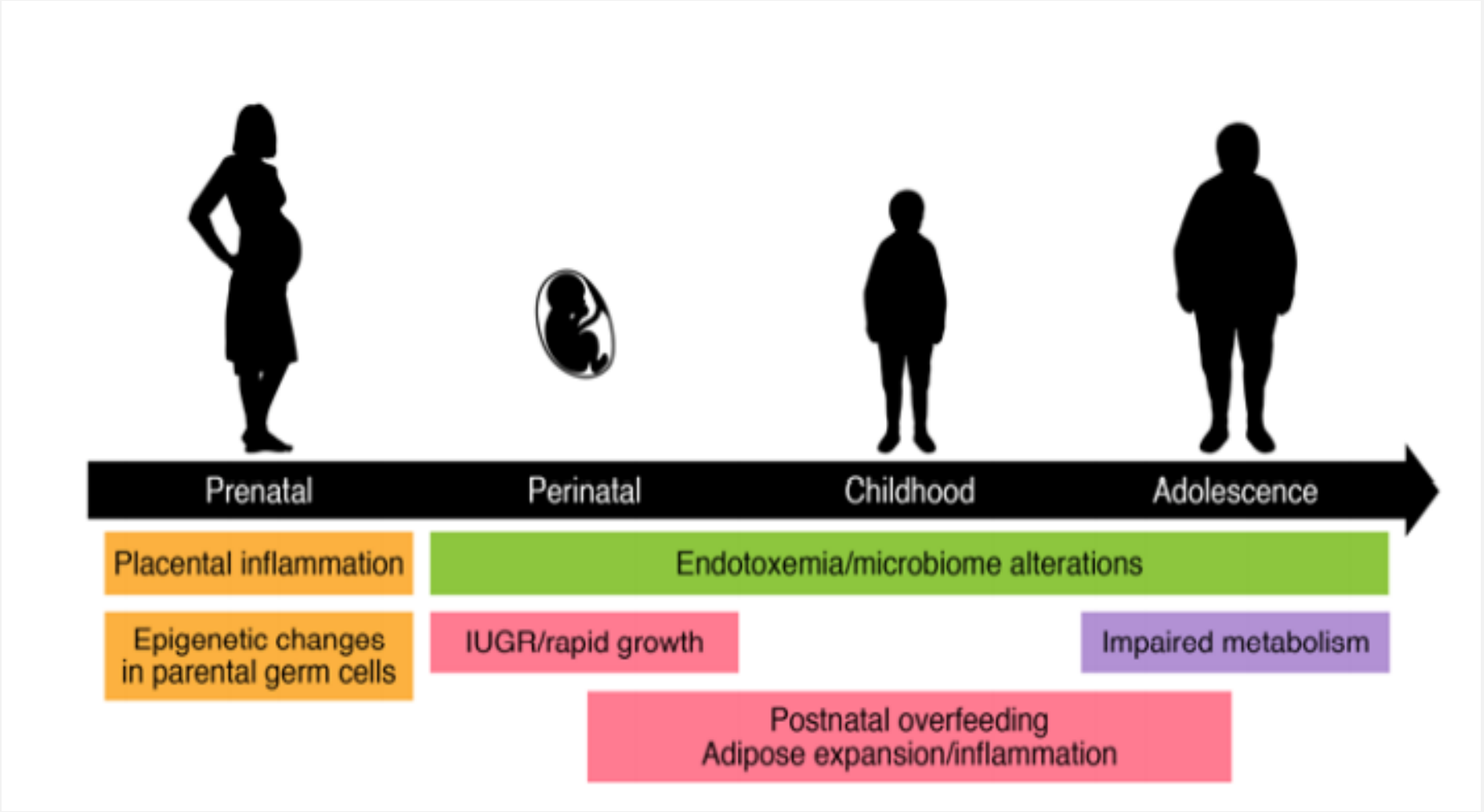


Metaflammation Contributors

- STRESS
- Caused by AND leads to “diabesity”:
  - Insulin resistance; type 2 diabetes
  - Obesity
  - Stress
  - Diet
  - LPS induced
  - Liver / kidney issues
- GUT microbiome issues – Leaky GUT

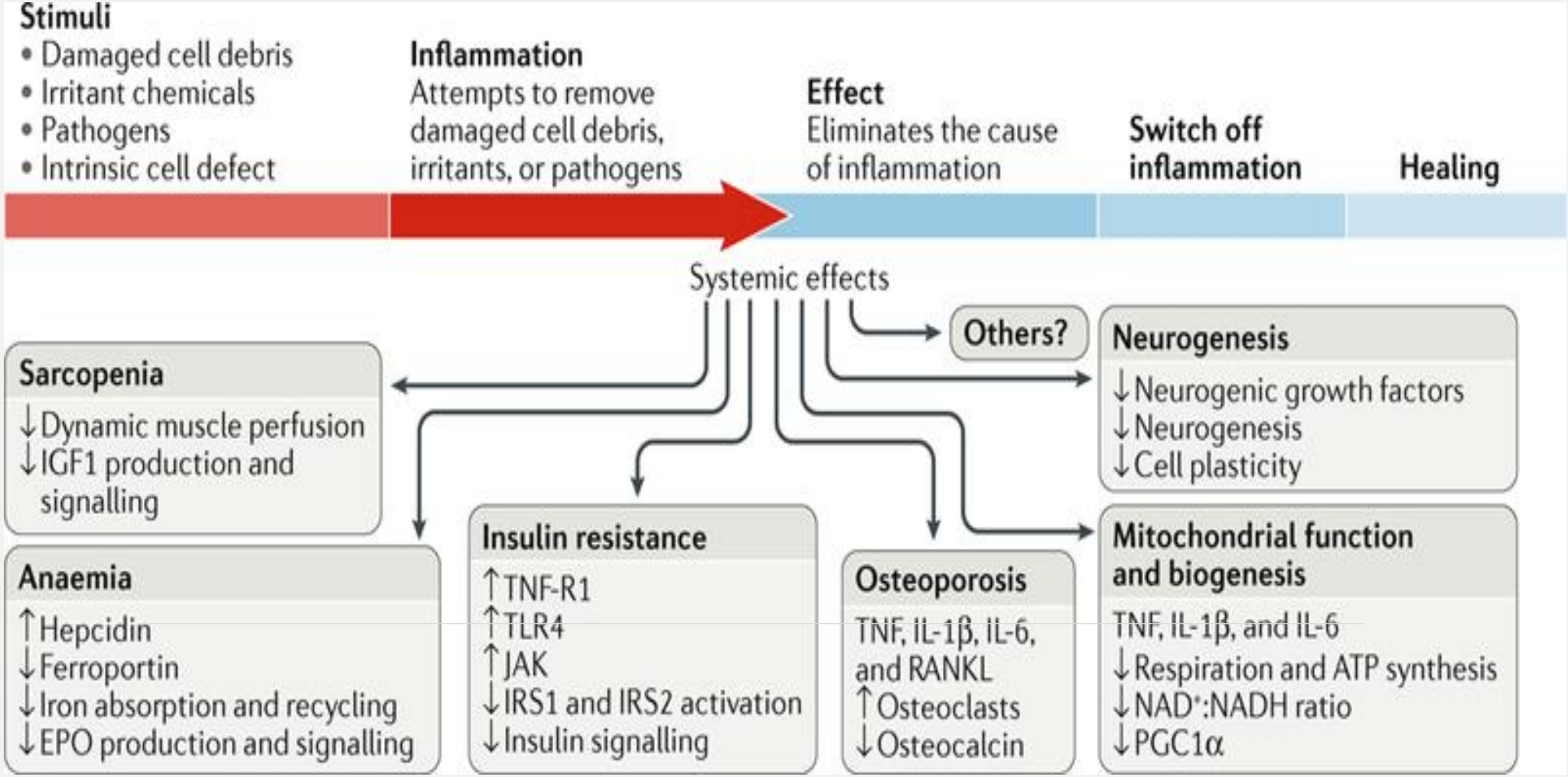
Prattichizzo F, et al. Inflammageing and metaflammation: the yin and yang of type 2 diabetes. Ageing Res Rev. 2018;41:1-17.

Metaflammation Constructs Begin in the Womb and Can Follow the Individual Through Life – Unless the Cycle is Broken



Kanakadurga S, et al. The initiation of metabolic inflammation in childhood obesity. J Clin Investig 2017;127(1):65-73.

Metaflammation Induces Catabolic State



Ferruci L, et al. Inflammageing: chronic inflammation in ageing, cardiovascular disease and frailty. Nat Rev Cardiol. 2018;15(9):505-22.

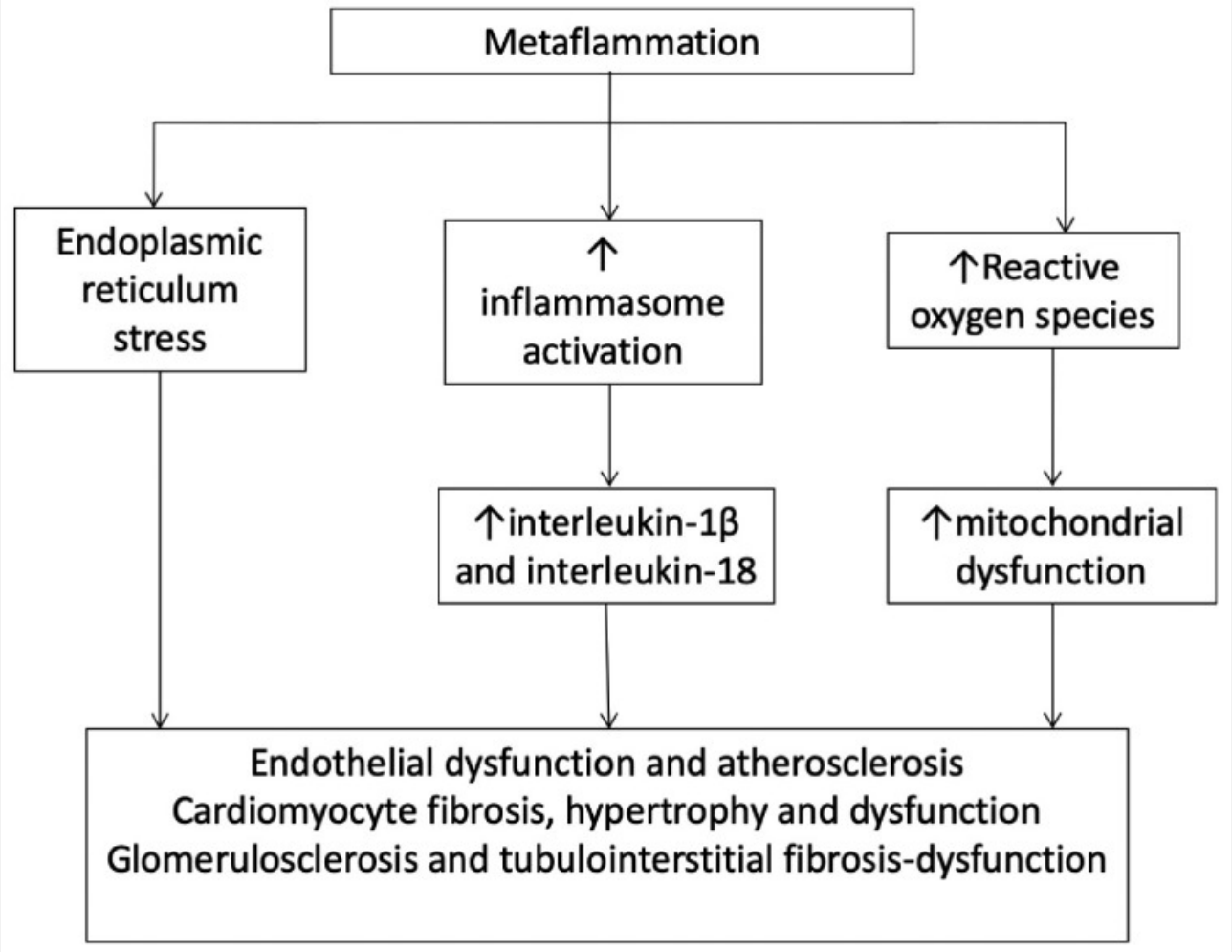
Metaflammation

Results in co-morbid conditions:

- Altered methylation patterns
- Cardiovascular issues – lipid, vascular
- Hormonal imbalances
- Liver and kidney diseases
- Immune dysfunction
- Thyroid, fatigue
- Sleep problems
- Cognitive and mood problems
- Sarcopenia
- Osteoporosis
- Cancer

Prattichizzo F, et al. Inflammageing and metaflammation: the yin and yang of type 2 diabetes. Ageing Res Rev. 2018;41:1-17.

Metaflammation and the Cardiovascular System

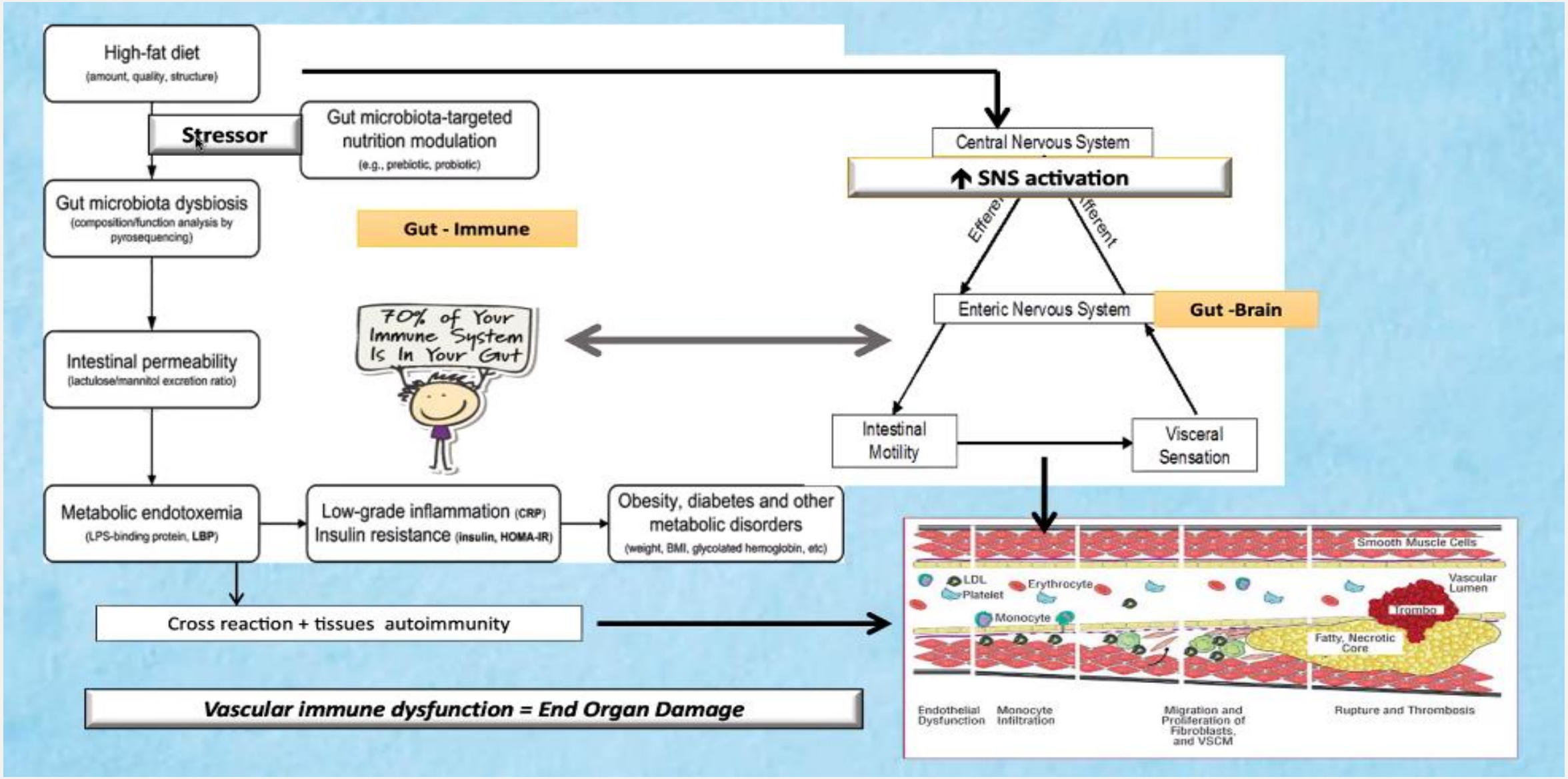


Kanbay M, et al. A journey from microenvironment to macroenvironment: the role of metaflammation and epigenetic changes in cardiorenal disease. Clin Kidney. 2019;12(6):861-70.

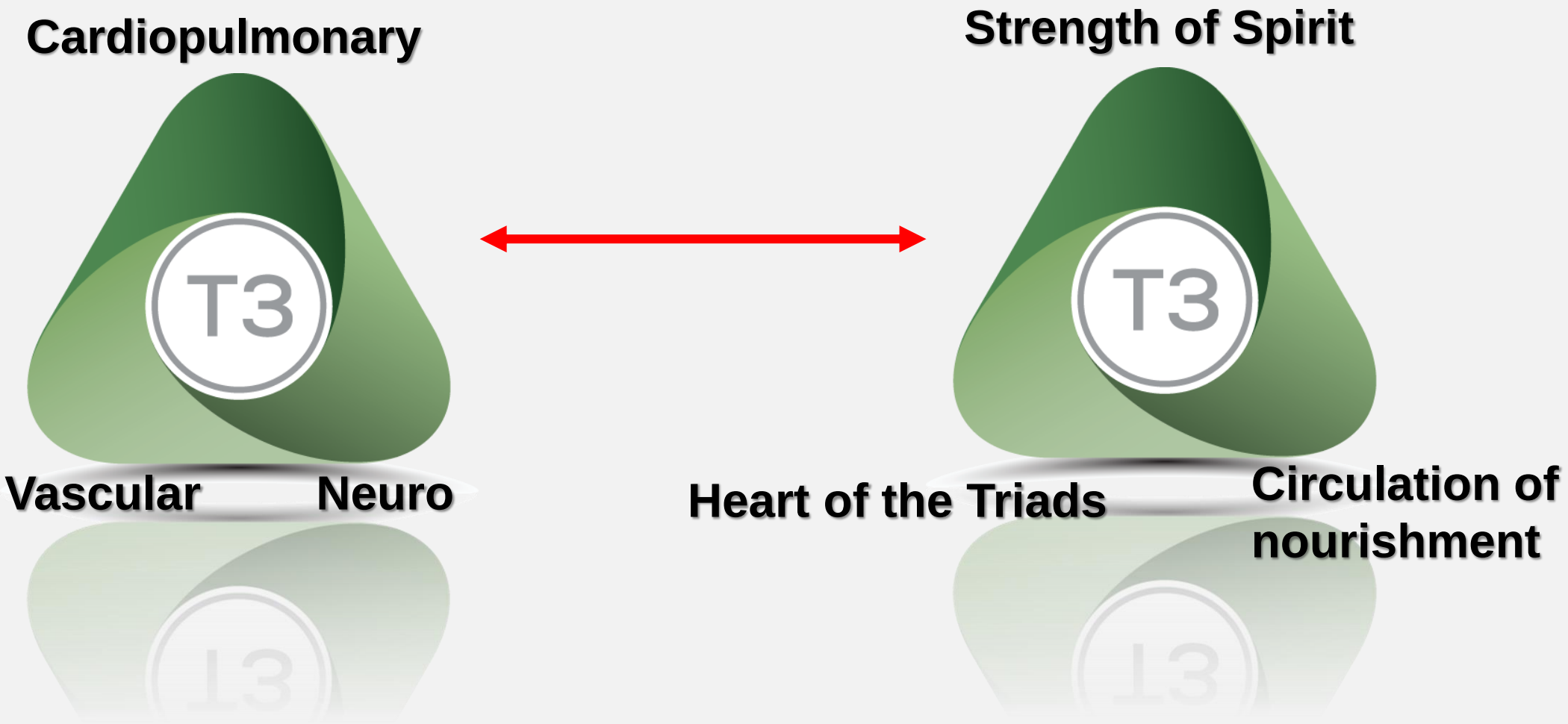
TRIAD 3

Cardio-Pulmonary-Neurovascular

Start With End In Mind



T3


Cardiopulmonary
Strength of Spirit
T3
T3
Vascular
Neuro
Heart of the Triads
Circulation of nourishment

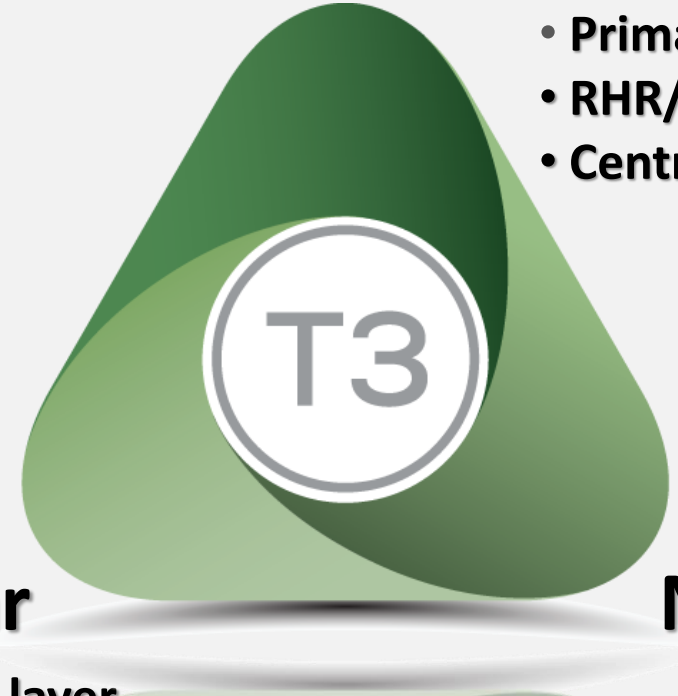
TRIAD 3
Cardiovascular – Neurovascular – Pulmonary

- Heart and blood vessels
- Respiratory system
- Neurovascular system
- Methylation support
- Bone/connective tissue also part of T3

T3

Cardiopulmonary

- Primary pump function
- RHR/RSA and HRV
- Central driver of physiology



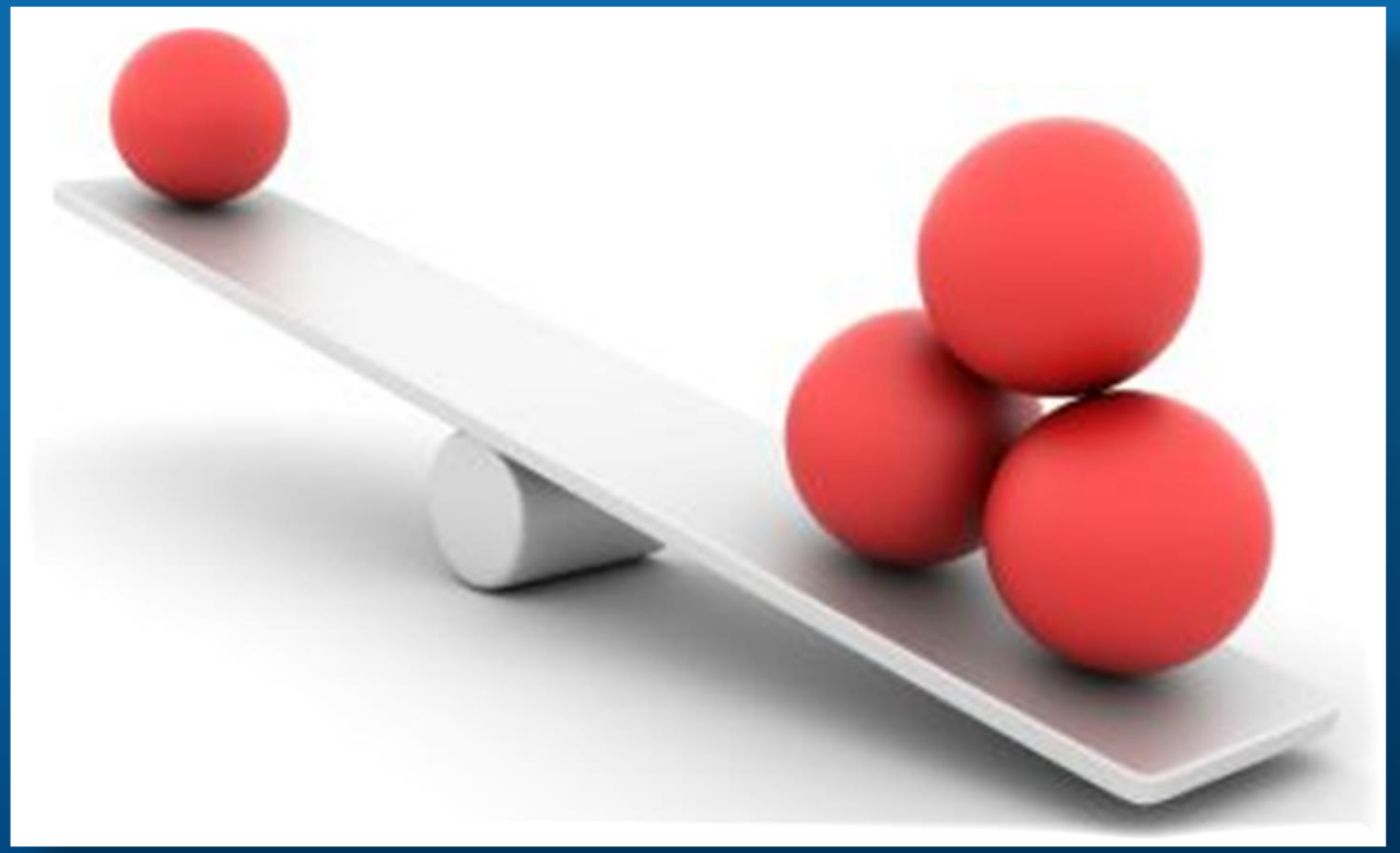
Vascular

- Single endothelial layer
- Architecture of circulation
- Major biological interface

Neuro

- SNS/PNS activity
- MTHFR, APO E
- Mind Heart Connection

T3 Imbalances



T3 Imbalance: Symptoms

Cardiopulmonary

- SOB, DOE
- Chest Pain → ACS/AMI
- Fatigue +/- exertional
- Resting tachycardia
- Edema
- Snoring

Vascular

- Leg pain
- Cold extremities
- Headaches
- Flushing

Neuro

- Depression +/- cognitive decline
- Stress, fatigue, anxiety
- TIA, CVA

T3 Imbalance: Lab Findings

Cardiopulmonary

- Abnl echo: Systolic and/or Diastolic fn
- Abnl sleep study: OSA
- Abnl ambulatory BP finding: dipping status
- Abnl non-invasive eval → Revascularization

Vascular

- HTN, HLD, ABI's, CAC, CIMT
- ↑ HS-CRP, ↑ Homocysteine
- ↑ Fibrinogen Ag, ↑ LP-PLA2
- ↑ MPO, ↑ OX-LDL
- Microalbuminuria, abnl EndoPat

Neuro

- APO E: 4/4, (+)(+) MTHFR
- Abnl neurotransmitters
- Abnl CIMT
- Abnl CTA, Angio: Revasc

