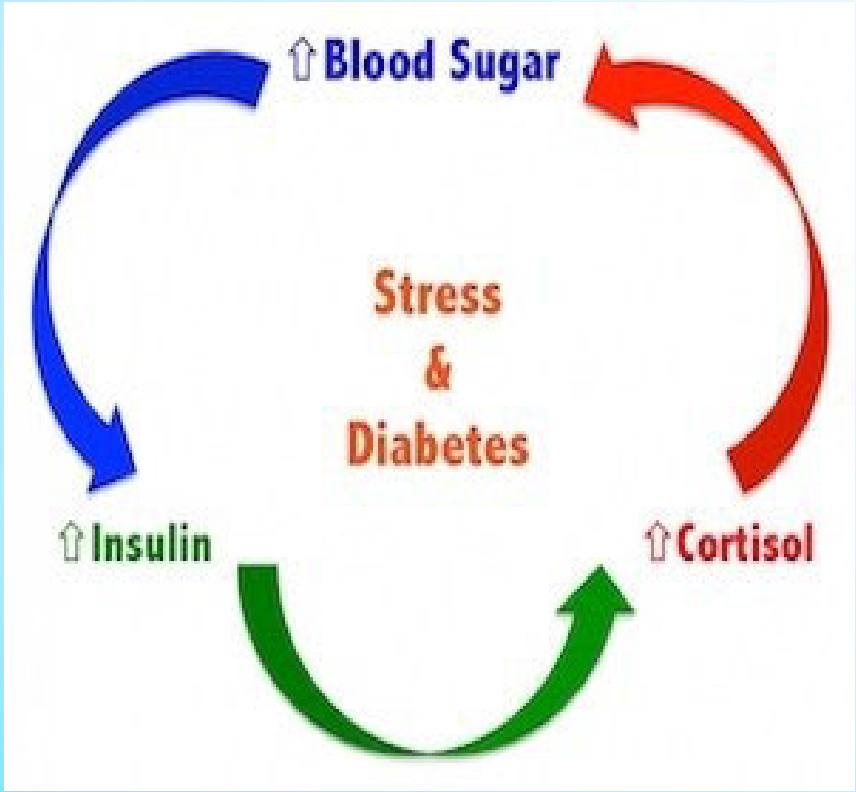
Advanced Diagnostics in DM and Stress



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Clinical Director Pro Football Hall of Fame Performance Health

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Laboratory Testing – Stress and Diabetes



- Successful management of blood sugar and successful weight loss depends on balancing the body's individual chemistry
- Can be done through laboratory testing of parameters in blood, urine and saliva
- General laboratory analysis panels + metabolic specialty markers
- Helps look at trends of imbalances in metabolism

Laboratory Testing

- Lab values (ranges) can be improved by:
  - Dietary and lifestyle changes
  - Exercise regimen
  - Dietary supplements
  - Rx medications/surgery when necessary

- Metabolic Balance = Improved Blood Glucose levels and Improved Cortisol/HPA axis

Factors Affecting Lab Results

- Sex
- Age
- Race
- Medical history including Rx, OTC and recreational drugs
- General health/metabolism
- Sleep and stress levels
- Not following testing requirements

Initial Lab Testing in T2D/Metabolic Patients

- CBC
- CMP
- Urinary pH
- Salivary pH
- Gamma glutamyl transferase (GGT)
- Fasting glucose
- Insulin
- Hemoglobin A1c
- 2 hr post prandial glucose
- HOMA-IR
- Thyroid panel
- Stress/Cortisol salivary and serum
- Comprehensive Vitamin D Test
- RBC Magnesium
- RBC Chromium
- NMR lipoprofile + LDL ox
- Adiponectin
- Leptin
- CRP
- Cystatin C
- GlycA
- Lpa
- ApoE 3,4 4,4
- ApoB

Add Ons

- Iron
- TIBC
- % Sat
- Ferritin
- Asymmetric Dimethylarginine (ADMA)
- C-Peptide
- 8OHdG
- F2 Isoprostane
- MSH
- MMA
- VEGF
- MMP-9
- Neopterin
- LPS