T3 Imbalances

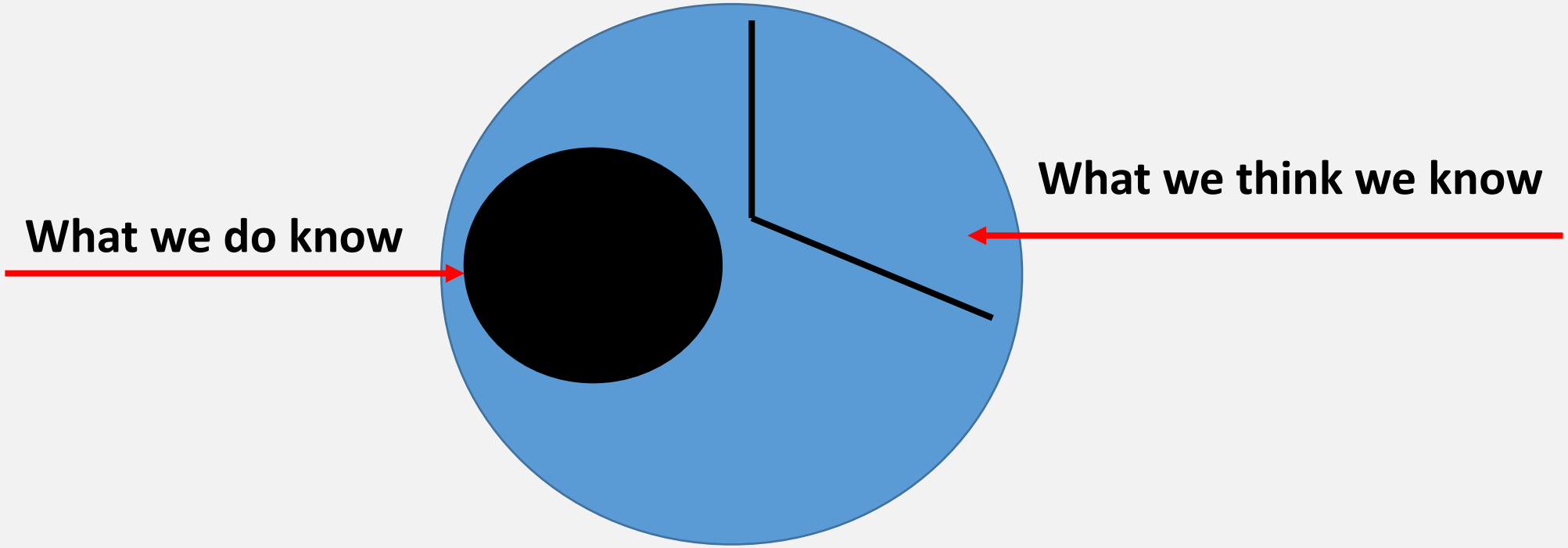
- Blood pressure problems
- Dyslipidemia and lipid imbalances
- Changes in heart rate; palpitations, tachycardia
- Loss of heart rate variability and autonomic tone
- Fatigue; shortness of breath
- Loss of stamina and endurance
- Erectile dysfunction
- Bone/connective tissue in TRIAD 3

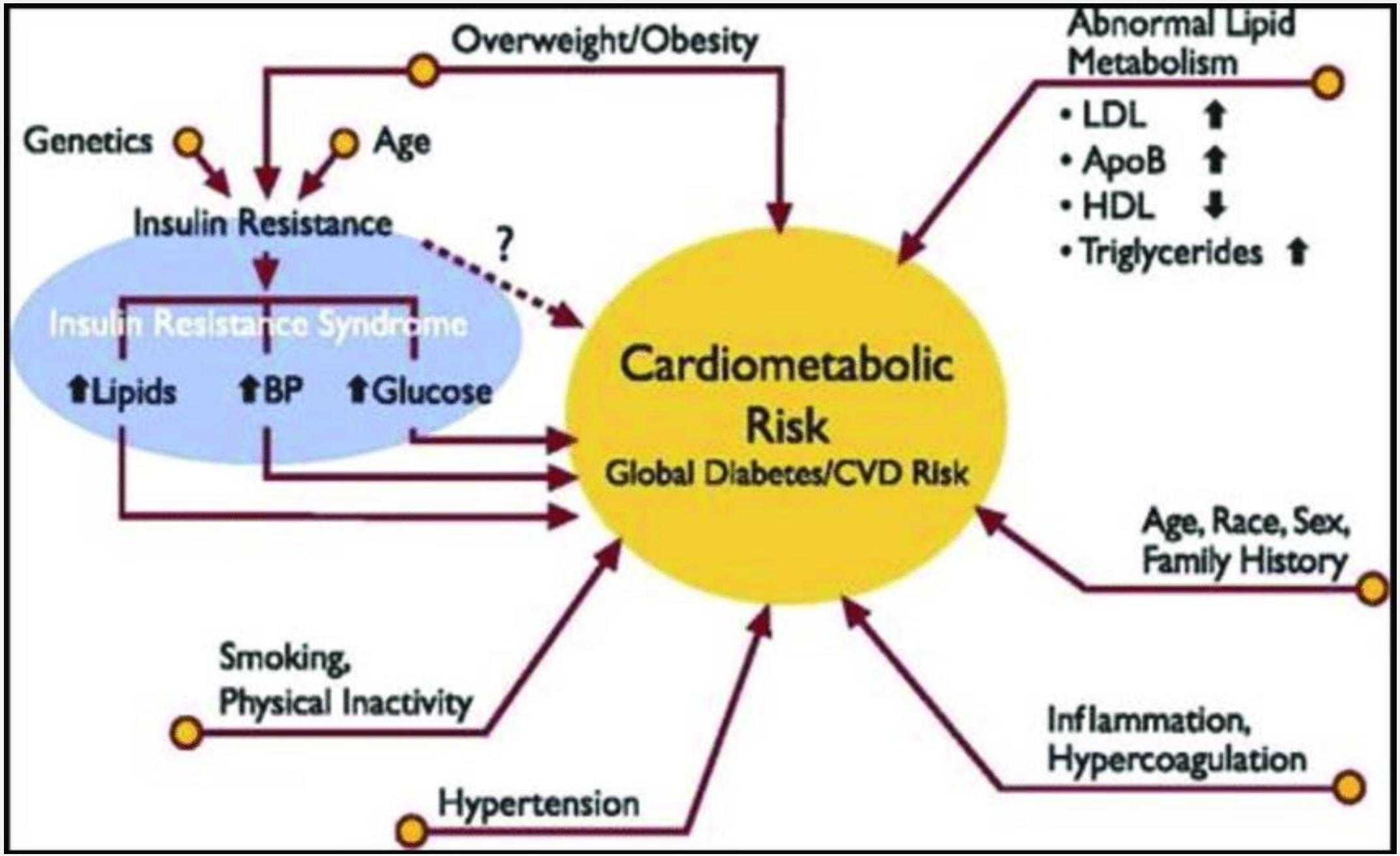
Lipids Matter But……

RISK FACTORS MATTER

LIPIDS MATTER

INFLAMMATION IS KEY




Overweight/Obesity
Abnormal Lipid Metabolism
• LDL ⬆
• ApoB ⬆
• HDL ⬇
• Triglycerides ⬆
Genetics
Age
Insulin Resistance
?
Insulin Resistance Syndrome
⬆Lipids
⬆BP
⬆Glucose
Cardiometabolic Risk
Global Diabetes/CVD Risk
Age, Race, Sex, Family History
Smoking, Physical Inactivity
Inflammation, Hypercoagulation
Hypertension

CAD is a Chronic Inflammatory Disease

- **Not a lipid storage disease**
  - Intensive lipid lowering therapy would eliminate CAD morbidity and mortality
  - Studies linked high cholesterol to CV events, and statins decreased LDL and CV events
- **A systemic inflammatory disease**
  - Hs-CRP and/or IL-6 strongly associated with, and an independent predictor of, future events in both 1^0 and 2^0 prevention
  - **Physicians Health Study** a primary prevention trial (1997) and the **Women's Health Study**: primary prevention (2000)
    - hs-CRP independently predicted future heart attacks, stroke, and CV deaths among "apparently" healthy individuals

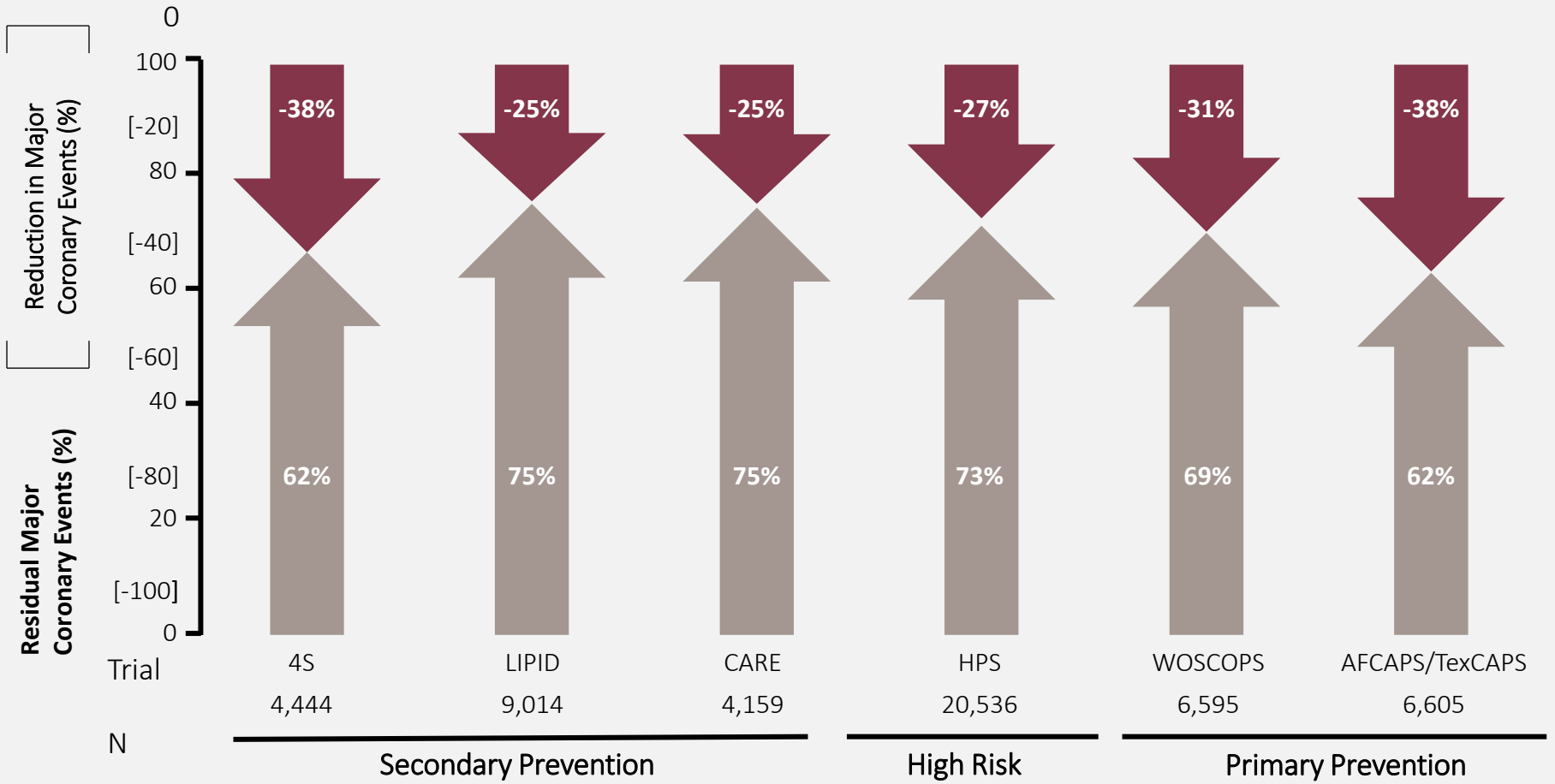
Libby P. Arterioscler Thromb Vasc Biol. 2012; 32(9): 2045-2051.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.
Libby P, Hanson GK. J Am Coll Cardiol. 2018. 71(2): 173-176.
Libby P, et al. J Am Coll Cardiol. 2018; 72(17): 2071-2081.

CAD is a Chronic Inflammatory Disease

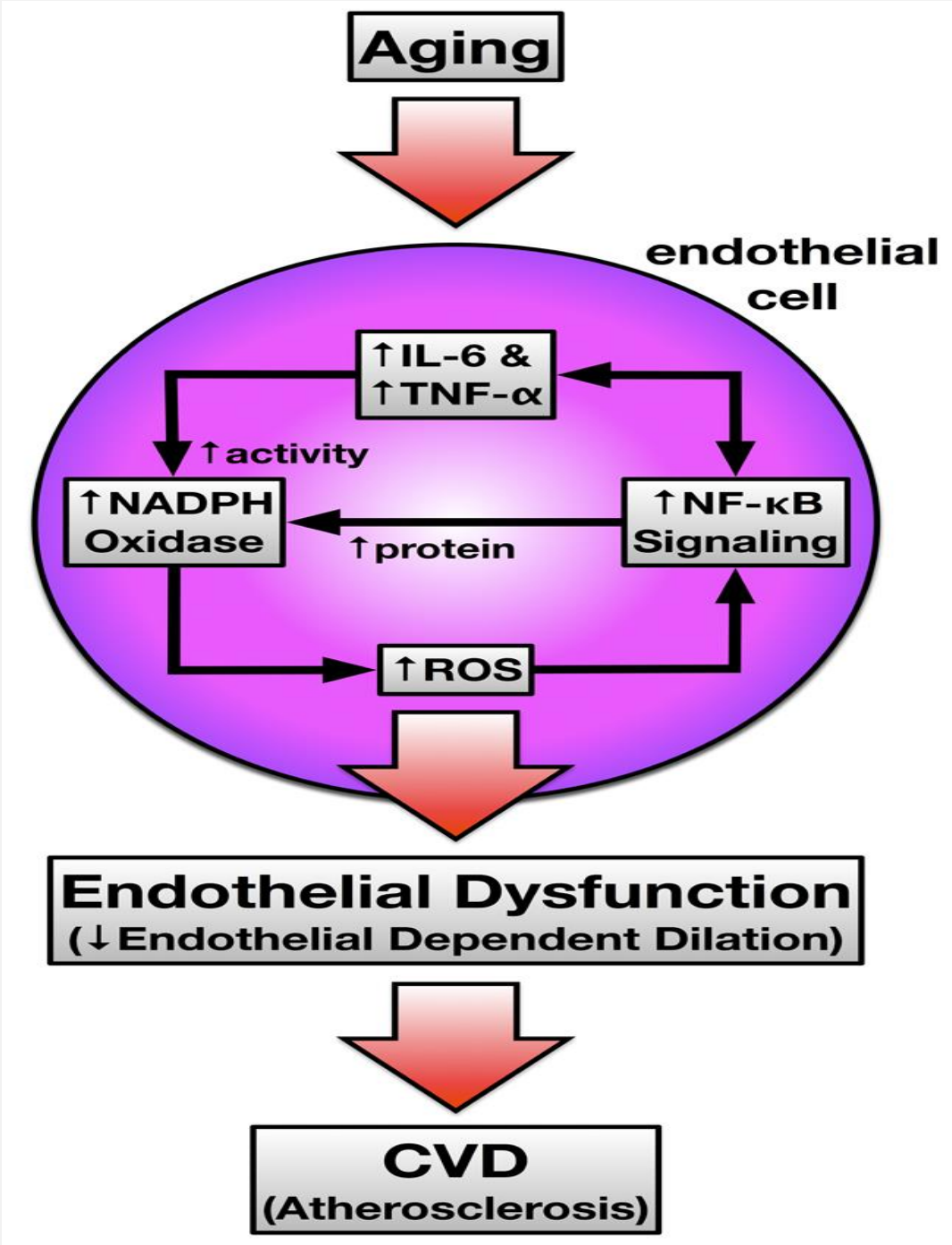
- **A systemic inflammatory disease**
  - Still doubt that therapies targeting inflammation per se would reduce CV event rates
  - No evidence that reducing inflammation w/o lowering lipids reduces events
- **CANTOS secondary prevention trial (2017): hs-CRP ≥ 2.0**
  - RCT with stable previous MI patients (at least 1 month post event) randomized to IL-1β monoclonal antibody (canakinumab)
    - Already on aggressive 2^0 preventive therapies
  - Results:
    - Lipids did not change
    - **15% decrease in 1^0 endpoint** (non-fatal MI, stroke, CV death); **17% decrease in expanded endpoint** (unstable angina requiring urgent revascularization)
    - **60% decrease in hs-CRP**

Libby P. Arterioscler Thromb Vasc Biol. 2012; 32(9): 2045-2051.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.
Libby P, Hanson GK. J Am Coll Cardiol. 2018. 71(2): 173-176.
Libby P, et al. J Am Coll Cardiol. 2018; 72(17): 2071-2081.

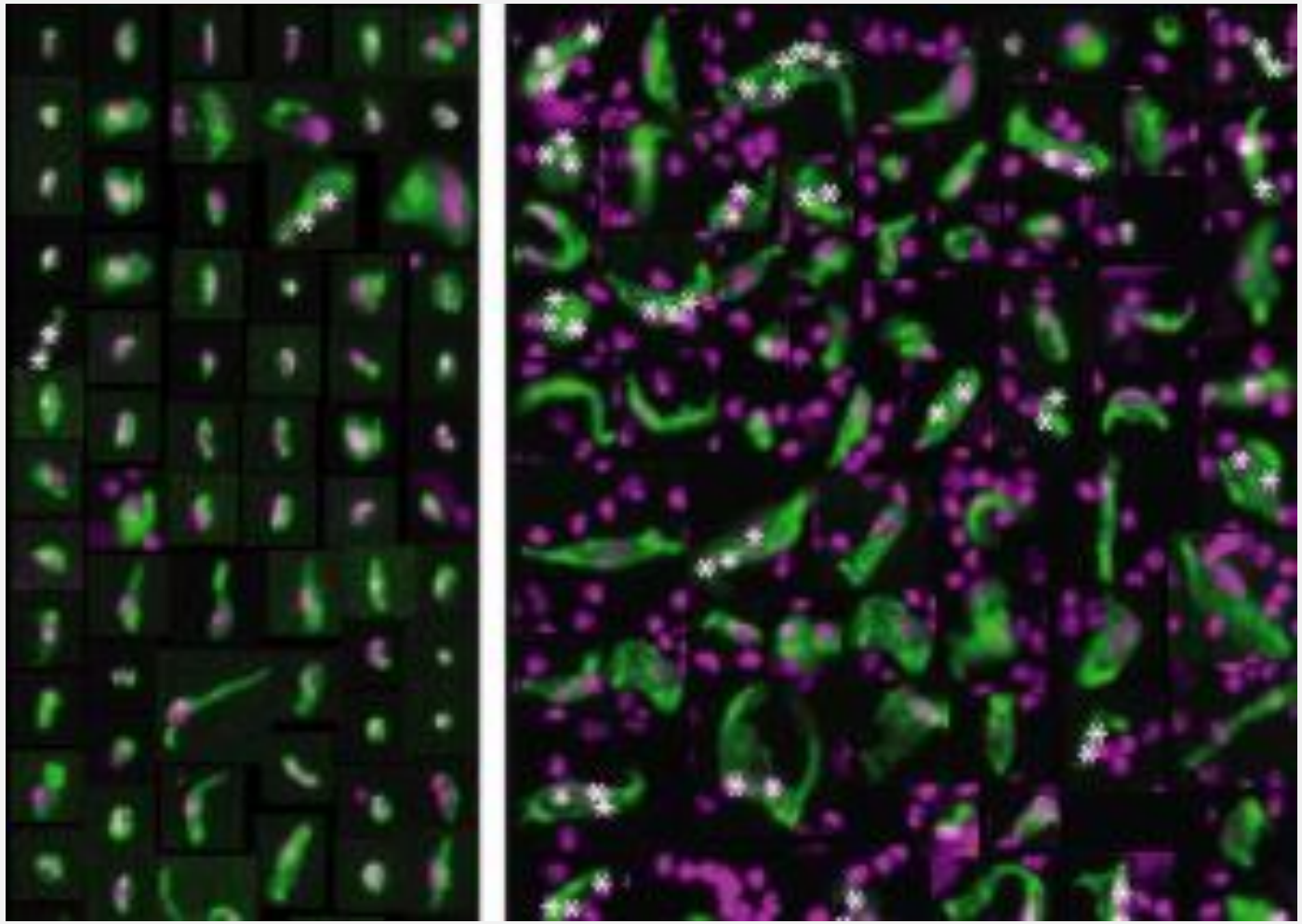
Majority of residual risk remains despite LDL lowering treatment



Libby P. J Am Coll Cardiol. 2005; 46(7): 1225-1228.
Libby P. J Am Coll Cardiol. 2017; 70: 2278-2289.
Libby P, et al. J Am Coll Cardiol. 2018; 72:2017-2081.


Aging
endothelial cell
↑IL-6 &
↑TNF-α
↑activity
↑NADPH Oxidase
↑protein
↑NF-κB Signaling
↑ROS
Endothelial Dysfunction
(↓Endothelial Dependent Dilation)
CVD
(Atherosclerosis)

Endothelial Cells Normal vs Pre-Heart Attack



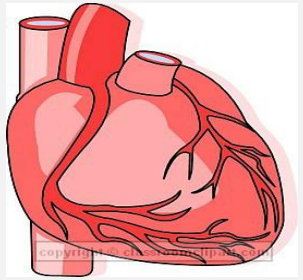
Endothelial Cells Pre-Risk Identification for Heart Attack

- Circulating Endothelial Cells (CEC)
- Cells become irregular with multinuclear area only in the Acute MI cases vs controls.

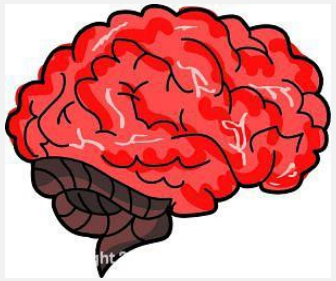
S. Damani, A. Bacconi, O. Libiger, A. H. Chourasia, R. Serry, R. Gollapudi, R. Goldberg, K. Rapeport, S. Haaser, S. Topol, S. Knowlton, K. Bethel, P. Kuhn, M. Wood, B. Carragher, N. J. Schork, J. Jiang, C. Rao, M. Connelly, V. M. Fowler, E. J. Topol, Characterization of Circulating Endothelial Cells in Acute Myocardial Infarction. Sci. Transl. Med. **4**, 126ra33 (2012).

Impact of Dysglycemia-Stress-Thyroid Disturbances

Inflammation
Oxidative stress



Endothelial dysfunction



Cortisol (T1) and T3 (CVD)

- Known association between high cortisol levels and increased CV morbidity and mortality
- Chronic stress (work, home, financial, SES) is associated with a 40–50% increase in CAD
- Chronic stress with elevated cortisol associated with lipid abnormalities
  - Increased: total cholesterol, LDL, oxidized LDL, and triglycerides
  - Decreased: HDL

Fioranelli M, et al. Front Immunol. 2018; 9:2031.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Steptoe A, Kivimaki M. Nat Rev Cardiol. 2012; 9(6): 360-370.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.

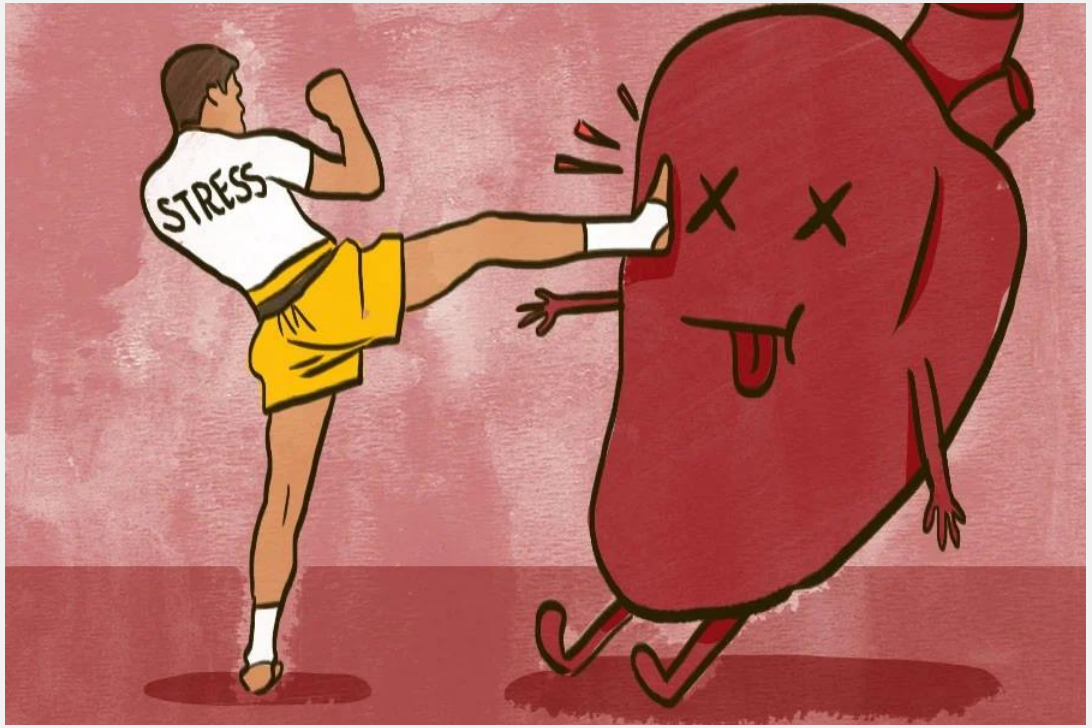
Cortisol (T1) and T3 (CVD)

- In patients with known CAD, chronic stress is associated with a poor prognosis and increased recurrent event rates and mortality
- In ACS and acute stroke survivors, stress:
  - Impairs recovery after an event,
  - Accelerates disease progression, and
  - Contributes to CV death among patients who have survived an acute coronary syndrome or stroke

Fioranelli M, et al. Front Immunol. 2018; 9:2031.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Steptoe A, Kivimaki M. Nat Rev Cardiol. 2012; 9(6): 360-370.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.

Cortisol (T1) and T3 (CVD)

- INTERHEART Study (2004)
- CARDIA Study (2006)
- WHITEHALL II Study (2011)
- InCHIANTI Study (2010)
- Mendelian Studies (2019)
- Hair Cortisol Studies (2011, 2013)

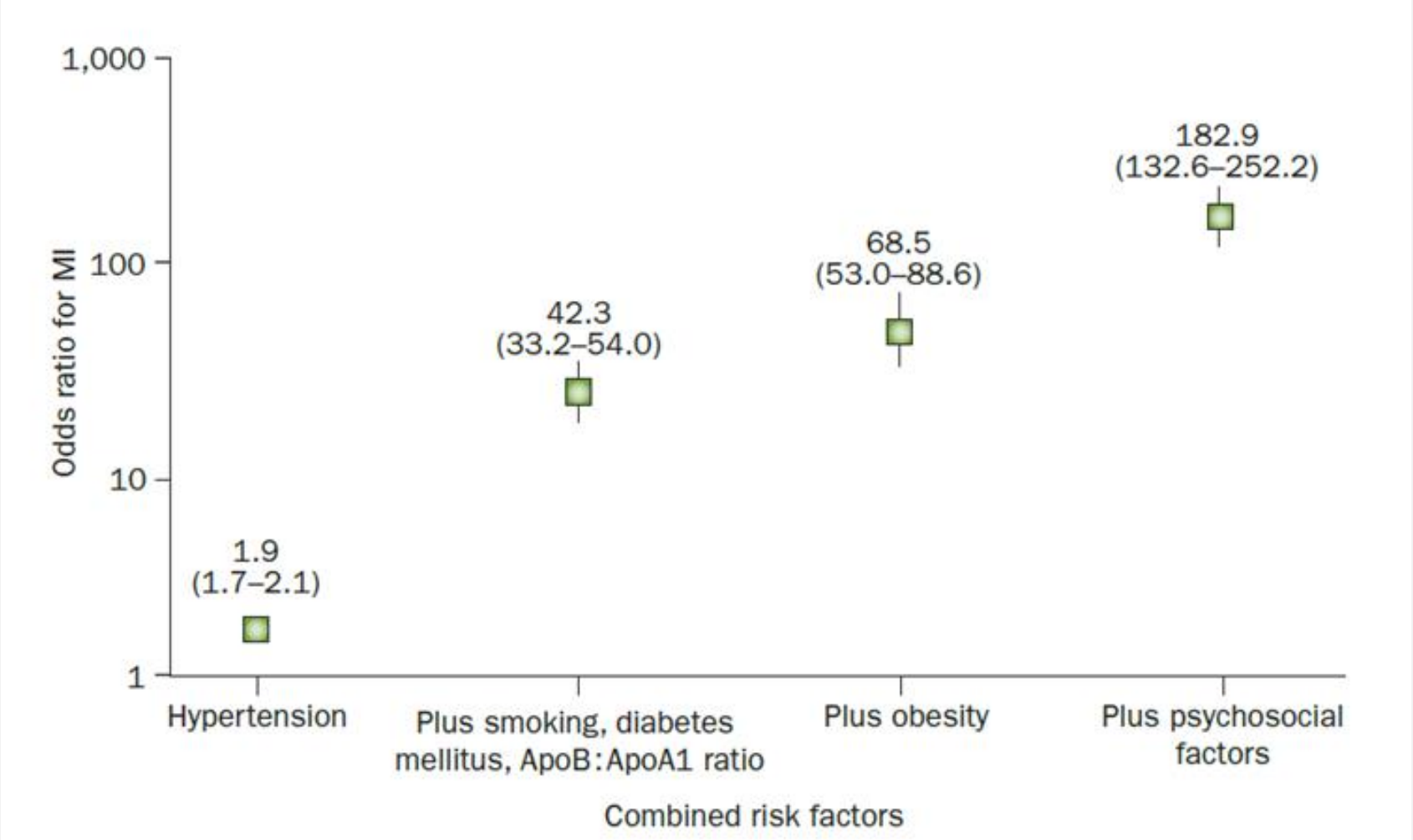


INTERHEART STUDY: [1] Yusuf S, et al. Lancet. 2004; 364(9438): 937-952. [2] Fioranelli M, et al. Front Immunol. 2018; 9:2031.
CARDIA STUDY: Mathews K, et al. Psychosom Med. 2006; 68(5): 657-661.
WHITEHALL II STUDY: Kumari M, et al. J Clin Endocrinol Metab. 2011; 96(5): 1478-1485
URINE CORTISOL AND CVD MORTALITY: Vogelzangs N, et al. J Clin Endocrinol Metab. 2010; 95(11): 4959-4964.
MENDELIAN STUDIES: Crawford AA, et al. Eur J Endocrinol. 2019; 181(4): 429-438.
HAIR CORTISOL: [1] Pereg D, et al. Stress. 2011; 14(1): 73-81. [2] Manenschijn L, et al. J Clin Endocrinol; 98(5): 2078-2083. [3] Iob E, Steptoe A. Current Cardiol Rep. 2019; 21(10): 116.

Cortisol (T1) and T3 (CVD)

- **INTERHEART case-controlled study (2004)**
  - Largest study to assess long-term stress and CAD
  - Study: n=15,152 MI patients, 14,820 controls from 52 countries world-wide between 1999-2003
  - Objective: determine the strength of the association between RF and AMI
  - Results:
    - The odds ratio of an MI was more than doubled in individuals with chronic stress in addition to conventional risk factors when compared to stress-free individual
    - A similar pattern of associations was found in men and women, old and young, across all continents
  - **Concluded that psychosocial factors significantly related to AMI risk**

Cortisol (T1) and T3 (CVD)



Steptoe A, Kivimaki M. Nat Rev Cardiol. 2012; 9(6): 360-370.

Cortisol, Testosterone, and Coronary Heart Disease

Prospective Evidence From the Caerphilly Study

George Davey Smith, DSc; Yoav Ben-Shlomo, BSc, MBBS, MRCP, FFPHM, PhD; Andrew Beswick, BSc; John Yarnell, MBChB, DPH, MSCM, MD, MFPHM (Ire), FFPHM; Stafford Lightman, MBChB, PhD, FMedSci; Peter Elwood, DSc, MD, FRCP, FFPHM

Background—There is a popular belief that chronic stress causes heart disease through psychoneuroendocrine mechanisms. We have examined whether an elevated circulating cortisol-to-testosterone ratio increases the risk of ischemic heart disease.

Methods and Results—We undertook a prospective cohort study of 2512 men aged 45 to 59 years between 1979 and 1983 from Caerphilly, South Wales, with a mean follow-up of 16.5 years. Subjects underwent a clinical examination, and morning fasting blood samples were taken for analysis of cortisol levels, testosterone levels, and other cardiovascular risk factors. The ratio of cortisol to testosterone showed weak associations with potential confounding factors but strong positive associations with components of the insulin resistance syndrome ($P<0.001$). A positive linear trend was seen across quintiles of cortisol:testosterone ratio for incident ischemic heart disease (age-adjusted OR per z score change in ratio 1.22, 95% CI 1.07 to 1.38, $P=0.003$). This was markedly attenuated after adjustment for components of the insulin resistance syndrome (age-adjusted OR per z score change in ratio 1.10, 95% CI 0.96 to 1.25, $P=0.18$). There was no association between the cortisol:testosterone ratio and other causes of death (age-adjusted hazard ratio 0.99, 95% CI 0.88 to 1.11, $P=0.81$).

Conclusions—This is the first population-based prospective study that has found a specific association between cortisol:testosterone ratio and incident ischemic heart disease, apparently mediated through the insulin resistance syndrome. Whether this reflects the effects of chronic stress, behavioral factors, or genetic influences remains to be determined. (***Circulation***. **2005;112:332-340.**)

Key Words: heart diseases ■ hormones ■ stress

Cortisol (T1) and T3 (CVD)

- **WHITEHALL II prospective cohort study (2011)**
  - First study to document daily diurnal cortisol patterns are predictive of subsequent cardiovascular mortality in men and women; saliva
  - Study: n=4047 men and women, average age 61, part of phase 7 (2000-2002)
  - Objective: to examine the association between cortisol patterns, CV and non-CV mortality
  - Results:
    - Flattened cortisol curve associated with increased CV mortality
    - An elevated PM cortisol was independently predictive of subsequent CV mortality
    - No association between waking cortisol, CAR, and mortality, group with flattened curve and higher bedtime cortisol did have higher non-significant elevated CARs
    - Neither a flattened slope, nor bedtime cortisol SS associated with non-CV mortality
  - **Concluded that these findings suggest a cause-specific association between the (flattened) cortisol curve, bedtime cortisol, and CVD mortality**

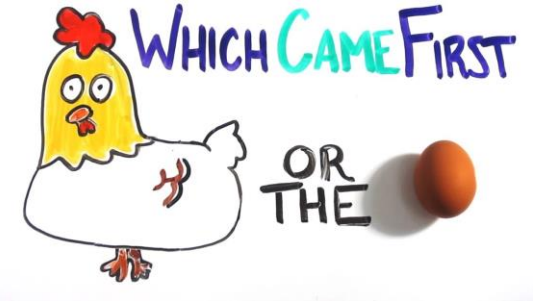
Cortisol (T1) and T3 (CVD)

- **InCHIANTI a prospective cohort study (2010)**
  - First urine study to document 24-hour urinary free cortisol (UFC) levels predict CV death
  - Study: n=861 older individuals randomly; age > 65 years old; 6 year study
    - UFC divided into 3 terciles
      - Low: < 78μg; moderate: 78-111μg; high: > 111μg
  - Objective: do 24-hour UFC levels predict all-cause and CVD mortality
  - Results:
    - UFC strongly predicts cardiovascular mortality, not non-cardiovascular mortality
    - Risk increased with increasing UFC levels
    - Those in the highest tercile (UFC > 111μg) had a 5x increased CVD mortality risk
  - **Concluded that UFC is a strong predictor of CVD mortality in persons with and without preexisting CVD**

Key Points

- **Chronic stress significantly associated with MI risk**
- **Cortisol is an acute and chronic stress marker**
- **Cortisol is a strong predictor of CVD risk, events, and mortality**
  - Elevated serum AM cortisol: strong association with incident CVD
  - Salivary flattened diurnal cortisol pattern with high PM cortisol
    - Associated with increased CAC deposition
    - Cause specific association between high flattened curve, PM cortisol, and CVD mortality
    - High bedtime cortisol independent CVD mortality predictor
  - Urinary Cortisol: elevated 24-hour UFC strong predictor of CVD mortality in persons with and without preexisting CVD
  - Hair cortisol is a chronic stress marker and there is a strong association between hair cortisol, CVD risk, and CV events (AMI)

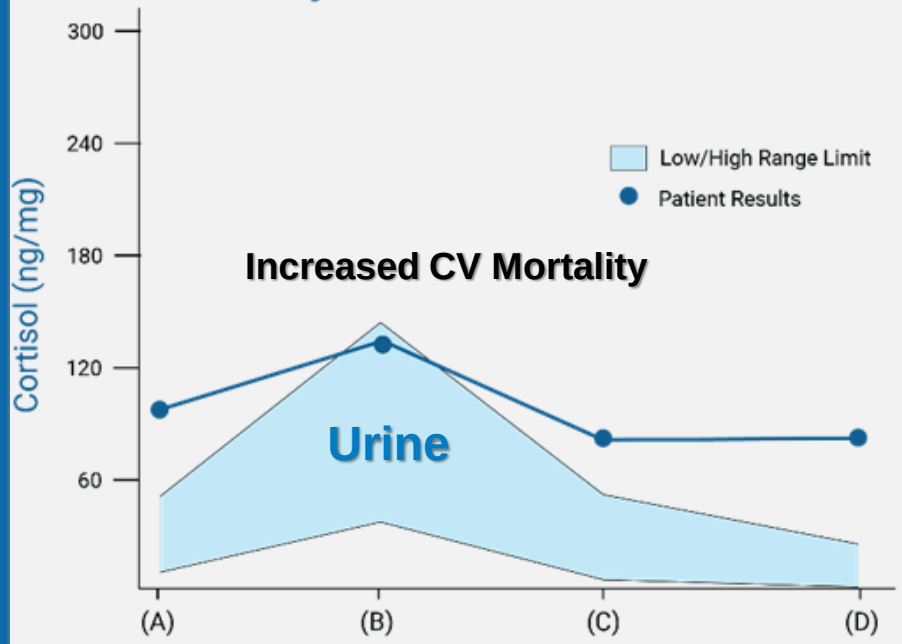
Cortisol (T1) and T3 (CVD)

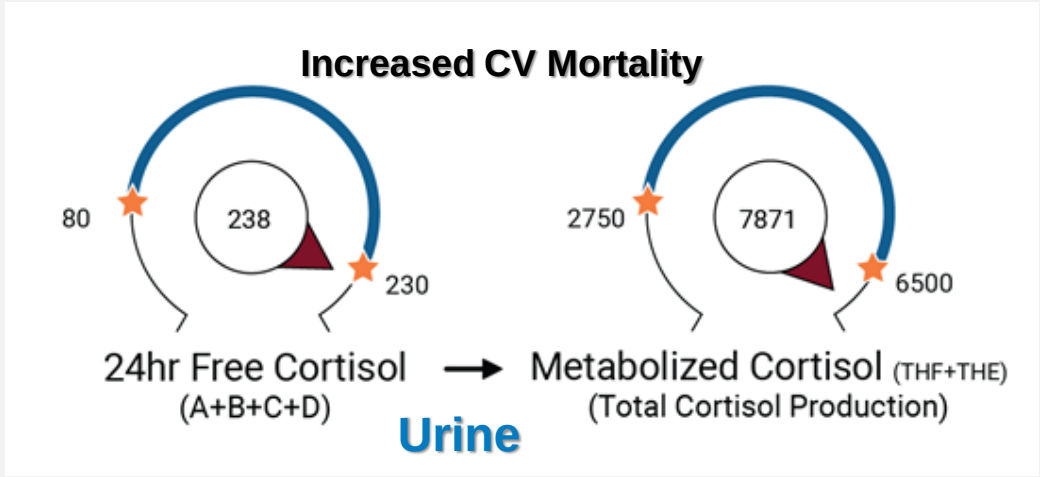


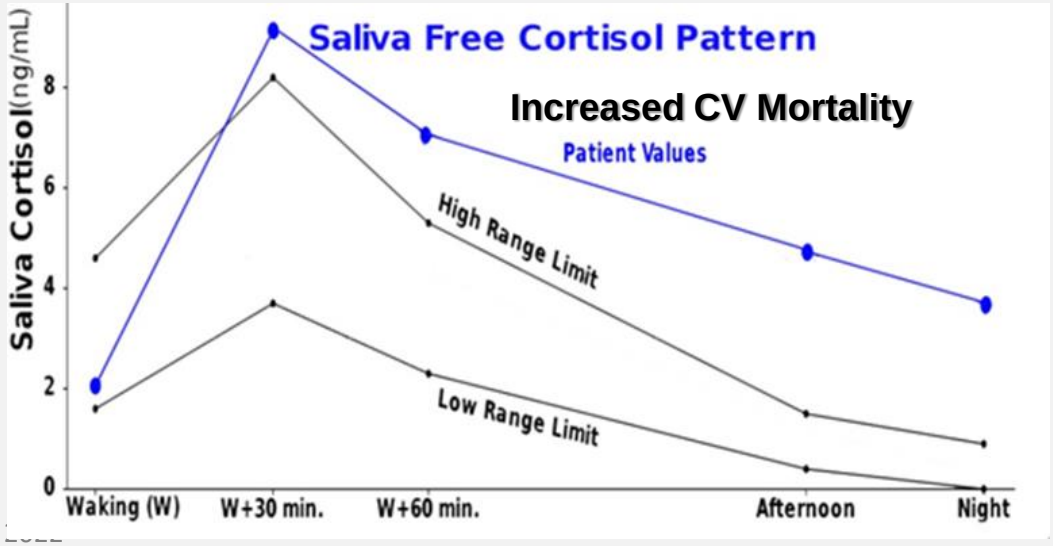
- **MESA Stress Study: a longitudinal prospective study (2012)**
  - Study: Multi-Ethnic Study Atherosclerosis n=869 adults, cortisol curves x 3 days
  - Objective: looked at associations between cortisol patterns and stable inflammatory markers (IL-6, TNF-α, and IL-10)
    - Was there a relationship between diurnal cortisol rhythms (awakening levels, CAR, or cortisol's decline over the day) and IL-6, IL-10, or TNF-α?
    - Was there an association between total cortisol output measured by area under the curve (AUC) and IL-6, IL-10, TNF-α?
  - Results:
    - A higher IL-6 was significantly associated with: lower CAR, flatter slope, and greater area under the curve; no association with waking cortisol or bedtime cortisol
    - A higher TNF- α was significantly associated with lower waking cortisol, non-significant flatter curve
    - IL-10: Non-significant flatter curve, no association with waking cortisol, CAR, HS cortisol
  - **Concluded that cortisol patterns and inflammatory cytokines are related**

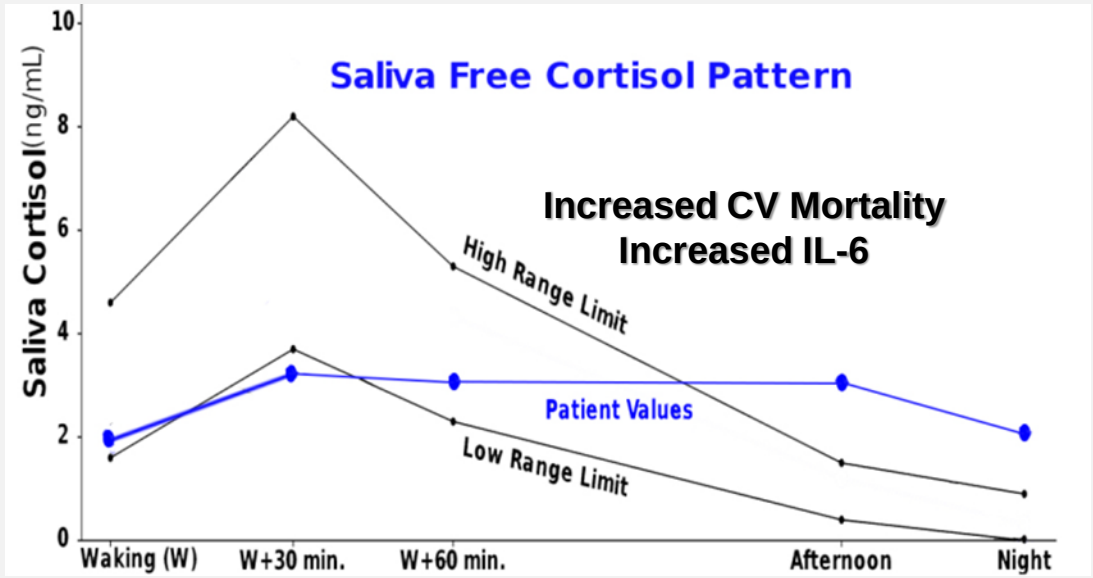
DeSantis AS, et al. Psychoneuroendocrinology. 2012; 37(7): 1009-1018.

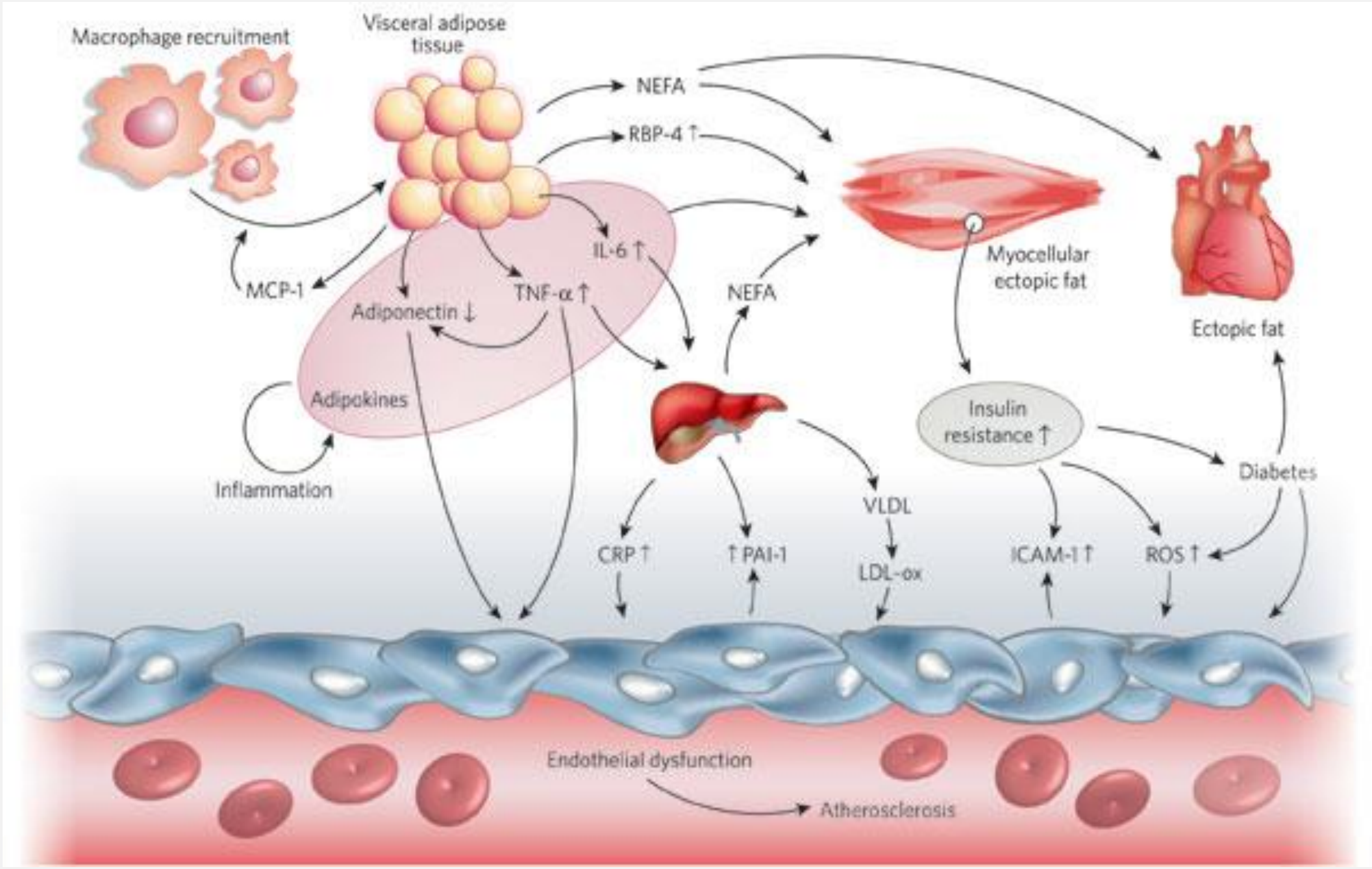
Daily Free Cortisol Pattern

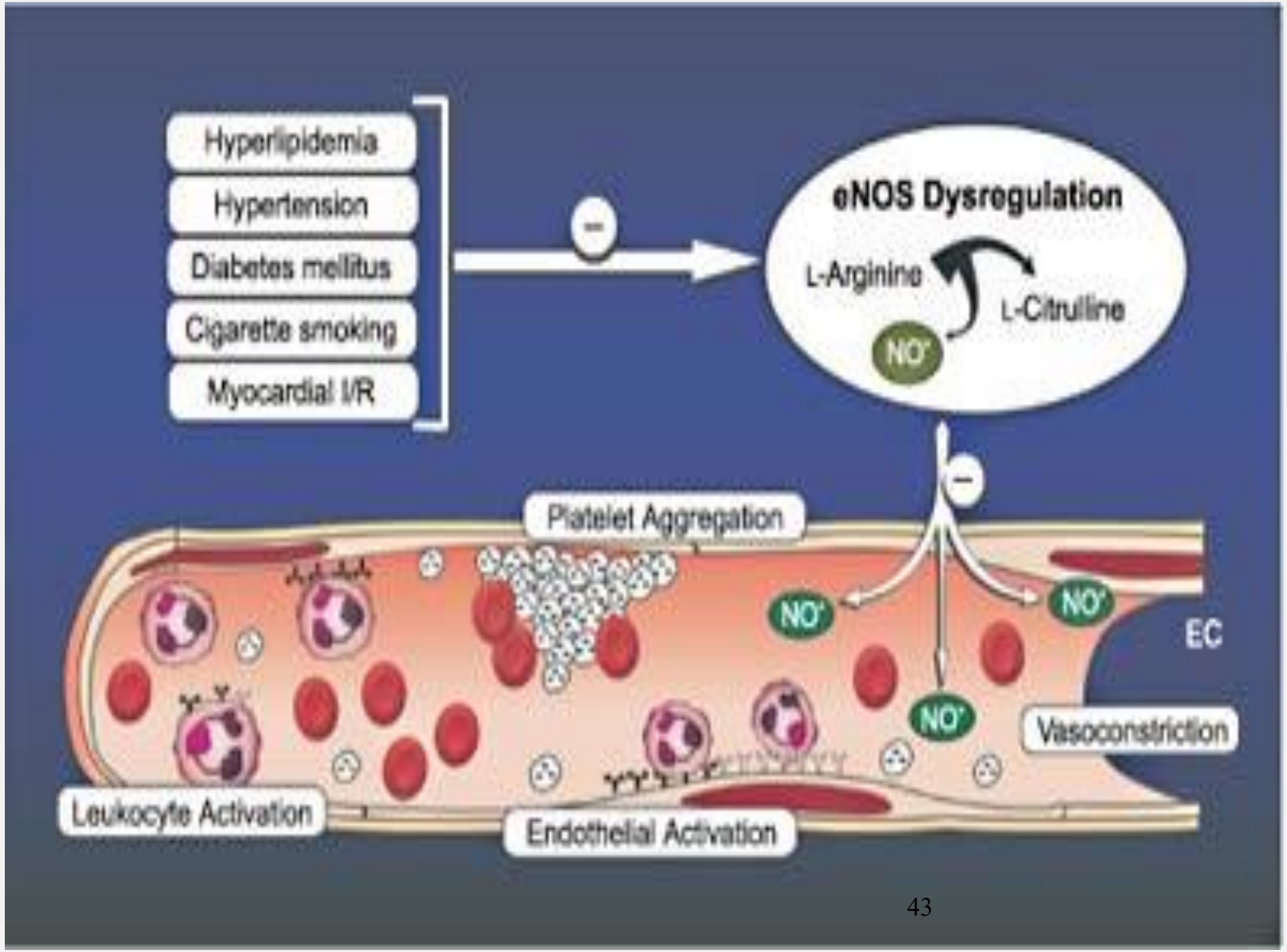




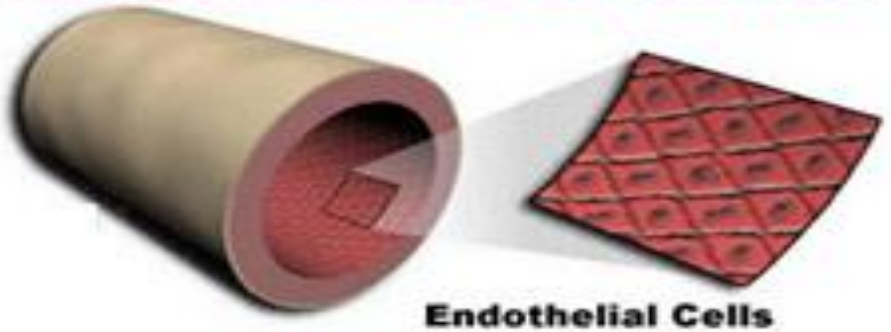





Macrophage recruitment
Visceral adipose tissue
NEFA
RBP-4 ↑
IL-6 ↑
MCP-1
Adiponectin ↓
TNF-α ↑
Adipokines
Inflammation
NEFA
Myocellular ectopic fat
Ectopic fat
Insulin resistance ↑
Diabetes
VLDL
CRP ↑
↑ PAI-1
LDL-ox
ICAM-1 ↑
ROS ↑
Endothelial dysfunction
Atherosclerosis

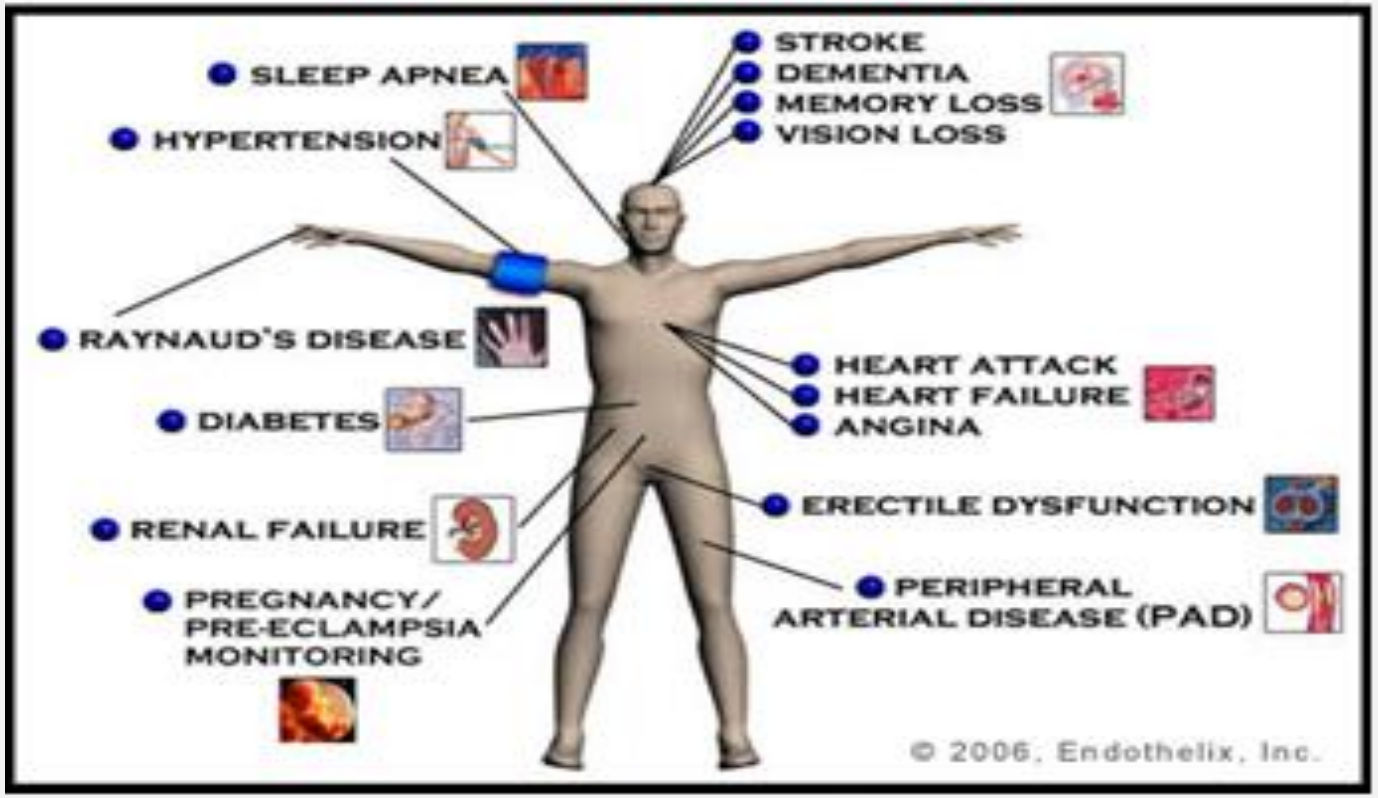

Hyperlipidemia
Hypertension
Diabetes mellitus
Cigarette smoking
Myocardial I/R
eNOS Dysregulation
L-Arginine
L-Citrulline
NO
Platelet Aggregation
NO
NO
NO
EC
Vasoconstriction
Leukocyte Activation
Endothelial Activation

ENDOTHELIAL DYSFUNCTION



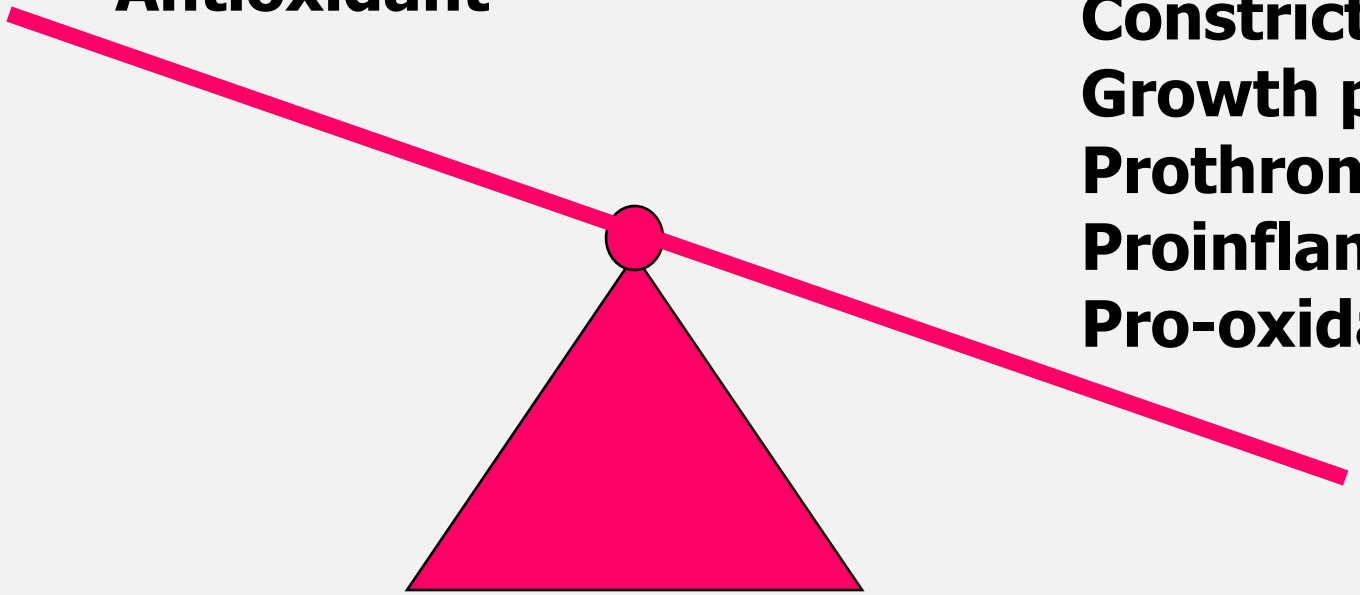
Endothelial Cells

IS THE PRECURSOR OF:



Endothelium Mediates Vascular Health

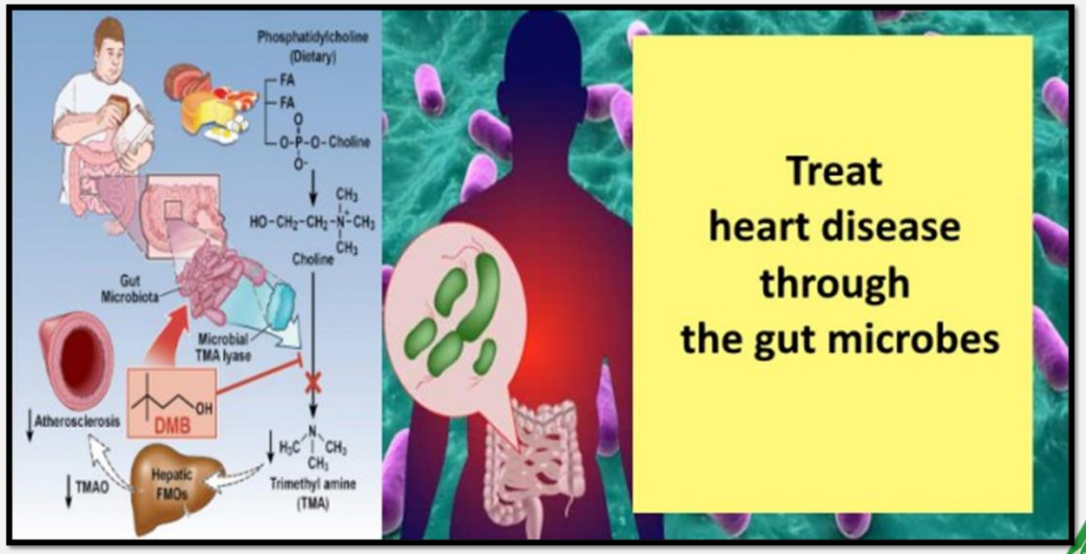
Dilatation
Growth inhibition
Antithrombotic
Anti-inflammatory
Antioxidant



Constriction
Growth promotion
Prothrombotic
Proinflammatory
Pro-oxidant

The Heart (T3) – Gut (T2) Connection

The Heart – Gut Connection

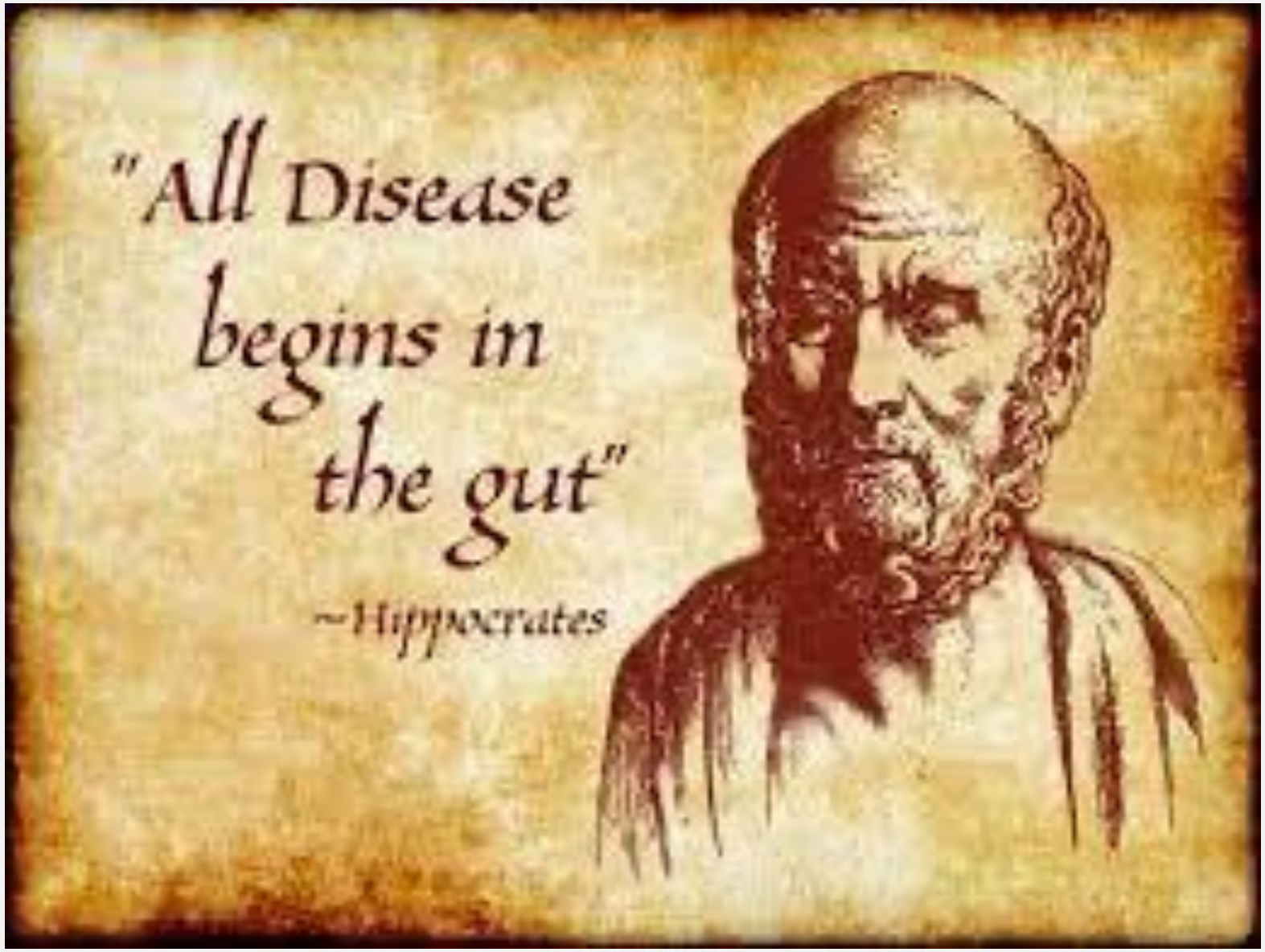


Gut – Heart Axis

- **Gut** imbalances directly linked **Heart** conditions
  - Hypertension
  - Hyperlipidemia
  - Atherosclerosis
  - Heart failure
  - CKDs
- Microbial metabolites – TMAO

Lam V, Su J, Hsu A, Gross GJ, Salzman NH, Baker JE. Intestinal microbial metabolites are linked to severity of myocardial infarction in rats. PLoS One. 2016;11:e0160840. Suzuki T, Heaney LM, Bhandari SS, Jones

DJ, Ng LL. Trimethylamine N-oxide and prognosis in acute heart failure. Heart. 2016;102:841–848.



The Heart (T3) – Gut (T2) Connection

- **An altered gut microbiome: Dysbiosis**
  - Dysbiosis is a change in the microbiome's composition 2^0 to:
    - Diet
    - Chronic stress leading to inflammation
    - Antibiotic overuse
  - Dysbiosis alters metabolic homeostasis contributing to:
    - Obesity
    - IR and DM
    - Met-S
    - CVD, HF
    - Cancer, etc.
- **Dysbiosis leads to decreased tight junction protein expression and increased gut permeability**

Tang WH, et al. Circ Res. 2017; 120(7): 1183-1196.
Brown JM, Hazen SL. Nat Rev Microbiol. 2018; 16(3): 171-178.

The Heart (T3) – Gut (T2) Connection

- **A healthy gut microbiome (GM)**
  - There is no single definition for a healthy gut microbiome
- **Gut microbiome is an immune organ**
  - 70-80% of the immune system lives in the gut – 1st line of defense
  - GM is involved in T cell and B cell lymphocyte differentiation
  - GM modulates mucosal IgA production – critical player in maintaining intestinal barrier function
  - It ferments complex carbohydrates producing SCFA
    - Butyrate inhibits NFκβ
    - Butyrate and propionate inhibit LPS-induced cytokine release (IL-6)

Tang WH, et al. N Engl J Med. 2013; 368(17): 1575-1584.
Tang WH, et al. Circ Res. 2017; 120(7): 1183-1196.
Yamashita T. J Atheroscler Thromb. 2017; 24(2): 110-119.
Peng J, et al. Life Sci. 2018; 214: 153-157.
Troseid M, et al. EBioMed. 2020; 52: 102649.
Zhao Y, Wang Z. Curr Opin Cardiol. 2020; 35(3): 207-218.

The Heart (T3) – Gut (T2) Connection

- **Dysbiosis, increased gut permeability, and LPS**
  - LPS is part of the gram-negative (-) bacteria's outer membrane
  - With a permeable gut, LPS enters circulation, binds to TLR-4
    - On macrophages, enterocytes, endothelial cells [ECs], dendritic cells [DCs]
  - Leads to increased pro-inflammatory cytokine (IL-1β, TNF-α), chemokines, and cell-adhesion molecules (VCAM-1) secretion
  - Leads to plaque formation

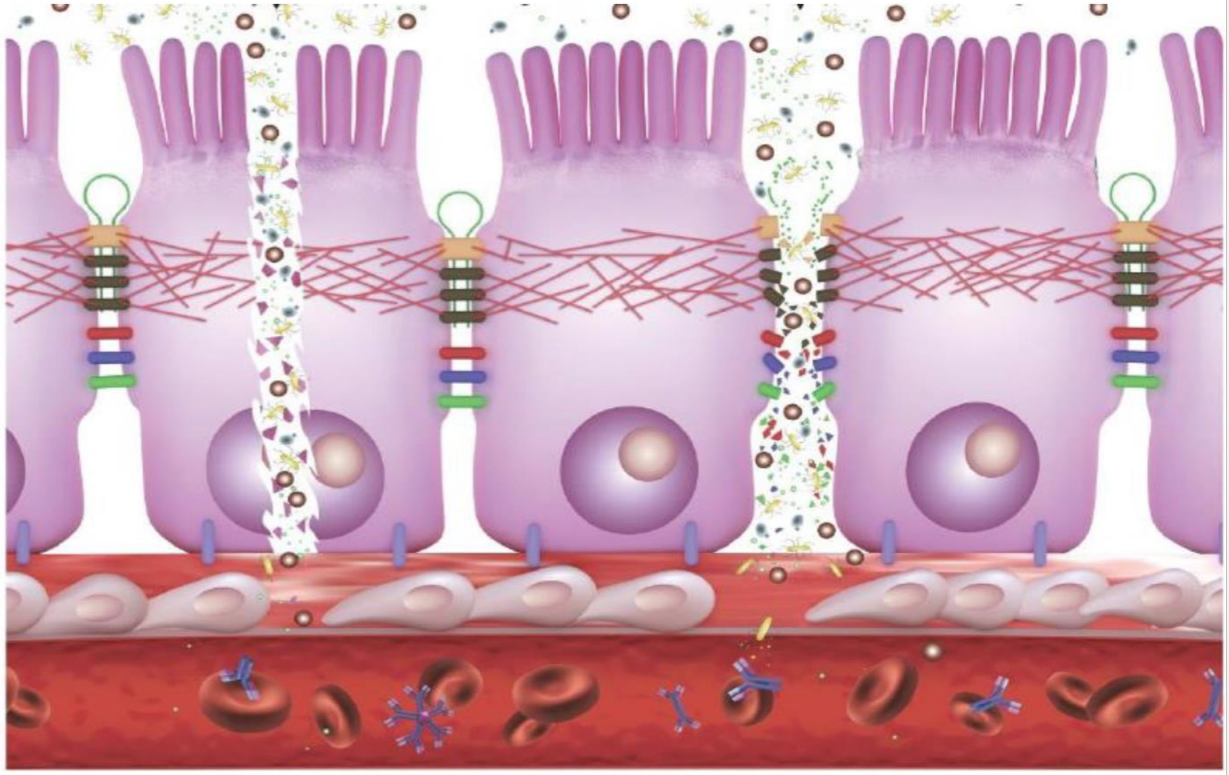
Tang WH, et al. N Engl J Med. 2013; 368(17): 1575-1584.
Tang WH, et al. Circ Res. 2017; 120(7): 1183-1196.
Yamashita T. J Atheroscler Thromb. 2017; 24(2): 110-119.
Peng J, et al. Life Sci. 2018; 214: 153-157.
Brown JM, Hazen SL. Nat Rev Microbiol. 2018; 16(3): 171-178.
Zhao Y, Wang Z. Curr Opin Cardiol. 2020; 35(3): 207-218.

What is Lipopolysaccharide (LPS)?

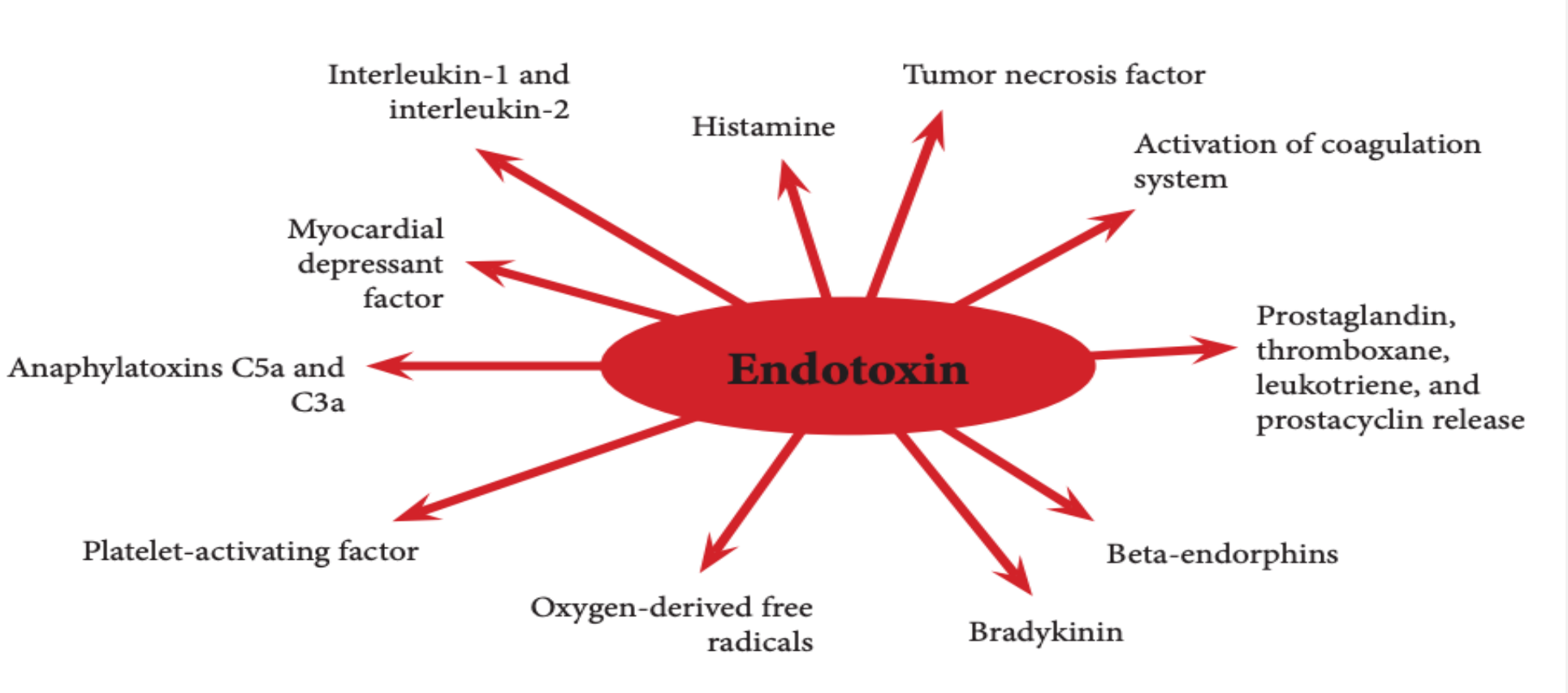
Lipopolysaccharides

LPS can cause damage to epithelial cells and tight junctions.

Increased intestinal permeability allows more LPS to enter the blood stream

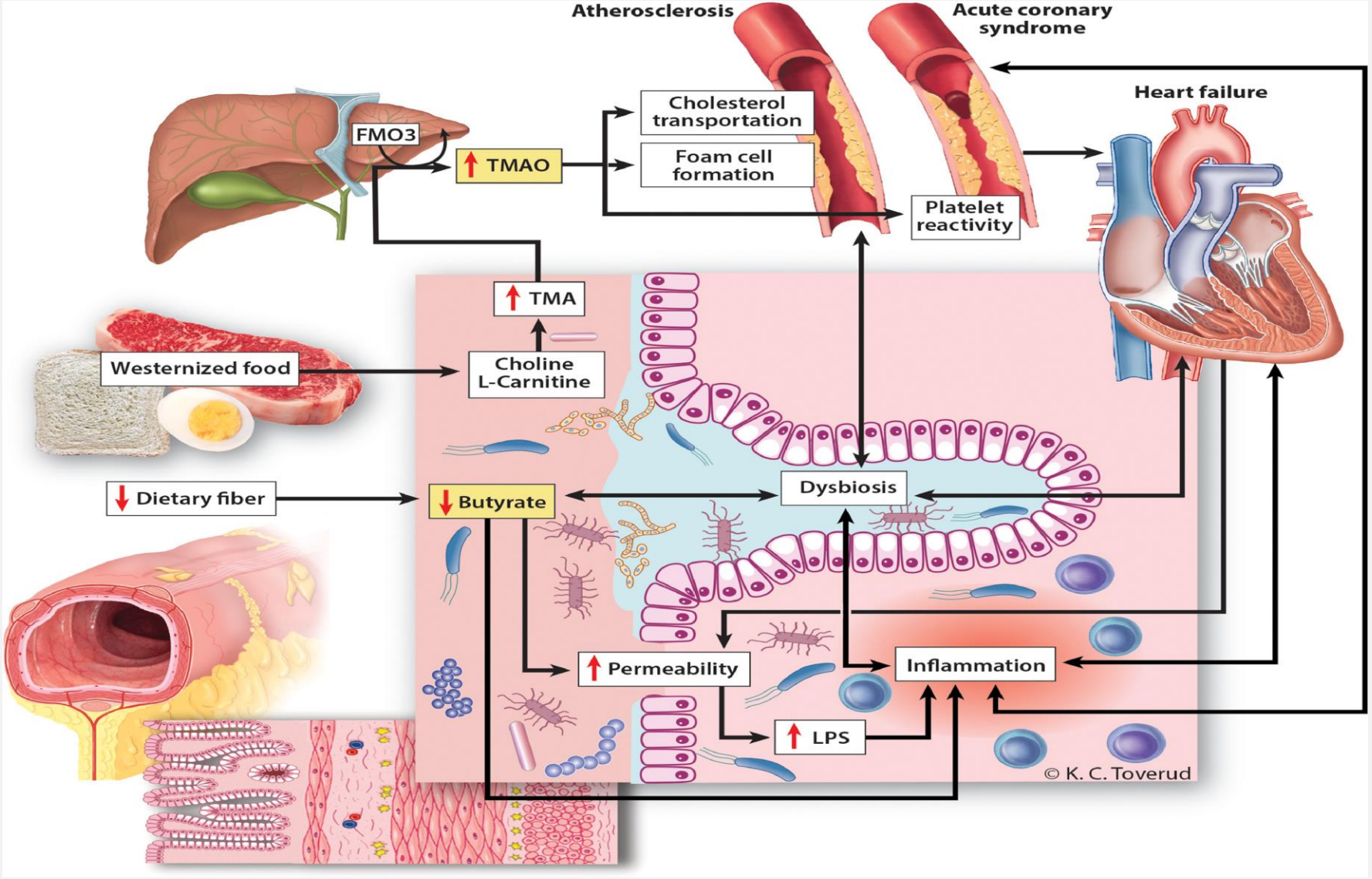


Metabolic Effects of Endotoxin



Pizzorrno J. Toxins from the GUT. Integr Med.(Encinitas. 2014;13(6):8-11.

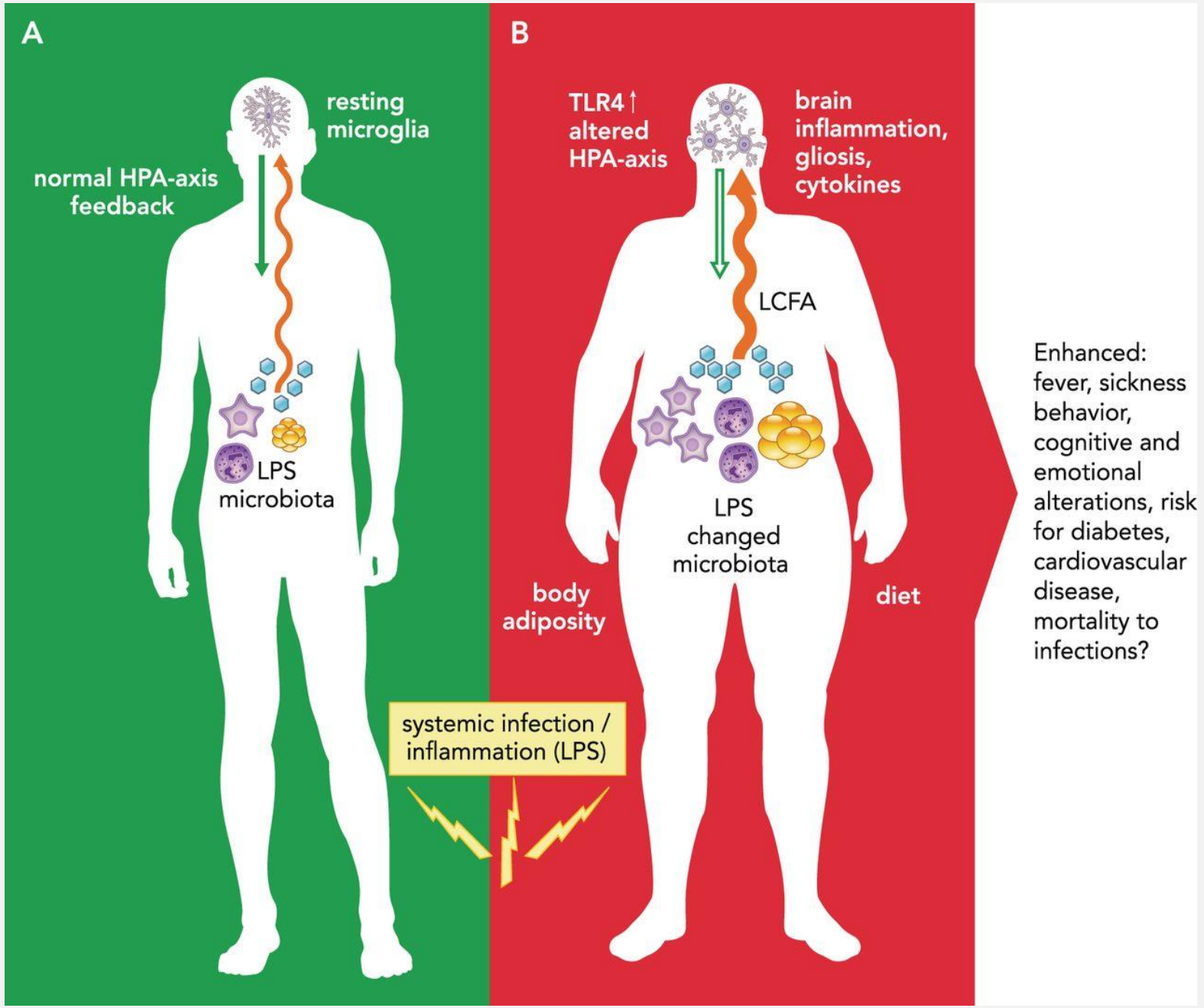
Putting it All Together



The Heart (T3) – Gut (T2) Connection

- **LPS exposure dose is important**
  - High concentrations ➔ robust inflammatory response + inhibitory feedback mechanisms
  - Persistent low concentrations ➔ ongoing expression of proinflammatory mediators ➔ chronic inflammation, oxidation, end-organ damage, i.e., increased CVD events
  - First indication may be thyroid Ab; the greater the exposure the higher the Ab
- **An appropriate response to a maladaptive environment**

Tang WH, et al. N Engl J Med. 2013; 368(17): 1575-1584.
Tang WH, et al. Circ Res. 2017; 120(7): 1183-1196.
Yamashita T. J Atheroscler Thromb. 2017; 24(2): 110-119.
Peng J, et al. Life Sci. 2018; 214: 153-157.
Brown JM, Hazen SL. Nat Rev Microbiol. 2018; 16(3): 171-178.
Zhao Y, Wang Z. Curr Opin Cardiol. 2020; 35(3): 207-218.


A
resting microglia
normal HPA-axis feedback
LPS microbiota
B
TLR4 ↑ altered HPA-axis
brain inflammation, gliosis, cytokines
LCFA
LPS changed microbiota
body adiposity
diet
systemic infection / inflammation (LPS)
Enhanced: fever, sickness behavior, cognitive and emotional alterations, risk for diabetes, cardiovascular disease, mortality to infections?

LPS and the Oral Cavity - The Pathway to Inflammation

Endotoxin translocates (leaks) into systemic circulation by:

- Binds to LPS binding protein(LBP)
- Bactericidal/permeability-increasing protein (BPI)
- Carried by serum VLDL and HDL

Monocytes respond to increase LPS presenting to TLR$/MD2 complex (toll receptor 4 myeloid differentiation factor 2

Storm of TNFα inducing TNF-NF-kappa B inducing kinase promoting transcription of several hundred genes increasing clotting elements, complement and other acute phase proteins, cytokines, chemokines and nitric oxide synthase.

Endotoxin, Toll Receptor-4 and Atherosclerotic Heart Disease

***Current Cardiology Reviews,* 2017, *13,* 86-93**

The Heart (T3) – Gut (T2) Connection

- **Dysbiosis, increased gut permeability, and TMAO**
  - TMAO, in addition to SCFAs, is a major gut microbiome metabolite
  - When large quantities of choline and carnitine are ingested, specific bacteria metabolize them to TMA
    - Specific bacteria not yet identified
  - TMA transported to liver via the portal circulation where oxidized by FO3 (flavin-containing monooxygenase 3) to TMAO
- **TMAO**
  - TMAO is a cardiovascular risk predictor that correlates with atherosclerosis
  - Higher baseline plasma TMAO levels directly linked to increased **m**ajor **a**dverse **c**ardiovascular **e**vents (MACE) in patients with and without underlying CAD, including those at low risk

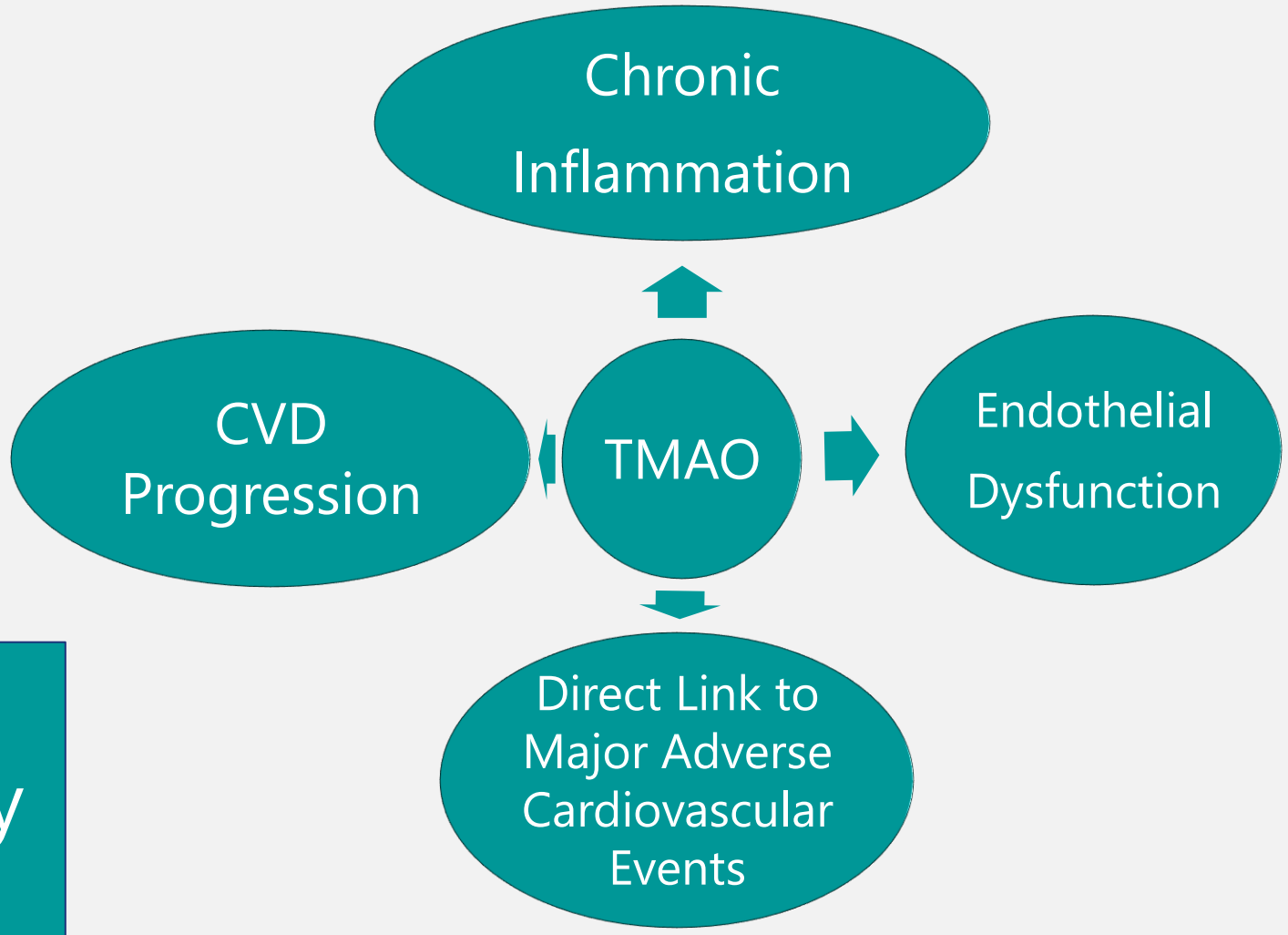
Tang WH, et al. N Engl J Med. 2013; 368(17): 1575-1584.
Tang WH, et al. Circ Res. 2017; 120(7): 1183-1196.
Peng J, et al. Life Sci. 2018; 214: 153-157.
Brown JM, Hazen SL. Nat Rev Microbiol. 2018; 16(3): 171-178.
Zhao Y, Wang Z. Curr Opin Cardiol. 2020; 35(3): 207-218.

Trimethylamine-N-Oxidase (TMAO)

Bacterial metabolite

Marker for gut barrier permeability

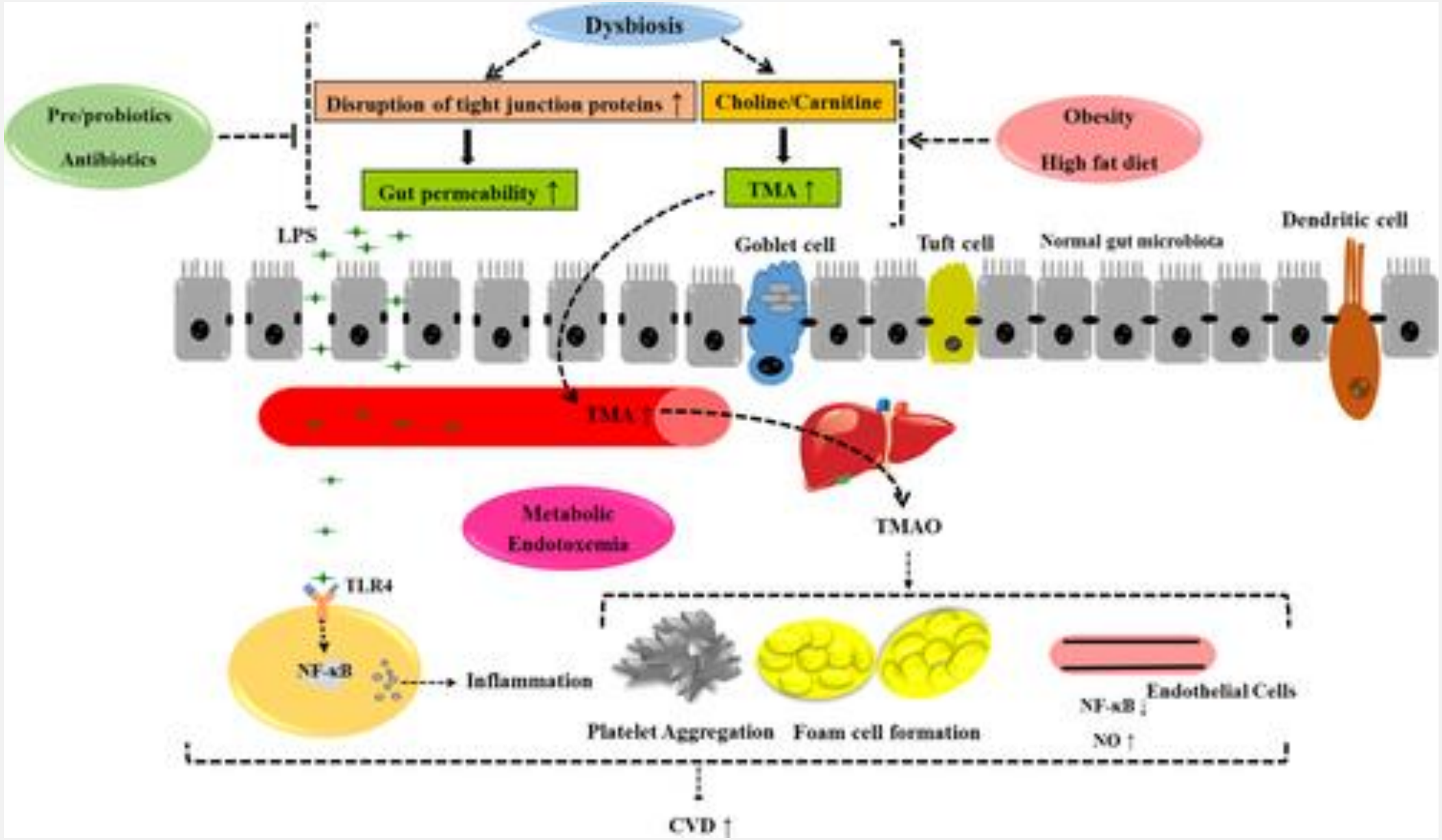
Prognostic in cardiomyopathy patients

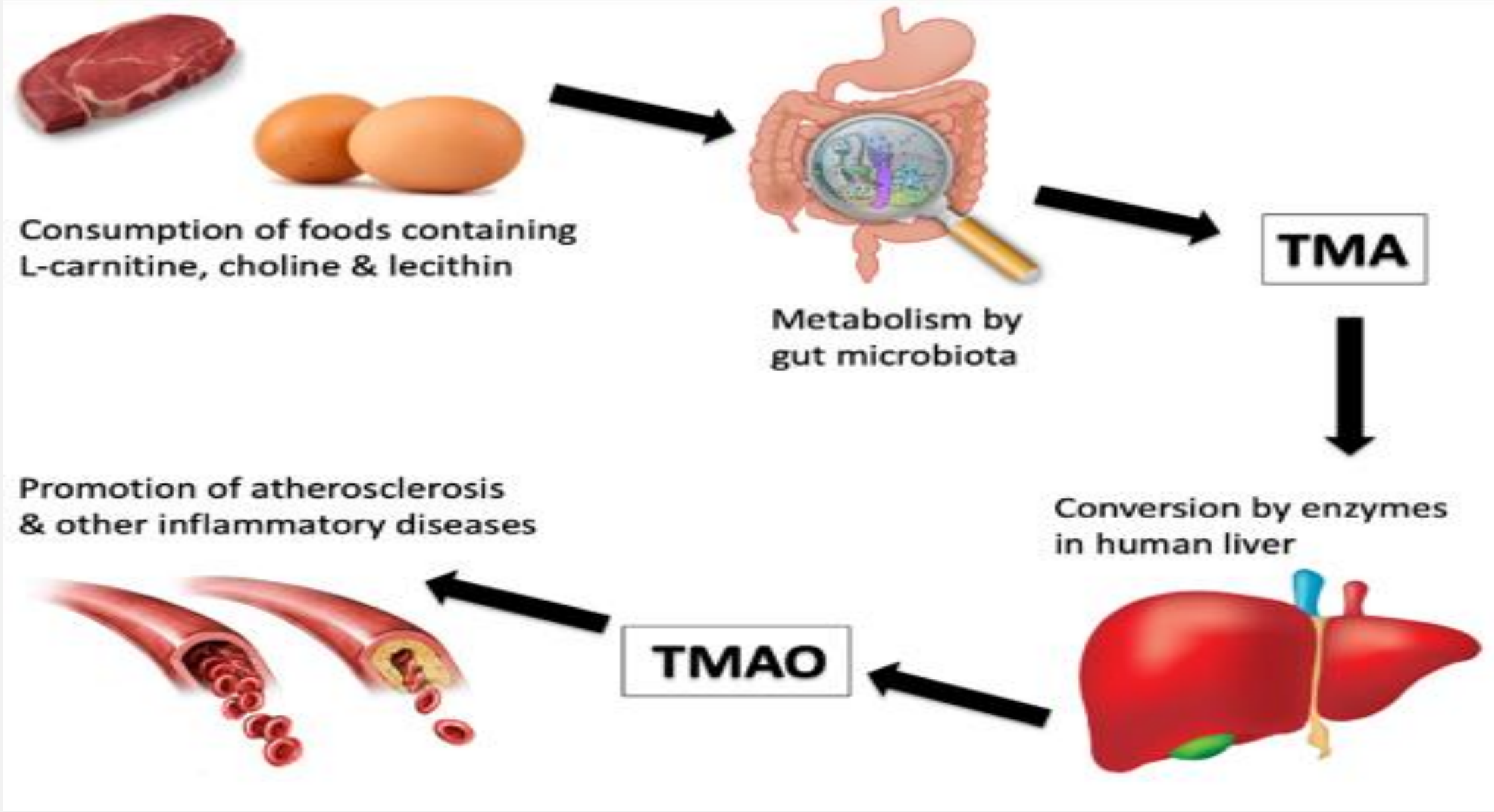


The Heart (T3) – Gut (T2) Connection

- **TMAO possible mechanisms leading to increased MACE**
  - TMAO increases forward cholesterol transport and inhibits reverse cholesterol transport
  - TMAO decreases hepatic bile acid formation leading to decreased cholesterol elimination
  - TMAO increases platelet responsiveness, thus increasing thrombotic potential
  - TMAO may upregulate macrophage scavenger receptors resulting in cholesterol deposition in macrophages and foam cell formation
  - TMAO can induce endothelial dysfunction, the 1st step in the atherosclerosis process

Tang WH, et al. N Engl J Med. 2013; 368(17): 1575-1584.
Tang WH, et al. Circ Res. 2017; 120(7): 1183-1196.
Peng J, et al. Life Sci. 2018; 214: 153-157.
Brown JM, Hazen SL. Nat Rev Microbiol. 2018; 16(3): 171-178.
Zhao Y, Wang Z. Curr Opin Cardiol. 2020; 35(3): 207-218.


Dysbiosis
Disruption of tight junction proteins ↑
Choline/Carnitine
Pre/probiotics
Antibiotics
Obesity
High fat diet
Gut permeability ↑
TMA ↑
LPS
Goblet cell
Tuft cell
Normal gut microbiota
Dendritic cell
TMA ↑
Metabolic
Endotoxemia
TMAO
TLR4
NF-κB
Inflammation
Platelet Aggregation
Foam cell formation
Endothelial Cells
NF-κB ↓
NO ↑
CVD ↑



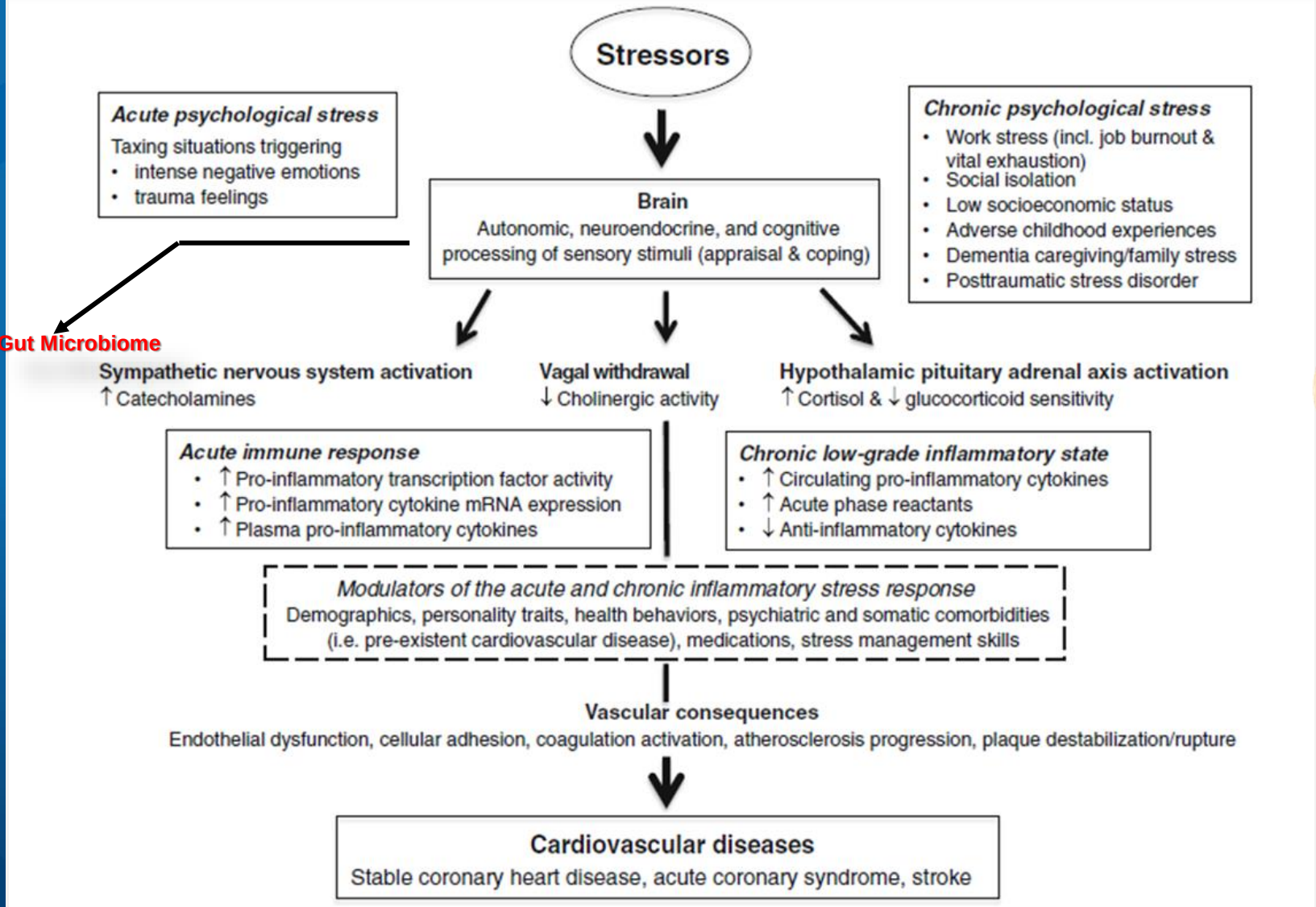
Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013;368:1575–1584.

Key Points

- Since 70-80% of the immune system lives in the gut and CVD is an inflammatory disease, a healthy gut is key to preventing cardiovascular morbidity and mortality
- Dysbiosis and increased gut permeability lead to increased LPS translocation and metabolic endotoxemia; first sign may be TPO antibodies
- TMAO, a western diet metabolite, is a CVD risk predictor that is associated with increased MACE in individuals with and without CAD, including those at lower risk

Key Points

A leaky gut = leaky blood vessels = increased inflammation ➔ increased CVD risk, increased CVD events, and increased CVD mortality



Putting it all together

Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.

Heart – Gut – Brain Connection

Stressors

High-fat diet
(amount, quality, structure)

Gut - Immune

Gut microbiota-targeted nutrition modulation
(e.g., prebiotic, probiotic)

Gut microbiota dysbiosis
(composition/function analysis by pyrosequencing)

Intestinal permeability
(lactulose/mannitol excretion ratio)

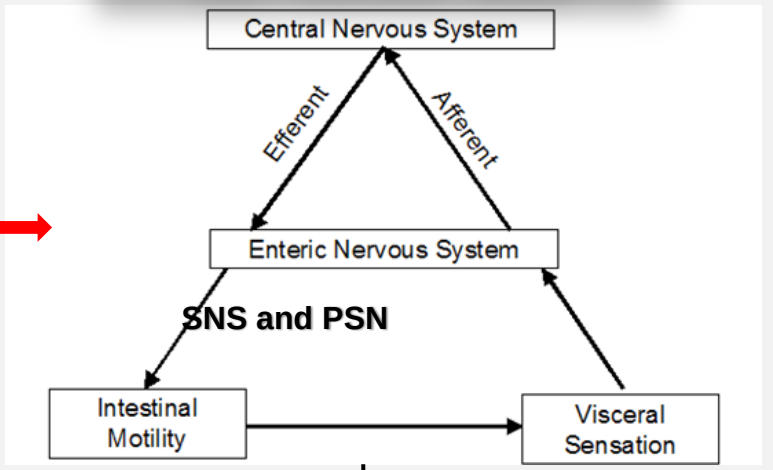
Metabolic endotoxemia
(LPS-binding protein, **LBP**)

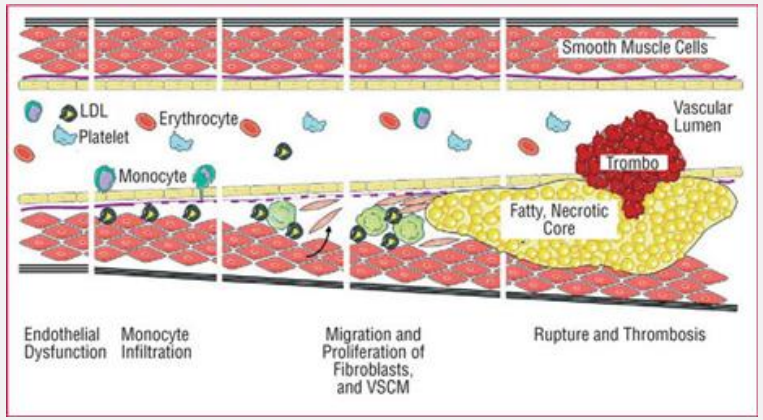
Low-grade inflammation (**CRP**)
Insulin resistance (**insulin, HOMA-IR**)

Obesity, diabetes and other metabolic disorders
(weight, BMI, glycolated hemoglobin, etc)

Cross-reactivity and autoimmunity

Gut – Immune - Brain



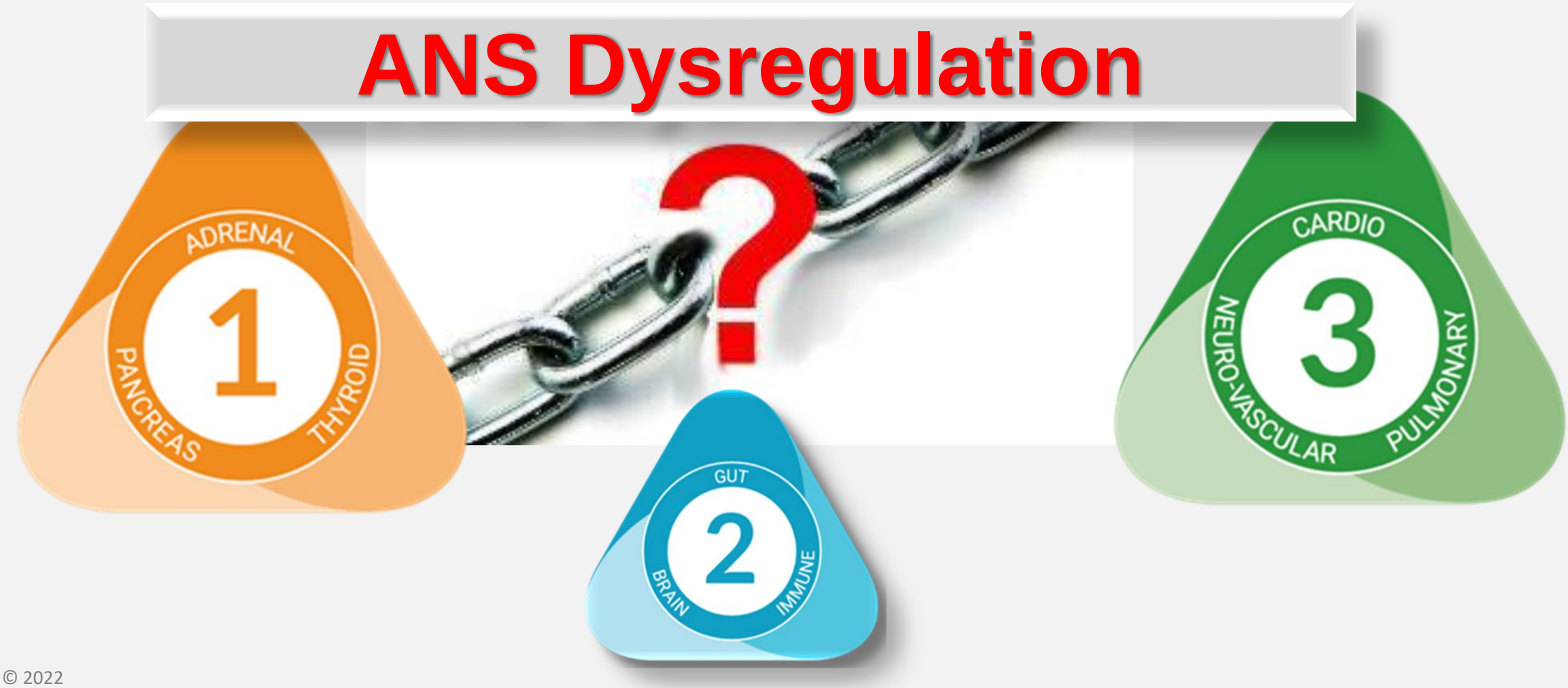


Gut ↔ Immune ↔ Brain ↔ CVD

Cortisol Links Metaflammation to CVDs

What is the mechanism?

Cortisol Links Metaflammation to CVDs



The Heart (T3) – Brain (T2) Connection

The Heart (T3) – Brain (T2) Connection

- A healthy ANS is dynamic and flexible: it adapts and is resilient
- Stress leads to ANS imbalance, i.e., sustained sympathetic activity and/or reactivity, delayed sympathetic recovery, reduced vagal activity
- Resulting in a dramatic shift toward enhanced sympathetic firing and/or withdrawal of vagal tone and higher risks of hypo and hyperkinetic arrhythmias, platelet aggregation, and vascular thrombosis

Tsigos C. J Psychosom Res. 2002; 53(4): 865-71.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.

Sympathetic Overdrive

- Imbalance between sympathetic and parasympathetic nervous systems
- Direct innervation of sinus node
- Dysregulation of vagus nerve in obesity = chronic inflammation
- Increased SNS due to affective disorders, chronic stress, neuroinflammation

Leads to elevation in resting heart rate (RHR)

Pavlov VA, et al. The vagus nerve and the inflammatory reflex – linking immunity and metabolism. Nat Rev Endocrinol. 2012;8(12):743-754.

The Heart (T3) – Brain (T2) Connection

- **The sympathetic nervous system**
  - Directly ↑ HR, ↑ BP, promotes insulin resistance and lipolysis, and stimulates the immune system
- **The parasympathetic nervous system**
  - Assists sympathetic functions by "withdrawing" and/or antagonizing them by increasing its activity
  - Vagal withdrawal and decreased parasympathetic activity give rise to increased pro-inflammatory cytokines, i.e., IL-1β, TNF-α
  - In addition, adipose tissue is also a major contributor to stress-induced increases in proinflammatory markers
- **PNS stimulation decreases proinflammatory cytokines and inflammation**

Tsigos C. J Psychosom Res. 2002; 53(4): 865-71.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.

The Heart (T3) – Brain (T2) Connection

Resting Heart Rate

Heart Rate Recovery Time

Each is a strong predictor of CV risk and all-cause mortality

Heart Rate Variability

The Heart (T3) – Brain (T2) Connection

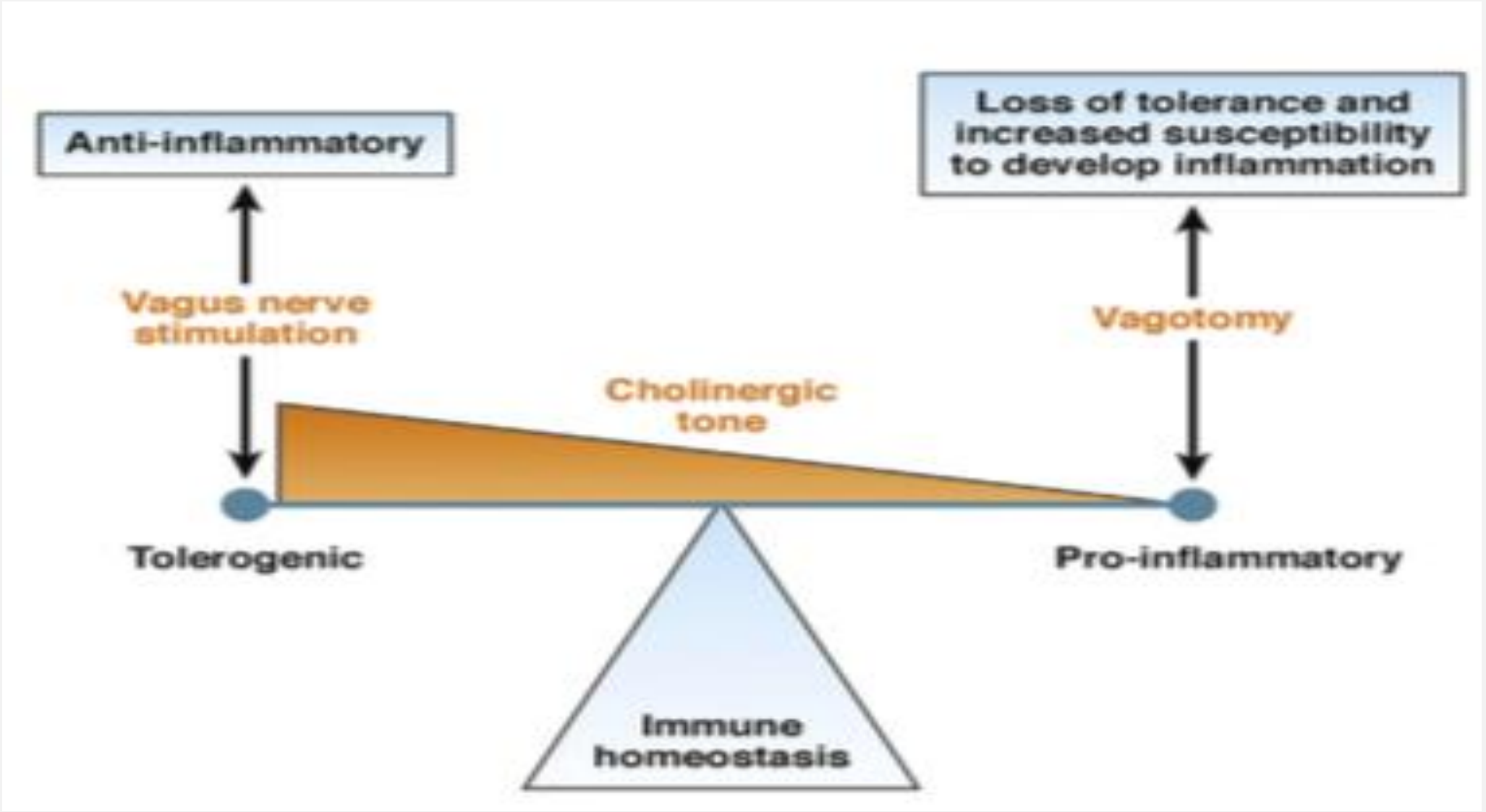
- **Resting Heart Rate (RHR)**
  - There is a remarkably strong association between heart rate and survival
  - A normal resting heart rate is 62 beats/min
  - As heart rate ↑ to 75 to 80 b/min, there are marked increases in total mortality and CAD mortality
  - For every increase in HR by 4 beats/minute the CHD risk increases 7-10%
- **Beta Blockers (BBs) and RHR**
  - The evidence is clear: BBs reduces mortality in AMI and in MI survivors
  - The magnitude of mortality reduction with BBs is directly proportional to the magnitude of heart rate decrease

Lauer M. Cleve Clin J Med. 2009; 76 Suppl 2:S18-22.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.

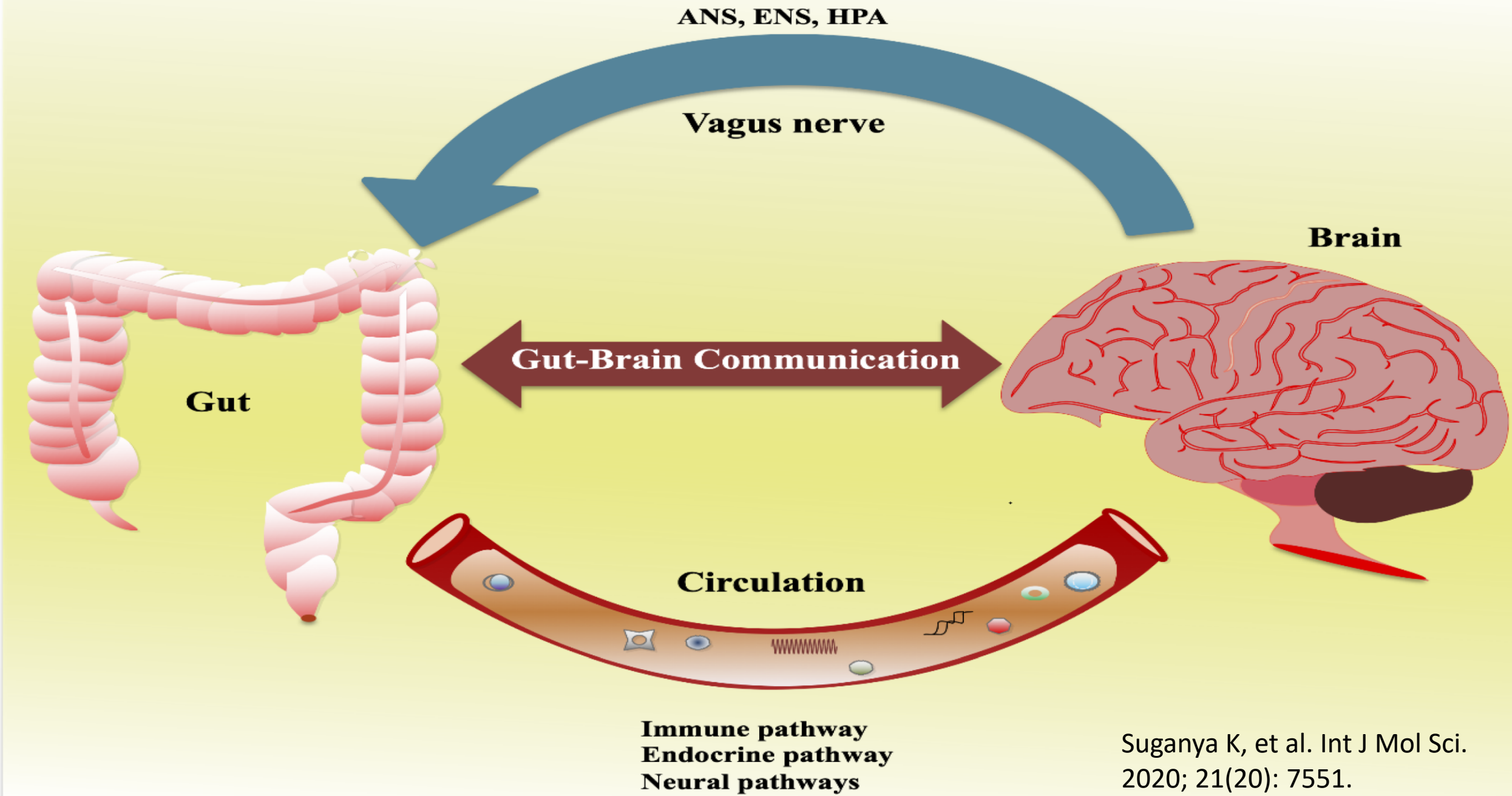
The Heart (T3) – Brain (T2) Connection

- **Heart Rate Recovery Time**
  - An optimal HRRT is a decrease in HR from peak to 1 minute by > 12 b/min
    - Peak HR = 160 b/min, an abnormal HRRT at 1 min = HR > 148 b/min
  - Low HRRT strongly predictive of SCD, not non-sudden cardiac death
  - PVCs early in recovery associated with increased mortality
  - The link between HRRT, mortality, and CV prognosis appears to be independent of symptom status, exercise protocol, LVEF, and CAD severity
  - The evidence suggests that the link between HRRT and mortality may be a reflection of the PSN system's antiarrhythmic properties
- **HRRT is thought to be a function/measure of PSN activity**

Lauer M. Cleve Clin J Med. 2009; 76 Suppl 2:S18-22.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.



Browning, et al. Gastroenterology. 2016; pii: S0016-5085(16)35487-7.



Suganya K, et al. Int J Mol Sci. 2020; 21(20): 7551.

The Heart (T3) – Brain (T2) Connection

- **Decreased PS tone linked to CVD risk factors and CVD**
  - Clinically, decreased PNS tone or increased SNS tone has been linked to
    - Obesity, IR, DM, HTN, HLD, depression, anxiety, heart failure, and PVD
  - Minor degrees of glucose intolerance associated with autonomic abnormalities
    - Among patients enrolled in a population-based cohort, the likelihood of an abnormal HRRT increased linearly as fasting plasma glucose increased from 70 to 80 to ≥ 90 mg/dL
    - Even at levels of plasma glucose that would be considered normal, the likelihood of an abnormal HRRT increased as plasma glucose increased
    - Strong association between FBS and HRRT at RHR ≥ 80 b/min, minimal association < 80 b/min
- **FBS is strongly and independently associated with abnormal HRRT, even at nondiabetic levels, particularly when RHR ≥ 80 b/min**

Lauer M. Cleve Clin J Med. 2009; 76 Suppl 2:S18-22.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.

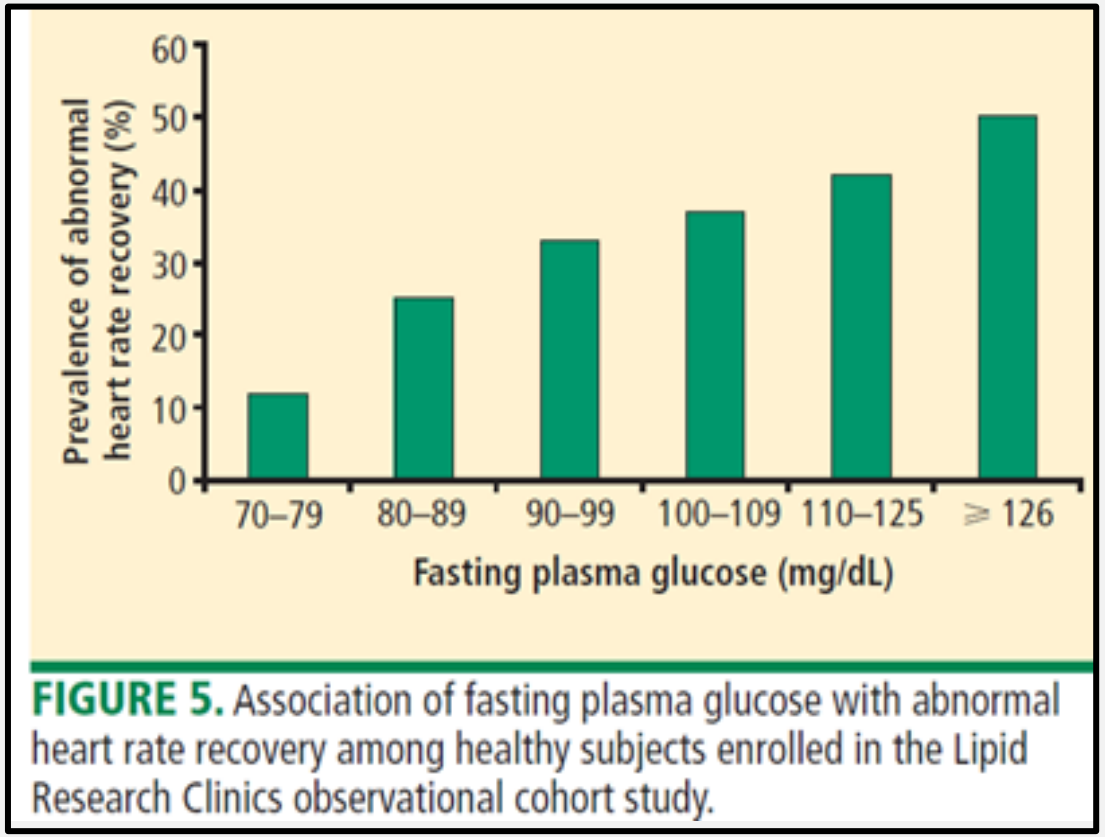


FIGURE 5. Association of fasting plasma glucose with abnormal heart rate recovery among healthy subjects enrolled in the Lipid Research Clinics observational cohort study.

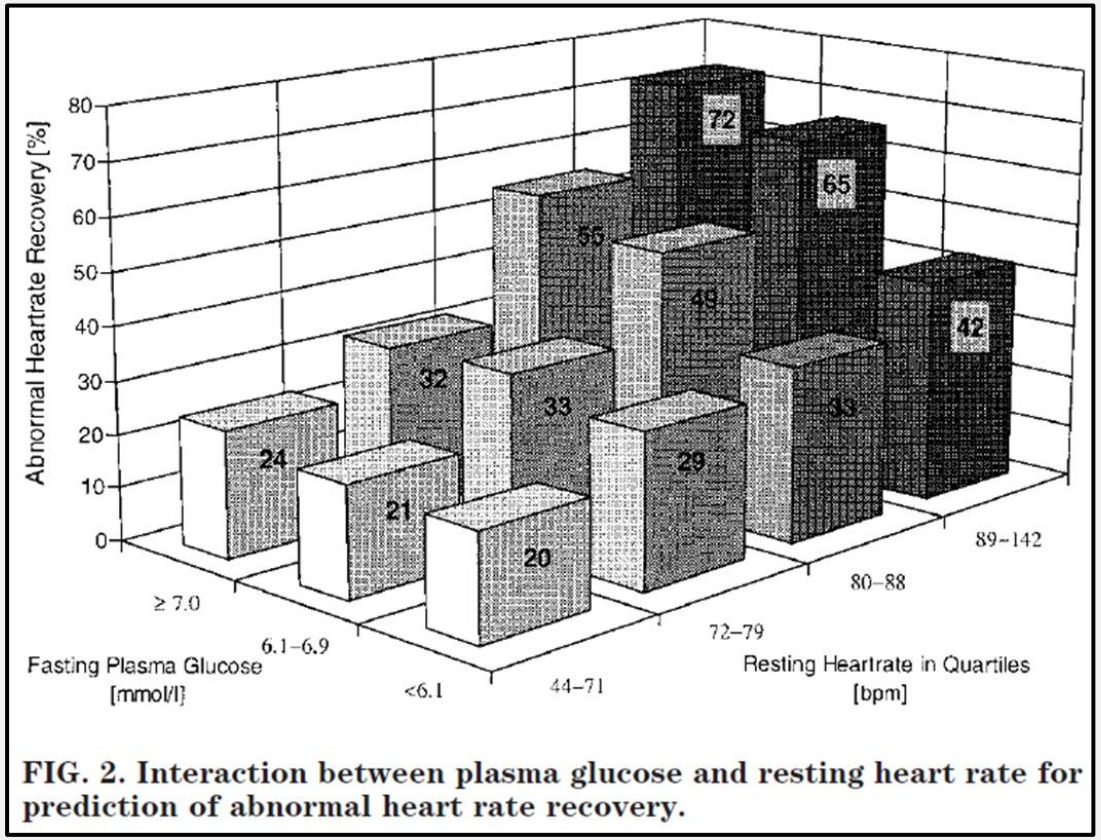


FIG. 2. Interaction between plasma glucose and resting heart rate for prediction of abnormal heart rate recovery.

- CCS, 5190 healthy adults w/o CVD, DM, PVD, arrhythmias
- 12-year study; 635 met criteria for impaired FBS or untreated DM
- HRRT was defined as the change from peak heart rate to that after 2 min of recovery; an abnormal value was <42 b/min
- Those with low HRRT had more CVD RF
- FBS associated with impaired HRV and RHR

Lauer M. Cleve Clin J Med. 2009; 76 Suppl 2:S18-22.
Panzer C, et al. Diabetes. 2002 Mar;51(3):803-807.

Heart Rate Variability

- Decreased HRV linked to cardiac events and mortality
- Among healthy elderly subjects enrolled in the **Framingham Heart Study**, decreased HRV associated with increased major cardiac events

LAUER M S Cleveland Clinic Journal of Medicine 2009;76:S18-S22

The Heart (T3) – Brain (T2) Connection

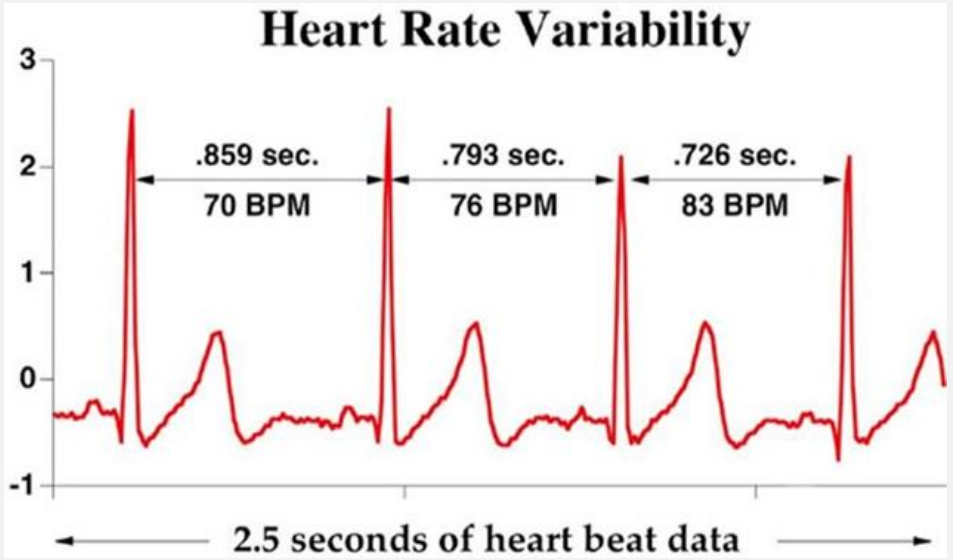
- **Heart Rate Variability negatively affects health outcomes**
  - Independent predictor of MI and SCD
    - Low HRV: SNS predominance and increased CV events and mortality
    - High HRV: PNS predominance and decreased CV events and mortality
  - HRV and HTN
    - SNS hyperactivity and PNS underactivity are central components in the etiology of early and borderline hypertension, as well as sustained essential hypertension
    - A hyperactivity SNS is found in hypertensive males and females, in the young and elderly
  - Associated with immune dysfunction and inflammation
    - PSN decreases inflammatory cytokines; decreased HRV (decreased vagal tone) leads to increased cytokine production and inflammation
- **HRV provides useful information on the body's adaptability to stressful situations**

Lauer M. Cleve Clin J Med. 2009; 76 Suppl 2:S18-22.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.
Guan L, et al. Front Neurol. 2018; 9: 90.

The Heart (T3) – Brain (T2) Connection

- Obesity
- IR and diabetes
- Borderline HTN and HTN
- Hyperlipidemia
- CAD
- CVD (TIA, stroke, HF)
- PVD
- Anxiety and depression

- Chronic ds associated with ↓ HRV



Lauer M. Cleve Clin J Med. 2009; 76 Suppl 2:S18-22.
Wirtz P, von Kanel R. Curr Cardiol Rep. 2017; 19(11): 111.
Fioranelli M, et al. Front Immunol. 2018; 9:2031.
Guan L, et al. Front Neurol. 2018; 9: 90.

The Heart (T3) – Brain (T2) Connection

- **The heart is not a metronome**
  - We were taught that the heart responds to signals from the brain
  - Actually, the heart sends more signals to the brain than vice versa
  - Heart signals significantly influence emotional processing and higher cognitive functions, i.e., attention, perception, memory, etc.
- **Psychosocial coherence leads to optimal health**
  - Cardiac coherence
    - The two branches of the ANS synchronize with one another, and there is an overall shift in autonomic balance toward increased parasympathetic activity
    - There is also increased physiological entrainment—a number of different bodily systems synchronize to the rhythm generated by the heart
- **Finally, there is ↑'ed synchronization between the heart and brain**

Thayer JF, et al. 2010; Int J Cardiol. 2010; 141(2): 122-131.
Schaffer F. Front Psychol. 2014 Sep 30; 5: 1040.

The Heart (T3) – Brain (T2) Connection

- **Stress and negative emotions lead to psychosocial incoherence**
  - Heart rhythm is erratic and disordered
  - Corresponding CNS signals inhibit higher cognitive function
  - Can't think clearly, remember, and/or make effective decisions leading to impulsive behavior
  - The heart's input to the brain also affects brain's emotional processing, reinforcing the emotional experience
- **Learning to generate and maintain psychosocial coherence will improve RHR, HRRT, and HRV and decrease cardiovascular morbidity and mortality!**

Thayer JF, et al. 2010; Int J Cardiol. 2010; 141(2): 122-131.
Schaffer F. Front Psychol. 2014 Sep 30; 5: 1040.

The Heart (T3) – Brain (T2) Connection

- **Psychosocial coherence is not relaxation**
  - At the physiological state
    - Relaxation is characterized by an overall reduction in autonomic outflow and a shift in ANS balance towards increased parasympathetic activity, not heart – brain synchrony
    - Coherence is physiologically distinct from relaxation in that the system oscillates at its natural resonant frequency and there is increased harmony and synchronization in nervous system and heart–brain dynamics
  - **At a psychological level**
    - Relaxation is a low-energy state: the individual rests both the body and mind, disengaging from cognitive and emotional processes
    - Coherence involves the active engagement of positive emotions

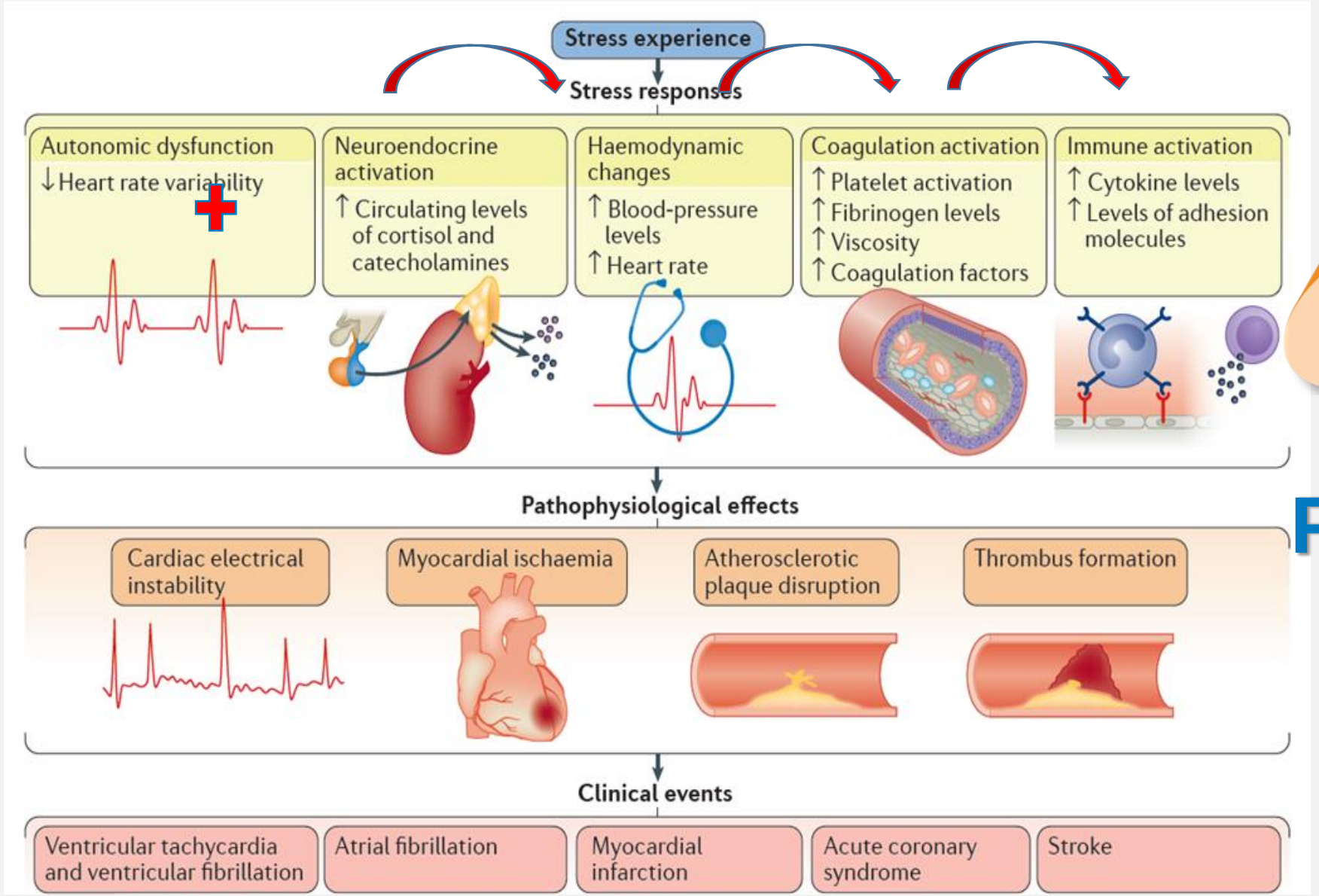
Thayer JF, et al. 2010; Int J Cardiol. 2010; 141(2): 122-131.
Schaffer F. Front Psychol. 2014 Sep 30; 5: 1040.

Blood Pressure: Key to Global Dysfunction

- **120/80** ideal
- **119/65** is good
- Low blood pressure adrenal hypocortisolism Sodium serum <139
- **130 systolic**: Elevated blood pressure shows epi/norepi dominance reflective of insulin resistance and cortisol dominance

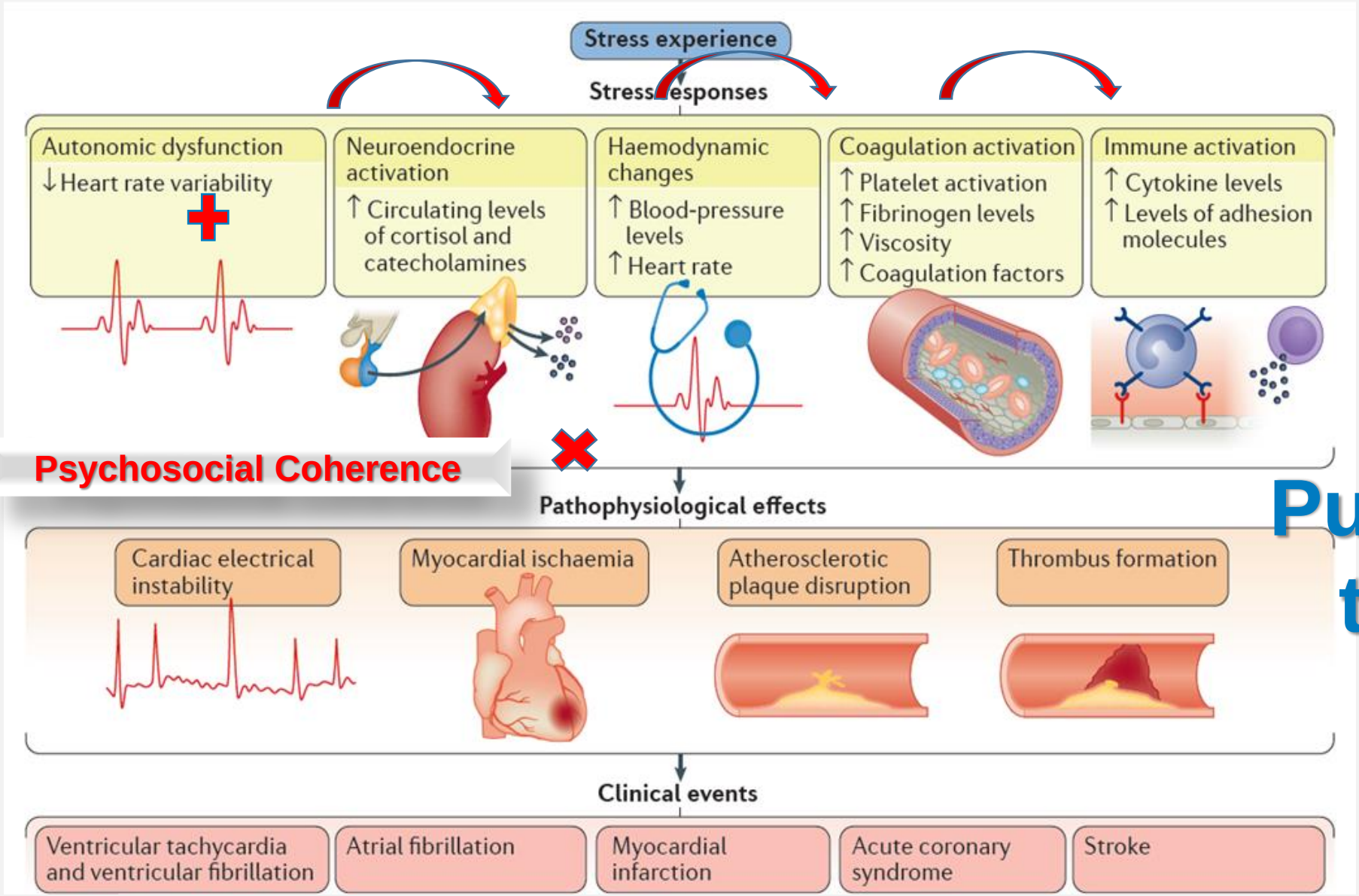
Decreased Vagal Tone and HRV

- Obesity
- Insulin resistance and diabetes
- Hypertension
- Hypercholesterolemia
- Depression and anxiety
- Heart failure
- Peripheral vascular disease



Putting it all together

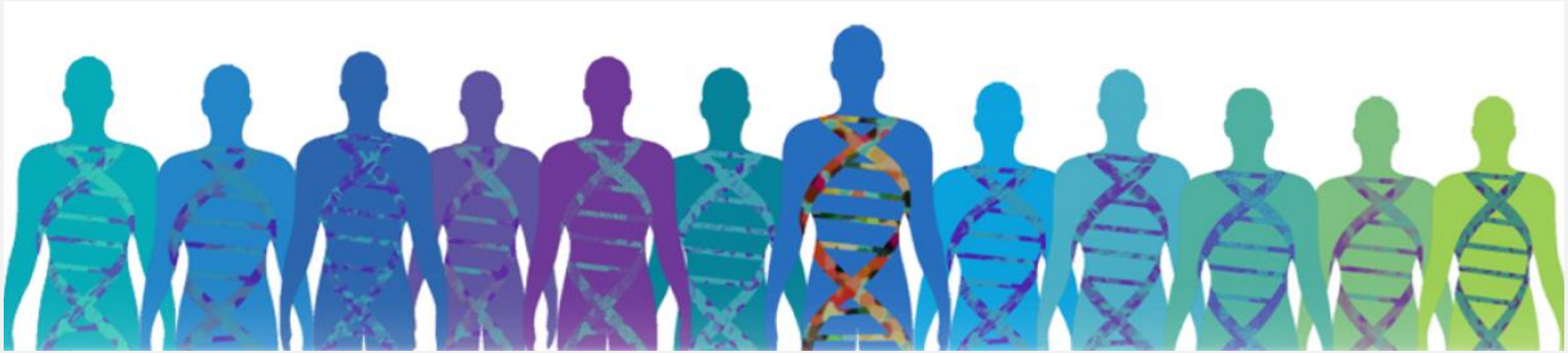
Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.



Putting it all together

Kivimaki M, Steptoe A. Nat Rev Cardiol. 2018; 15(4): 215-229.

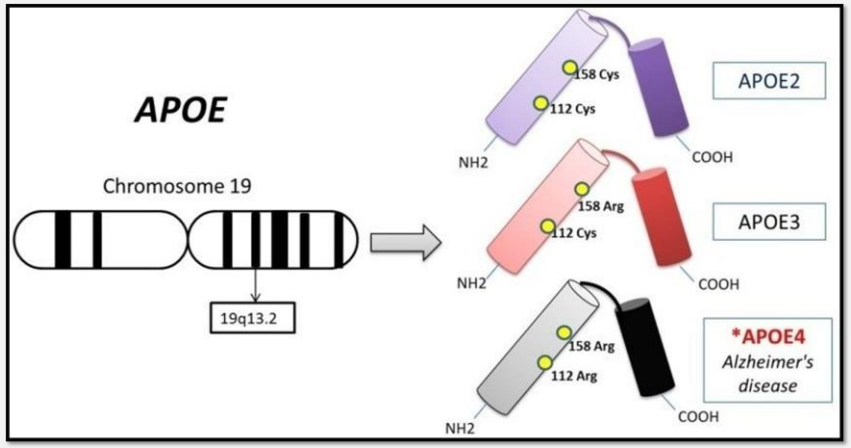
Selected LABS and Supplements



Apolipoprotein E (APOE)

- **Found on chylomicrons (CM), CM remnants, VLDL, HDL, NOT LDL**
- **3 Isoforms: Apo E 2, 3, 4**
  - Apo E3 is most common, occurring ~ 77% of the population
  - Apo E2 occurs in ~ 8%
  - Apo E4 occurs in ~ 15%

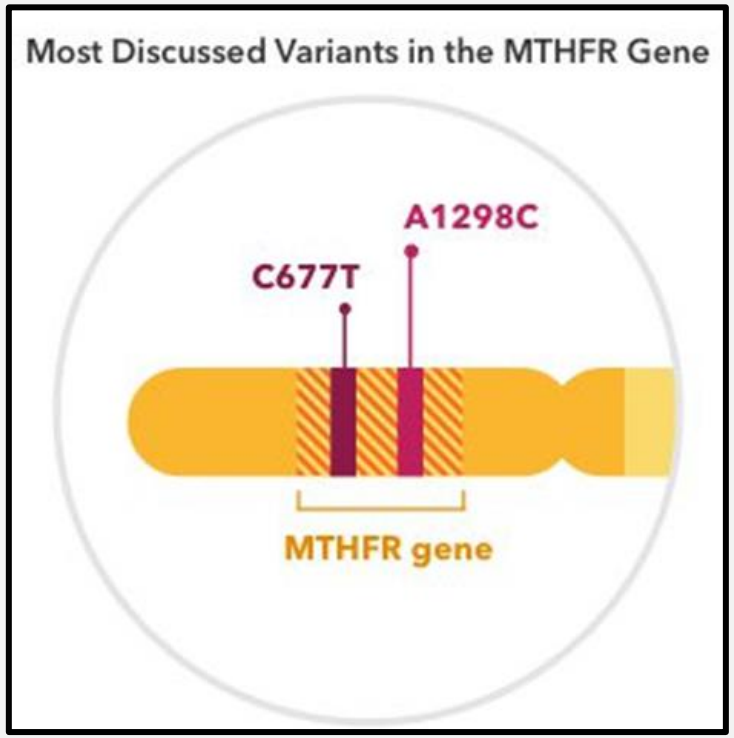
– **Apo E 4**
  - Apo E4 associated with increased CVD and AD
  - Those w/ E4 do not live as long as those with E2 or E3
    - Apo E4 carriers decrease with age
  - E4 is associated with an overactive proinflammatory response
  - Less effective at down-regulating microglia and peripheral macrophages → increased pro-inflammatory cytokine release



Dose J, et al. Lipids Health Dis. 2016; 15: 121.

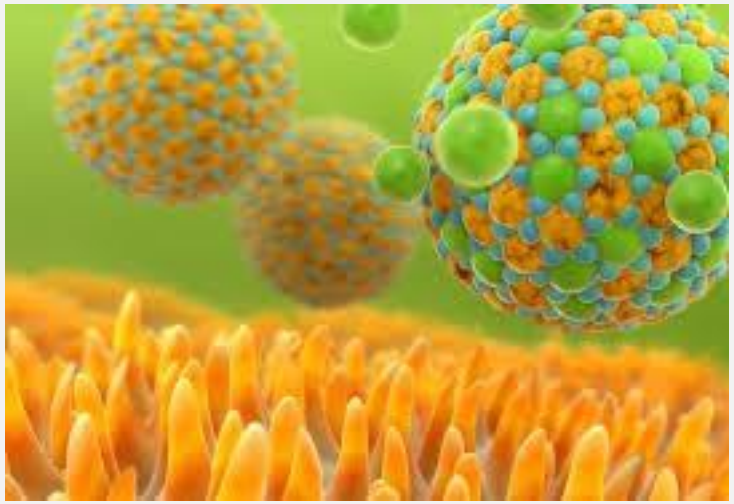
MTHFR

- **MTHFR C677T**
  - The most common cause of elevated homocysteine is a point mutation of the 677 nucleotide
  - Double SNP positive individuals have < 50% of the MTHFR activity and elevated homocysteine
  - Double SNPs associated with increased risk of CAD
- **MTHFR A1298C**
  - Unlike MTHFR C677T, the A1298C mutation does not usually lead to elevated homocysteine levels
  - This reaction helps generate (redox) BH_4
    - BH_4 is important for endothelial function as well as detoxification
    - BH_4 is a cofactor in the production of nitric oxide (NO)
    - BH_4 is also the rate limiting factor for the production of neurotransmitters and catecholamines (serotonin, melatonin, dopamine, norepinephrine, and epinephrine)
    - If limited BH_4, will prioritize detoxification

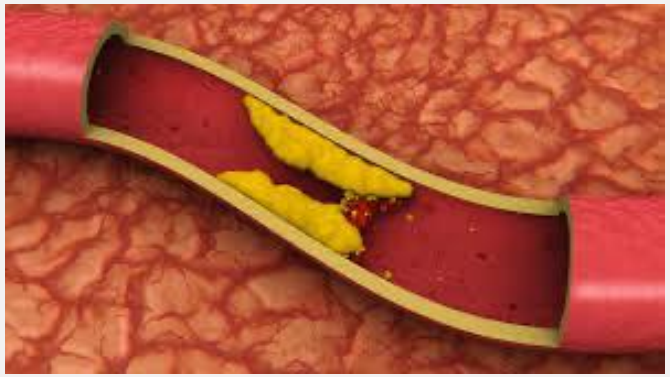


Blom HJ. Smulders Y. Inherit Metab Dis. 2011; 34(1): 75-81.
Crider KS, et al. Adv Nutr 2012; 31(1): 21-38.

Oxidized LDL (oxLDL)

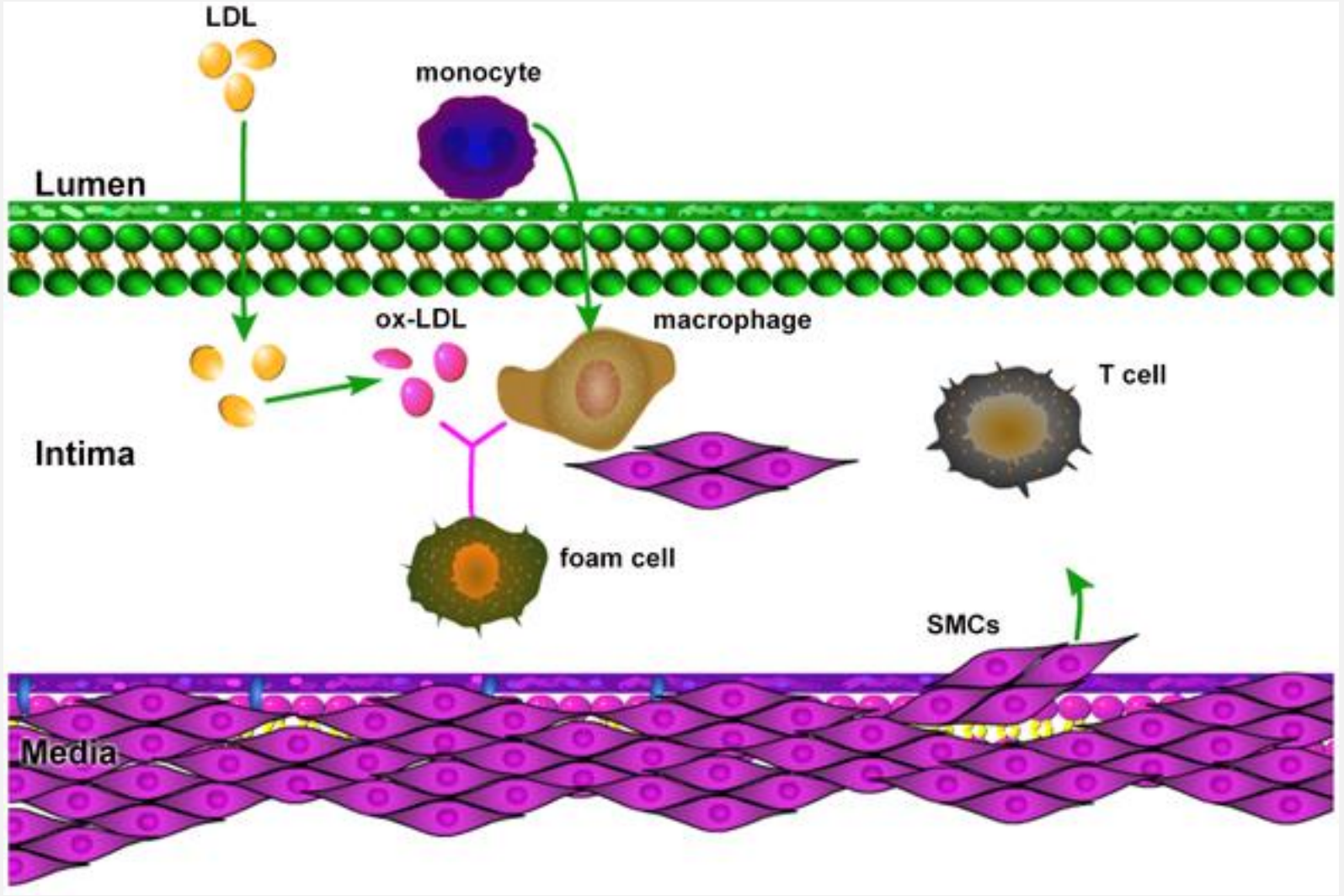


- Very proinflammatory
- Transforms macrophages into foam cells
  - Major constituent of arterial plaque
- Increased levels correlate with increased risk of coronary artery disease (CAD)
- MetS risk increased 4x w/ increase oxLDL
- Levels increase as CAD severity increases
- Range < 60 U/L



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

oxLDL


LDL
monocyte
Lumen
ox-LDL
macrophage
T cell
Intima
foam cell
SMCs
Media

LDL-Particle Number (LDL-P)

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

Reference Values

LDL Particle Number

LDL-P <1000 nmol/L

Low:	<1000 nmol/L
Moderate:	1000 - 1299
Borderline-High:	1300 - 1599
High:	1600 - 2000
Very High:	>2000

Mayo Clinic, 2018

Lipoprotein(a) (Lpa)

- LDL-like particle consisting of ApoA moiety and 1 molecule of $ApoB_{100}$
- Elevated Lpa associated with increased CV disease risk
- T2D patients and Lpa risk inverse relationship
- Low Lpa = increase T2D risk

Gubjartsson DF, et al. Lipoprotein(a) concentration and risks of cardiovascular disease and diabetes. J AM Coll Cardiol. 2019;74(24).

Lpa

- Quest/LabCorp - CV disease risk
  - < 75 nmol/L
  - 75-125 moderate risk
  - > 125 high risk
- T2D risk indicator also

Gubjartsson DF, et al. Lipoprotein(a) concentration and risks of cardiovascular disease and diabetes. J AM Coll Cardiol. 2019;74(24).

Apolipoprotein B (ApoB)

- Indicator of CAD
- Superior to LDL for marker of vascular disease
- Elevated even in presence of normal LDL
- Quest/LabCorp range
  - Optimal < 90 mg/dL
  - 90-129 moderate risk
  - 130 and > high risk
- Elevated levels also risk for T2D

Ley SH, et al. Association of Apolipoprotein B with incident type 2 Diabetes in an aboriginal Canadian population. Clin Chem. 2010;56(4):666-70.

GlycA

- Novel marker for systemic inflammation and CVD 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

- Zonulin family proteins 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

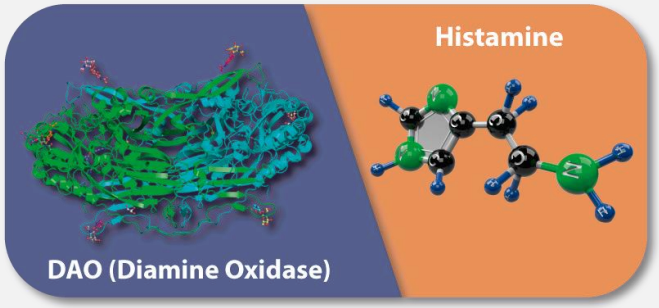
Drago S, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 2006;41(4):408-19.

Zonulin

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

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

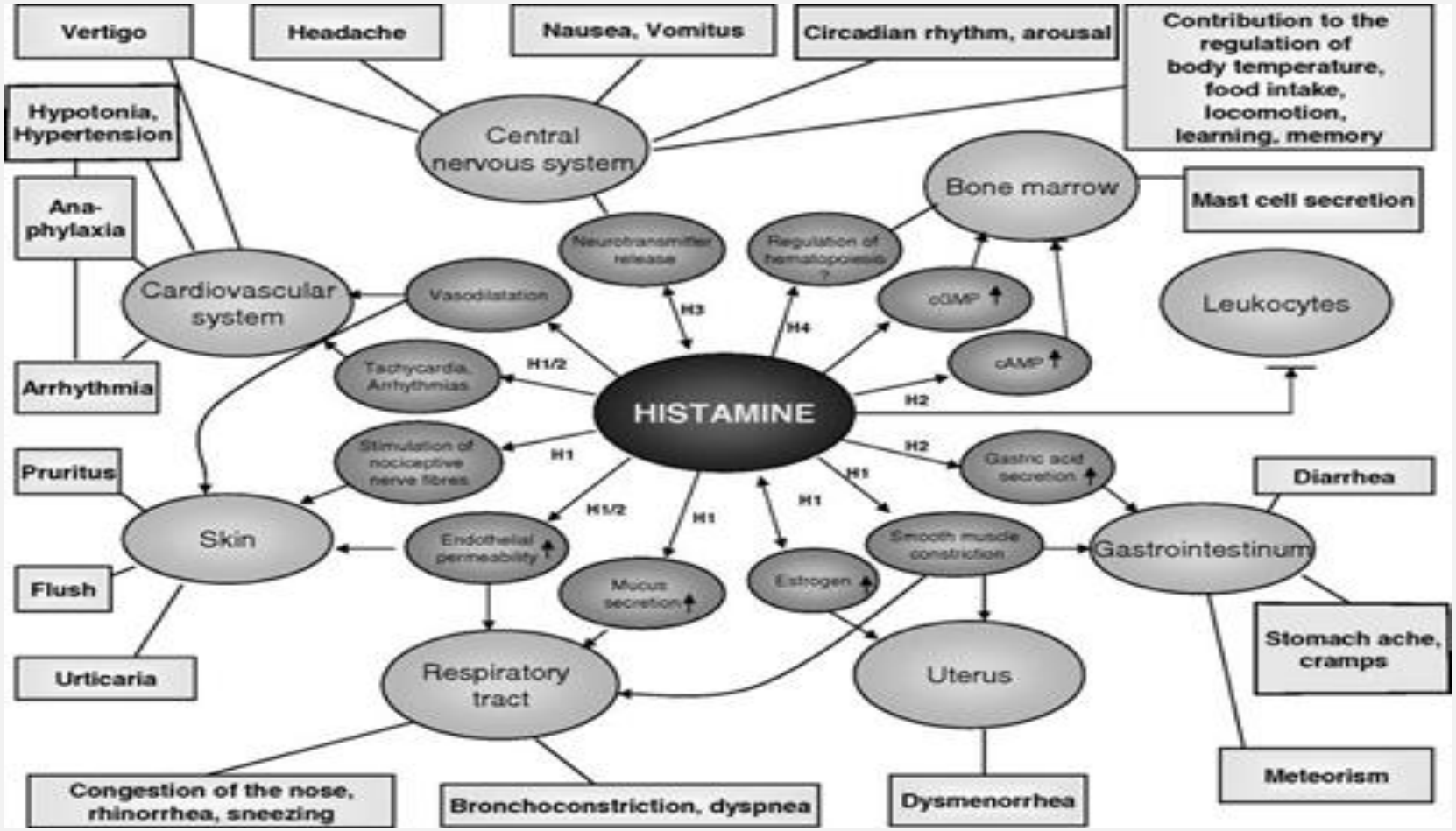
Histamine Testing



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

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

Histamine Intolerance Wide-Reaching Symptoms



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

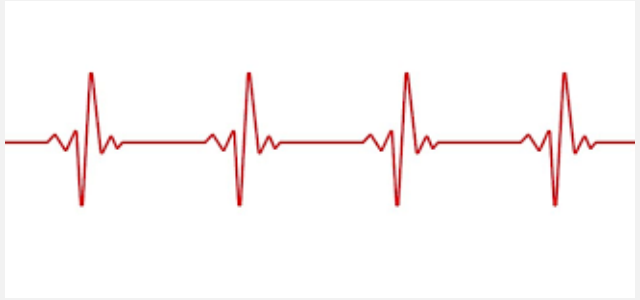
Histamine Testing

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

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

Supplement Support for TRIAD 3

- Supports the Cardiovascular system
- Supports blood pressure and vascular health
- Improved nerve impulses from brain to heart – supports vagal tone
- Lipid support



CardioPulmonary

Supplements

- Fish oil
- Magnesium Taurate
- Hawthorn
- Carnitine
- d-Ribose
- CoQ10
- Vitamin K2
- Grape Seed Extract
- Aged Garlic Extract
- Vitamin C buffered
- Chelation therapy
- Quercetin
- Bilberry
- Nattokinase

Vascular

- Tocotrienols
- Plant sterols
- Thai ginseng
- Hawthorn
- Cocoa
- Aged Garlic Extract
- Fish oil
- Krill Oil
- ER niacin
- Milk peptides
- Arginine
- Red Yeast Rise
- CoQ10
- Vitamin D
- Borage oil
- Pleo Muc
- Magnesium Taurate
- HCSE
- Quercetin
- Pomegranate
- EGCG

Neuro

- Huperzine/GPC
- Alpha Lipoic Acid
- Cistanche
- Ginkgo
- Vinpocetine
- Resveratrol
- Rg3
- αGPC
- Aged Garlic Extract
- St. John’s Wort
- Saffron
- SAMe
- Kava
- Holy Basil
- L-theanine
- Cerebrum compositum
- EGCG
- Breathing techniques
- Folate/B6/B12/MTHF

Aged Garlic Extract

- Proprietary garlic fermentation
- Supports cardiovascular health
- Over 900 clinical studies and papers supporting uses
- Clinical lowers blood pressure – 16%
- Supports blood vessel integrity
- Supports immunity
- Supports healthy blood lipid levels – LDL, HDL, LDL-P
- Decreases metaflammation
- Decreases coronary artery calcification
- Improves microbiome

Why Is Aged Garlic So Effective?: The Obvious and the Subtle

Obvious

- Improve Nitric Oxide
- Antioxidant effect ↓ox-LDL ↓LDL ↓Fibrinogen ↑HDL ↓homocysteine
- Improve endothelial dynamics

Subtle

- Reduce Epinephrine and Norepinephrine effects
- Antimicrobial effect
- Improve Nitric Oxide
- Reduce Epinephrine and Norepinephrine effects
- Antimicrobial effect
- Antioxidant effect
- Improve endothelial dynamics

Aged Garlic Extract

- Improves homocysteine levels
- Neuroprotective
- Improves gingival health – decreases inflammation and bleeding
- No odor or taste
- 1-2 caps 2 times daily (600-1,200mg)
- Does NOT interact with anticoagulants unlike other garlic preparations

New Research
AGE and Reducing Chronic Inflammation

- 2019 double-blind, placebo controlled randomized clinical study
- n= 51 healthy but obese adults
- 3.6gm AGE daily in divided doses x 6wk
- IL-6 and TNF-alpha significantly lower in AGE vs. placebo
- Increased gamma-delta T cells – modulated immunity
- Significant reduction in LDL cholesterol

Xu C, et al. Aged garlic extract supplementation modifies inflammation and immunity of adults with obesity: A randomized, double-blind, placebo-controlled clinical trial. Clin Nutr ESPEN. 2018;24:148-55.

New Research
AGE Reduces Plaque in Coronary Arteries

- Single-center, randomized, placebo-controlled, double-blind trial at Harbor UCLA Medical Center
- Published in Feb. 2020 Experimental and Therapeutic Medicine
- n= 66 final patients with diabetes mellitus
- 30-75 years, HbA1c>6.5%, Fasting blood glucose>125mg/dl)
- 2,400mg AGE or placebo x 12 months
- RESULTS:
  - AGE suppresses adverse cardiovascular events by reducing low-attenuation plaque, decreasing left ventricular mass and improving endothelial function

Shaikh K, et al. Aged garlic extract reduces low attenuation plaque in coronary arteries of patients with diabetes: A randomized, double-blind, placebo-controlled study
Exp Ther Med. 2020;19(2):1457-61.

New Research – Aged Garlic Extract Improves Microcirculation

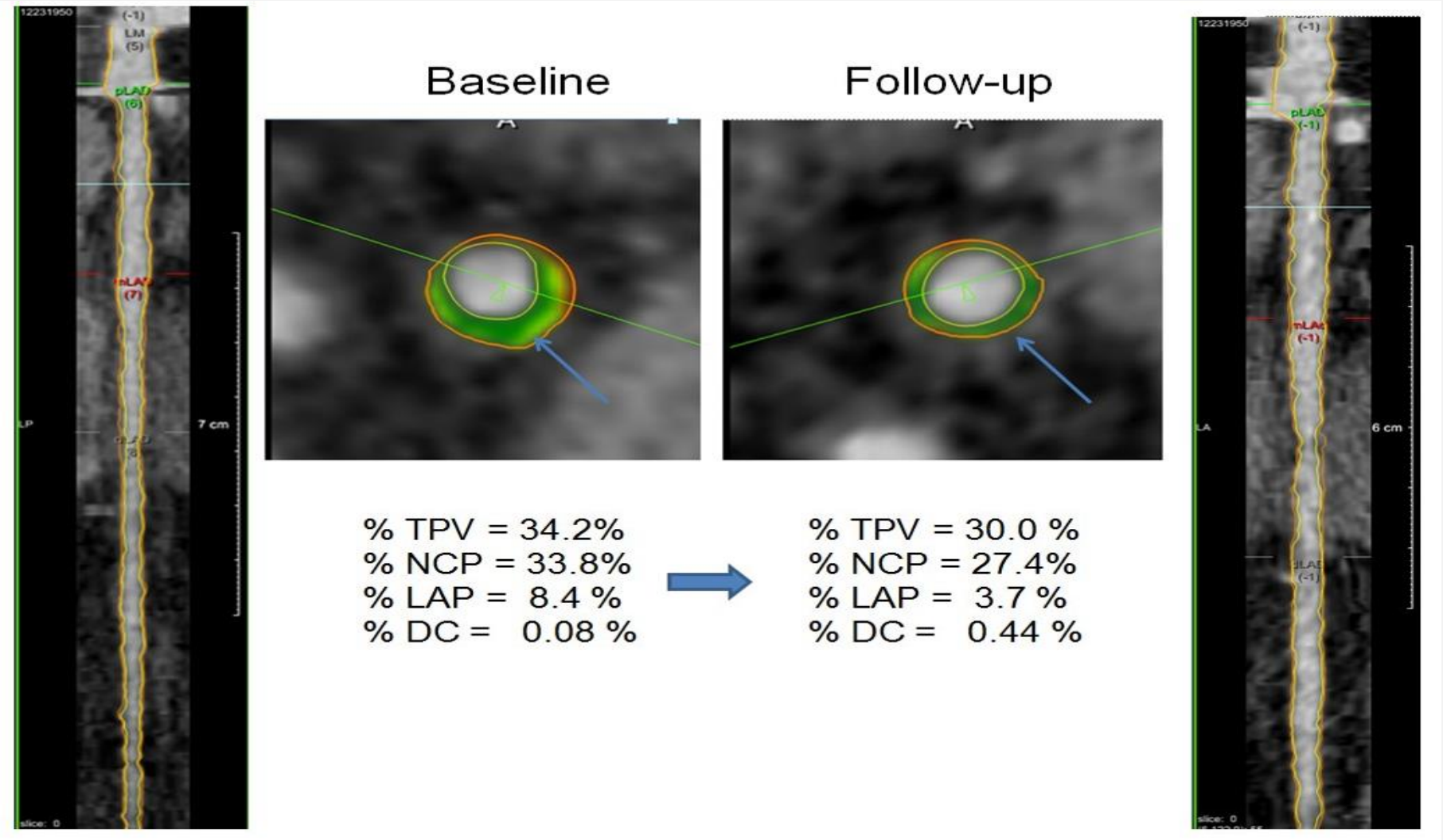
- 2019 double-blind, placebo-controlled study
- n= 93 patients aged 40-75 w/ Framingham Risk Score ≥ 10, CAC score > 10 completed test
- 2,400mg AGE daily for 12 months
- RESULTS:

★ *AGE suppresses adverse cardiovascular events by reducing CAC progression in not only non-Europeans but also European population*

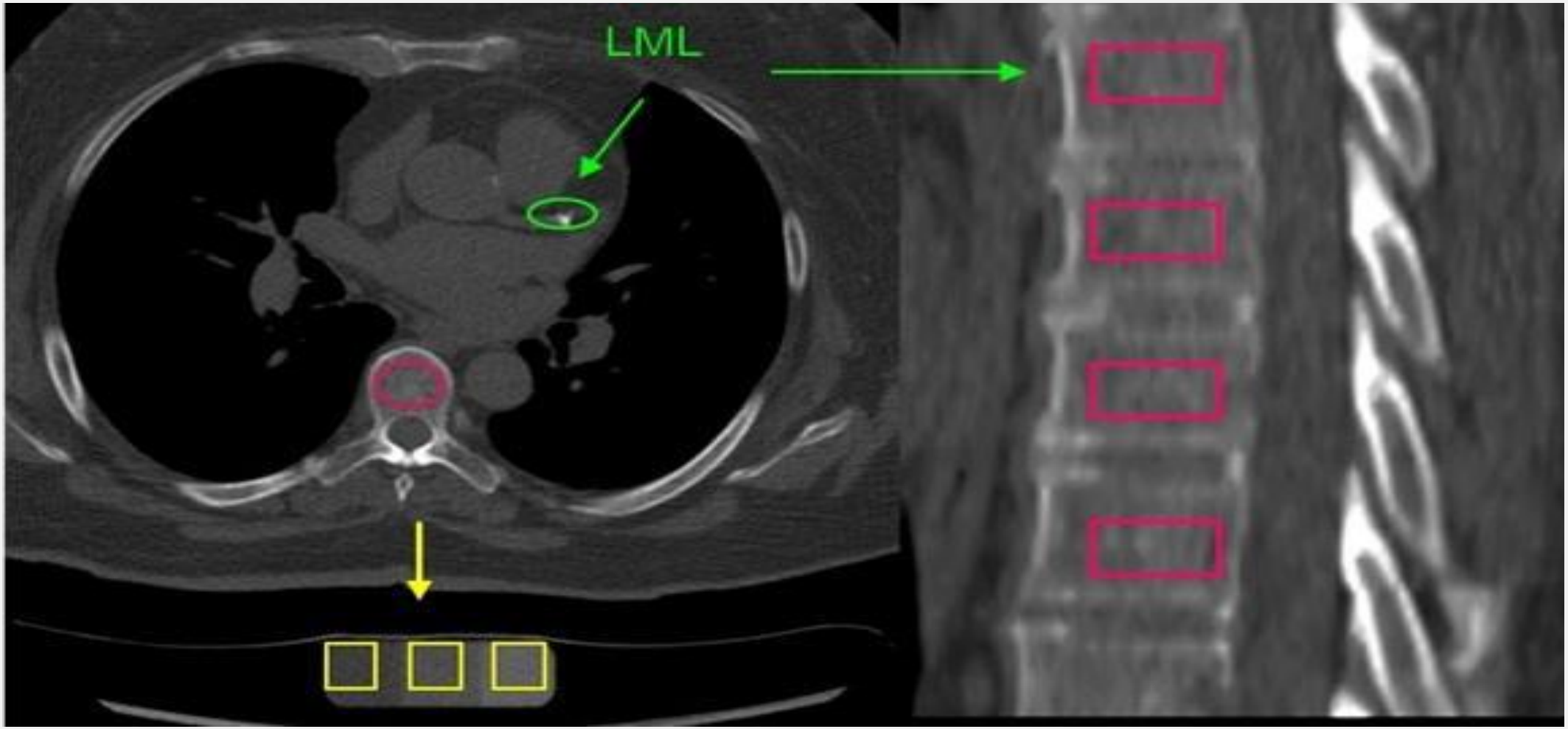
★ *AGE facilitates wound healing by increasing the level of microvascular blood flow*

Wlosinka M, et al. Aged garlic preserves cutaneous microcirculation in patients with increased risk for cardiovascular diseases: a double-blinded placebo-controlled study. Int Wound J. 2019;16(6):1487-93.

Age – Regression of Plaque



Bone Mineral Density and Age



New Research
Aged Garlic Extract Improves **Microbiome**

- 2018 double-blind randomized placebo-controlled 12 wk trial
- n= 49 patients w/ uncontrolled hypertension
- 1,200mg AGE daily
- RESULTS:
  - AGE significantly lowered central blood pressure (systolic 10± 3.6mmHg and diastolic 5.4 ± 2.3 mmHg compared to placebo
  - Decreased pulse pressure and arterial stiffness
  - Decreased inflammatory markers TNF-α and IL-6 – results need to be confirmed in larger trials
  - AGE improved gut microbiota, evident by higher microbial richness and diversity with a marked increase in *Lactobacillus* and *Clostridia* species after 3 months of supplementation

Ried K, et al. The Effect of Kyolic Aged Garlic Extract on Gut Microbiota, Inflammation, and Cardiovascular Markers in Hypertensives: The GarGIC Trial. Front Nutr. 2018;5:122.

Aged Garlic

Cardiovascular Benefits in Clinical Study Examples

Revised Jan., 2013

	Change (%)
Blood Pressure (Systolic) [1-4]	↓ 6-10
HDL (good) Cholesterol [5,6]	7-25 ↑
Homocysteine [6,7]	↓ 18-27
LDL (bad) Cholesterol [1,6,8-10]	↓ 5-26
Oxidized LDL (bad Cholesterol) [6] LDL Oxidation (resistance for bad cholesterol formation) [11]	↓ 35-69 100 ↑
Oxidative Stress [12]	↓ 29-48
Platelet Adhesion [8,13] (sticky blood)	↓ 30-58
Platelet Aggregation [8,13,14] (sticky blood)	↓ 10-25
Total Cholesterol [1,2,6,8-10,15,16]	↓ 6-18
Triglyceride [1,2,10]	↓ 10-23
Vascular function (vascular elasticity) [6,7,17,18]	15-111 ↑
Coronary artery calcification [6,19,20]	↓ 45-78

References:

1) Steiner et al., Am. J. Clin. Nutr. (1996).
2) Steiner et al. Shinyaku To Rinsho (New Drug Clin.) (1996).
3) Ried et al., Maturitas. (2010).
4) Ried et al., Eur. J. Clin. Nutr. (2012).
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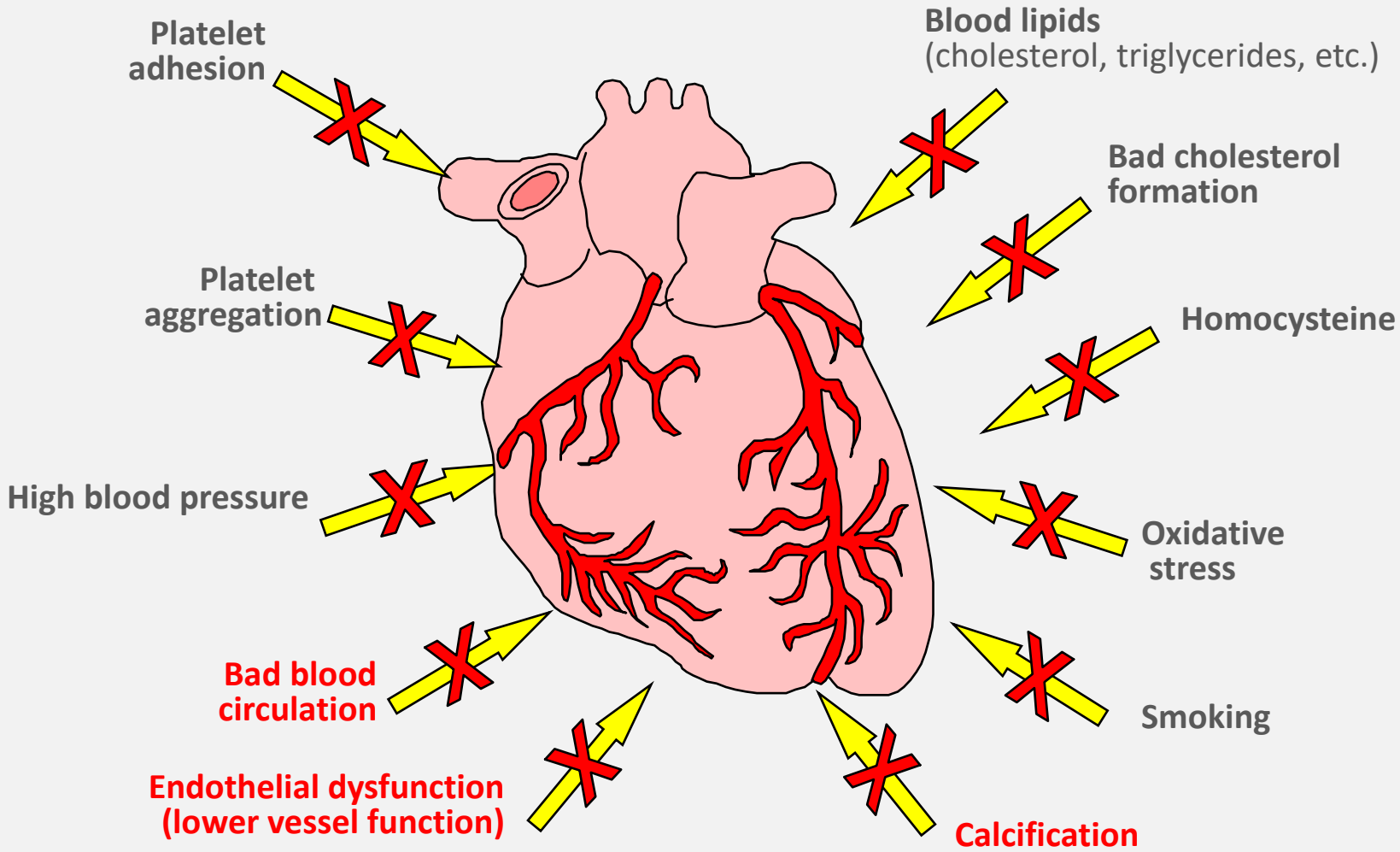
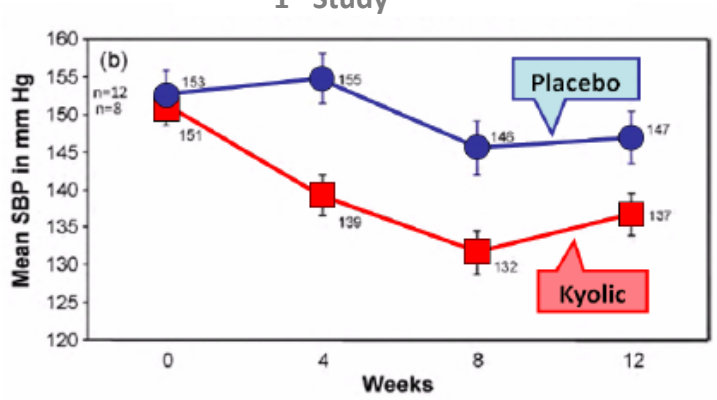
20) Zeb et al., J. Cardiovasc. Dis. Res. (2012).

AGE Reduces Multiple Risk Factors in Cardiovascular Diseases

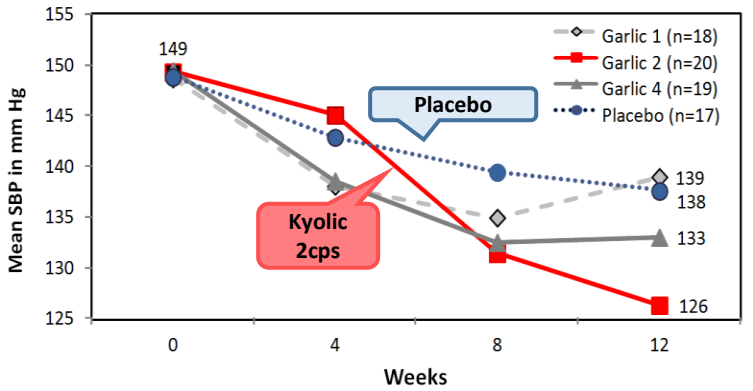


Summary of Clinical Studies Conducted by Dr. Ried at University of Adelaide / National Institute of Integrative Medicine, Australia

1st Study



2nd Study



Dr. Ried

Mean Systolic Blood Pressure (SBP) over the 12-weeks Period of Patients with Uncontrolled Hypertension (SBP>140 mmHg at baseline)

	Kyolic Formula Used & Dosage	Subjects	Outcome	Description
1st Study	AGE High Potency 4 capsules: 0.96g AGE/day for 12 weeks	• n=50 (AGE n=25; placebo n=25) , SBP ≥ 140: n=22 (AGE n=12; placebo n=10) • Patients with uncontrolled hypertension (SBP ≥ 140 or DBP ≥ 90 mm Hg) on anti-hypertensive medication	SBP: 6% ↓ (vs Placebo)	Ried K, et al. 2010. Maturitas. 67 (2): 144-50.
2nd study	AGE High Potency 1, 2 or 4 capsules: 0.24, 0.48 or 0.96g AGE /day for 12 weeks	• n=79 (AGE 1 cap: n=21, 2 caps: n=20, 4 caps: n=19; placebo n=19) • Patients with uncontrolled hypertension (SBP ≥ 140 mm Hg) on antihypertensive medication	8% ↓ (2caps) (vs Placebo)	Ried K, et al. 2012. Eur. J. Clin. Nutr.

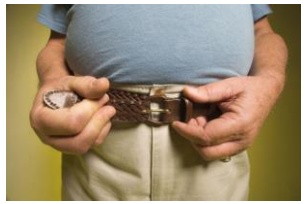
Clinical Finding on Metabolic Syndrome Patients and AGE

Summary of Clinical Study Conducted by Dr. Lopez at University of Santander, Colombia

Approximately 47 million Americans have metabolic syndrome

According to the American Heart Association and the National Heart, Lung, and Blood Institute, there are 5 risk factors involved:

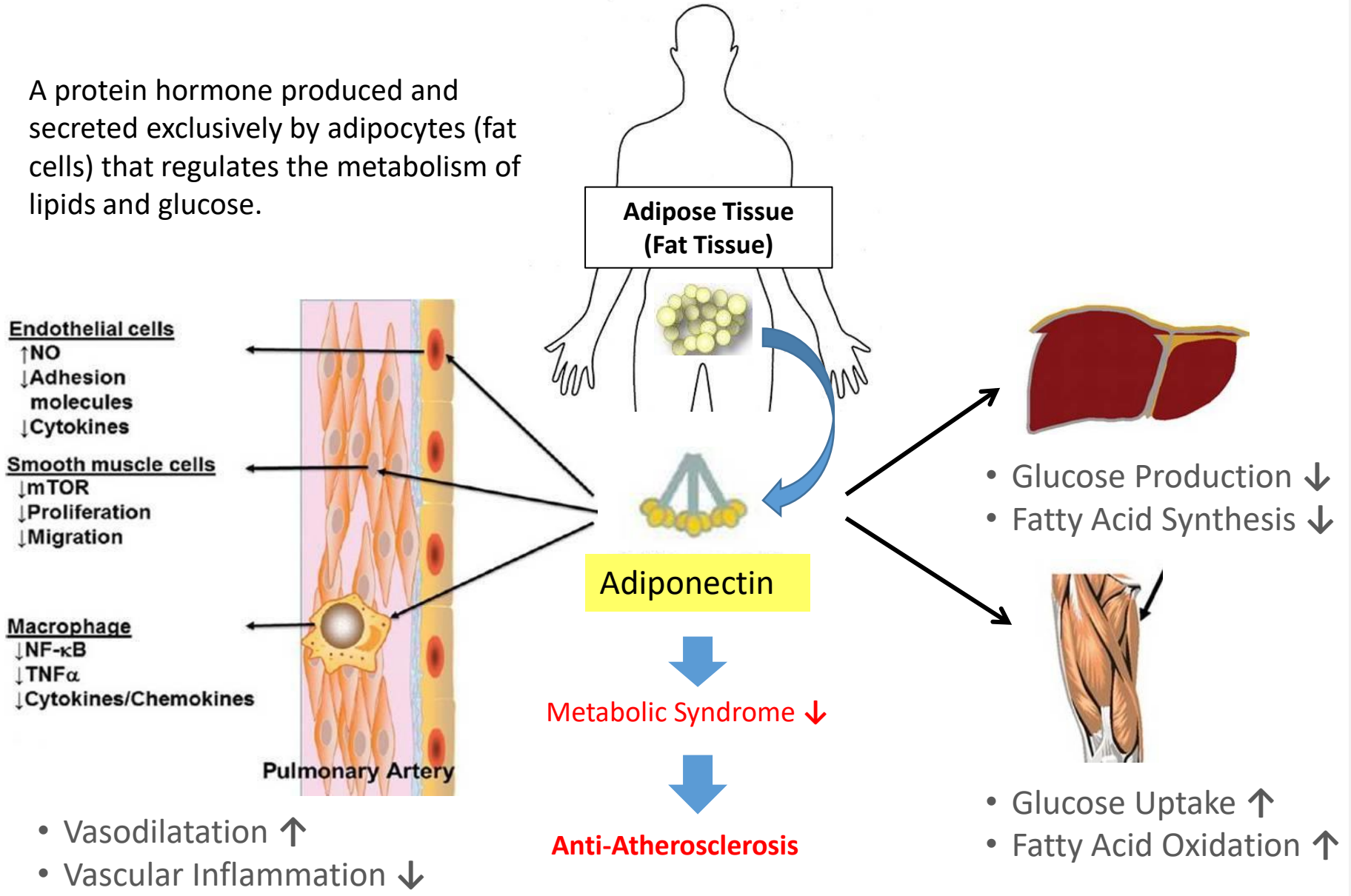
1) Large Waist:	Men with 40 inches or larger; Women with 35 inches or larger
2) Triglycerides:	higher than 150 mg/dL
3) Blood Sugar Level:	higher than 100 mg/dL
4) High Blood Pressure:	130/85 mm Hg or higher
5) Low HDL Cholesterol:	Men with less than 40 mg/dL Women with less than 50 mg/dL



AGE Dosage	Subjects	Outcome	Description
AGE 4 capsules: 1.2g AGE powder/day for 12 weeks	• n=86 (AGE n=43; placebo n=43) • Men and women over 18 years old with diagnosis of metabolic syndrome. • Double-blind, Crossover, Randomized, Placebo-controlled Clinical Trial.	Adiponectin 10% ↑ (vs Placebo)	Gomez-Arbelaez, D. et al. 2013. Mediators of Inflammation, Accepted for Publication

Adiponectin

A protein hormone produced and secreted exclusively by adipocytes (fat cells) that regulates the metabolism of lipids and glucose.



Summary of Clinical Study at University of Florida

By Prof. Dr. Percival

Clinical Design:

double-blind, placebo-controlled study on healthy volunteers

(n=120; 60 placebo, 60 AGE powder; 21-50 years old).

Treatment:

AGE powder or placebo capsule (4 capsules: 2.56 g per day) for 3 months

Results

① AGE significantly reduced the severity of cold/flu:

in the number of days a person suffers from cold/flu by 61%

in the number of symptoms of cold/flu by 21%

in the number of work days missed due to their illness by 58%

② AGE enhanced the activity of immune cell function compared to placebo, γδT cell (x8) and NK cell (x2).

③ AGE also enhanced lymphocyte antioxidant Glutathione.

④ AGE significantly modulates inflammatory marker INF –γ.

B Complex

- B6, B12, FOLATE
- Supports TRIAD 3 cardiovascular health
- Supports methylation reactions
- Decreases homocysteine formation
- Used for pertinent DIND

Joseph J, et al. Methoxistasis: Integrating the roles of homocysteine and folic acid in cardiovascular pathology. Nutrients. 2013;5(8):3235-56.

Coenzyme Q10

- Supports cellular energy production (ATP)
- Supports cardiovascular health
- Used for DIND
  - Statins/fibrates
  - Oral contraceptives
  - Beta blockers
  - Alpha blockers (clonidine/methyldopa)
  - Sulfonylureas and biguanides (metformin)
  - Thiazide diuretics
  - Bisphosphonates
  - Tricyclic antidepressants, phenothiazines
- 100mg daily should be sufficient
- CoQ10 labs can be analyzed

Liver/Bile Support

- Used for TRIAD 3 cholesterol
- Supports healthy liver and bile flow
- Helps remove toxins
- Artichoke (*Cynara scolymus*) leaf
  - Hepatoprotective
  - Choleretic – improves bile flow
  - 500-750mg daily std to 10% 3-caffeoylquininc acid and 2% luteolin-7-glycoside
- Dandelion (*Taraxacum officinale*) root
  - Choleretic
  - Useful in hepatic inflammation

Niacin Sustained Release

- B3
- Reported to
  - Increase HDL
  - Decrease triglycerides
  - Decrease lipoprotein(a)
  - Decrease LDL-C
  - Decrease apolipoprotein pattern B and increase pattern A.
- Sustained release = no “flush”
- Check liver enzymes (ALT, AST, LDH) every 6 months.
- 500mg SR TID

Salgado BJ, Salgado JV, Dos Santos AM, et al. Effects of low-dose of niacin associated to simvastatin in the treatment of mixed dyslipidemia Salgad. Minerva Cardioangiol. 2010;58(5):531-42.

Red Yeast Rice

- Monascus purpureus
- Contains HMGCoA compounds
  - Monacolin A
  - Monascin
  - Ankaflavins
- Reprorted to decarease total cholesterol, LDL, trigs, and increase HDL
- Improves ApoB to ApoA ratio
- TG/HDL ratio improve
- A meta-analysis report that red yeast rice compared to placebo lowers total cholesterol levels by 16%.
- 600mg , BID

Huang CF, Li TC, Lin CC, et al. Efficacy of Monascus purpureus Went rice on lowering lipid ratios in hypercholesterolemic patients. Eur J Cardiovasc Prev Rehabil. 2007;14(3):436-40.

Magnesium

- Critical in over 400 biochemical reactions
- Low levels correlate to insulin resistance/type 2 diabetes
- 2017 population-based cohort study using Rotterdam Study results, n = 8,555, mean age 64.7yr
- Decreased serum magnesium associated with increases risk of pre-diabetes (insulin resistance) and type 2 diabetes

Kieboom BCT, et al. Serum magnesium and the risk of prediabetes: a population based cohort study. Diabetologia. 2017;60(5):843-53.

Magnesium Intake

- Gallup poll 2004 commissioned by Purdue Products (makers of SloMag®)
  - 80% not getting RDA just from diet
  - 35% getting RDA between diet and supplements
    - July 21, 2004 PRNewswire
- Low levels Mg found in obese/overweight individuals
  - Huerta MG, et al. Magnesium deficiency is associated with insulin resistance in obese children.Diabetes Care. 2005 May;28(5):1175-81
  - Hassan SA, et al. Comparison of serum magnesium levels in overweight and obese children and normal weight children. Cureus. 2017;9(8):e1607.
- NHANES study 1999-2000 68% got less than RDA, 19% consumed less than 50%. Low Mg increases CRP and heart disease
  - JACN, Vol. 24, No. 3, 166-171 (2005)

Magnesium – CVD

- Correlations between low magnesium status and cardiovascular events

BMC Med. 2016; 14: 210.
Published online 2016 Dec 8. doi: 10.1186/s12916-016-0742-z
PMCID: PMC5143460
PMID: 27927203

Dietary magnesium intake and the risk of cardiovascular disease, type 2 diabetes, and all-cause mortality: a dose–response meta-analysis of prospective cohort studies

Xuexian Fang,[1] Kai Wang,[2] Dan Han,[1] Xuyan He,[1] Jiayu Wei,[1] Lu Zhao,[1] Mustapha Umar Imam,[3] Zhiguang Ping,[3] Yusheng Li,[4] Yuming Xu,[4] Junxia Min,[2] and Fudi Wang[1,3]

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- 40 cohort studies = 1 million participants
- Increasing magnesium in diet associated w/ 22% reduction in risk of heart failure

Li J, et al. Association of dietary magnesium intake with fatal coronary heart disease and sudden cardiac death.

Magnesium – IR

2016 meta-analysis, inception to 2015

- Does magnesium have positive effect on insulin sensitivity and glucose control in diabetic and non-diabetic individuals? YES
- Magnesium supplementation for ≥4 months significantly improves HOMA-IR index and fasting glucose in both diabetic and non-diabetic individuals
- Check RBC magnesium level
- 400-800mg daily chelates
- Dose at night, if sleep is a problem

Simental-Mendia LE, et al. A systematic review and meta analysis of randomized controlled trias on the effects of magnesium supplementation on insulin sensitivity and glucose control. Pharmacol Res. 2016;111:272-282.

Foods Rich in Magnesium

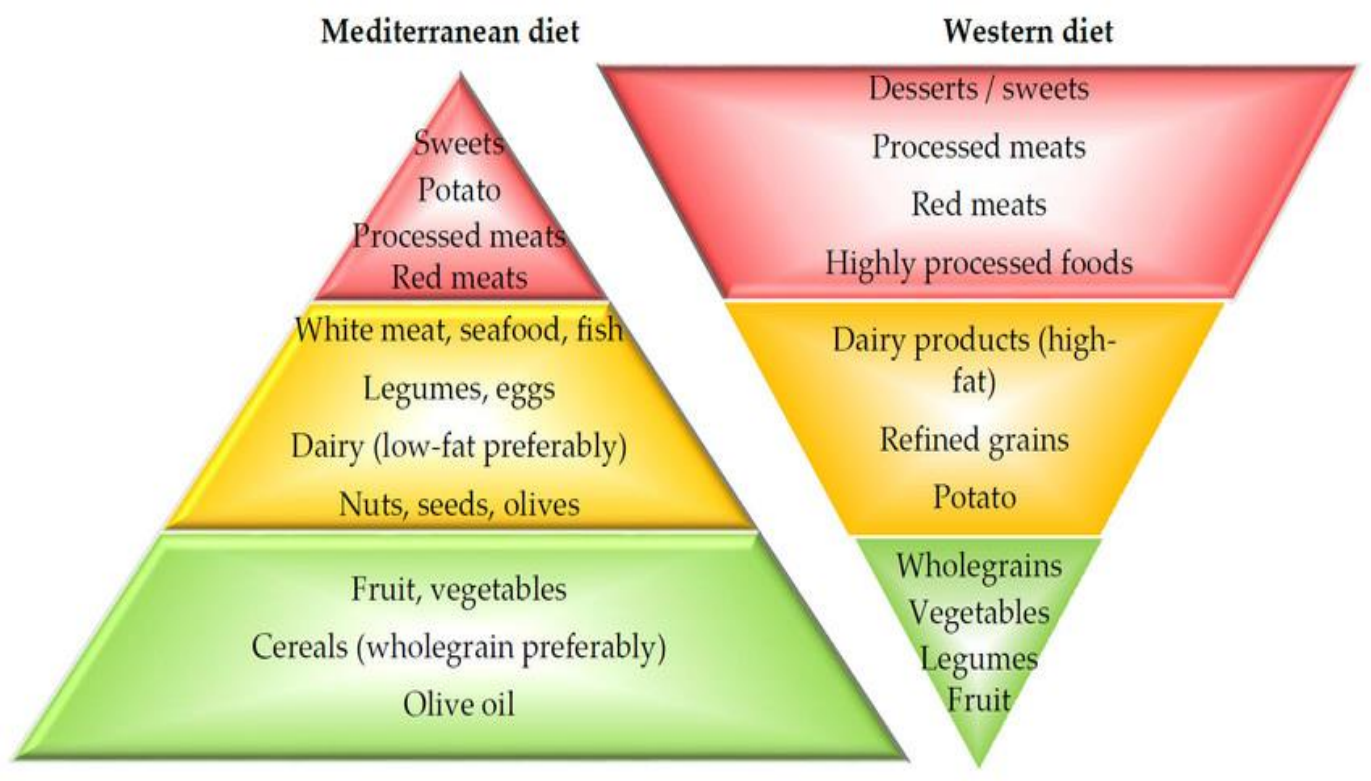
Magnesium-Rich Foods	Amount
• Pumpkin seeds (roasted)	532
• Almonds	300
• Brazil nuts	225
• Sesame seeds	200
• Peanuts (roasted, salted)	183
• Walnuts	158
• Rice	110
• Whole-grain bread	85
• Spinach	80
• Cooked beans	40
• Broccoli	30
• Banana	29
• Potato (baked)	25

(Milligrams per 100 grams). **Source: USDA nutrient database.**

Western Diet and CV Diseases

- Western dietary pattern associated with severe coronary artery disease

- WD =
  - ↑ Fat
  - ↑ Red meat
  - ↑ Carbohydrates
  - Minimal consumption of fruits and green leafy vegetables



Kanbay M, et al. A journey from microenvironment to macroenvironment: the role of metaflammation and epigenetic changes in cardiorenal disease. Clin Kidney J. 2019;12(6):861-70.

Thank You!

Questions?