Core Testing – CMP and CBC

- Where is the patient's basic metabolism?
- Includes:
  - CBC (complete blood count)
  - CMP (comprehensive metabolic panel)
- Looks at
  - blood parameters
  - organs of detoxification
  - blood sugar and insulin regulation
  - electrolyte balance

Basics - 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
- pH

MPV – Mean Platelet Volume

- Platelet size demonstrated to reflect platelet activity
- Useful predictive and prognostic biomarker of cardiovascular events
- Associated with prothrombotic and proinflammatory events
- Changes in MPV reported to be important biomarker for inflammatory processes
- Also neoplastic diseases

Korniluk A, et al. Mean platelet volume (MPV): New perspectives for an old marker in the course and prognosis of inflammatory conditions. Mediat Inflamm. 2019;2019:9213074/

Anemias - Overtraining

Decreases in:

- Hemoglobin
  - Oxygen carrying capacity
- Iron
  - Anemia
- Decreased Ferritin
- Hematocrit – Normal

Iron

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

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

Hemoglobin

- Iron-containing protein molecule in red blood cells
- Transports oxygen to cells, CO2 away from cells
- Low levels = anemia
- Can be
  - Genetic (as in sickle cell anemia)
  - Dietary and nutrient related
  - Related to your exercise intensity
  - Related to menstruation in women
  - Gastrointestinal tract bleeding (stomach, intestines, colon) or low red blood cells due to types of cancer.
  - Certain medications can deplete iron.

Iron Ranges

- MALE
  - Range 50-180 mcg/dL
  - 100-160 optimal
  - Trending low = 50-100
  - Alert low <50
- FEMALE
  - Range 45 – 160
  - Optimal = 75-140
  - Trending low = 45-75
  - Alert low = <45

Hemoglobin

- Low levels can lead to
  - Fatigue
  - Decreased endurance
  - Decreased performance
  - Shortness of breath
  - Poor memory/concentration
  - Heart palpitations
  - Dizziness
  - Pale skin
  - Loss of sex drive

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

Hemoglobin

- Ranges are gender specific
- MALE
  - Range = 13.2-17.1 g/dL
  - Optimal = 14.5-16
  - Trending low = 13.2 – 14.4
  - Alert low <13.2
- FEMALE
  - Range = 11.7-15.5
  - Optimal = 13.9-14.9
  - Trending low = 11.7-13.8
  - Altert low = <11.7

Ferritin

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

Ferritin

- Ranges gender and age specific
- Av. Male range = 20-380 ng/ml
  - Optimal = 100-320
  - Trending hi = 320.1-380
  - Trending low = 20-99.9
- Av. Female range = 10-232
  - Optimal = 40-200
  - Trending hi = 200.1-232
  - Trending low = 10-39.9

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)
- **More acidic (lower pH) = more inflammation**
- More lactic acid produced at lower pH
- Mitochondria less efficient
- Joints and tissues stressed
- Mineral Deficient Magnesium and Potassium

Urinary and Salivary pH

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

Low Urinary pH

Linked to

- Accumulation of Visceral fat
- IR
- Methylglyoxal concentration
- Hypertension
- Increased intrarenal oxidative stress
- Up-regulation of renin-angiotensin system

Ogawa S, Takiguchi J, Nako K, et al. Elucidation of the etiology and characteristics of pink urine in young healthy subjects. Clin Exp Nephrol 2014.

Urinary pH

- Renovascular disorder onset in lower urinary pH with Diabetes
- Lower urinary pH is commonly seen in obese and CKD leading to higher uric acid
- Alters 8-OHdG levels and pulse wave velocity
- 10yr observational study 350 participants

Ogawa S, Takiguchi J, Nako K, et al. Elucidation of the etiology and characteristics of pink urine in young healthy subjects. Clin Exp Nephrol 2014.

Alkalinize to Spare the Kidneys

- Bicarbonate has been used to reduce the need for dialysis
- Alkalinization can suspend proteinuria-induced oxidative damage (proximal tubular cells)
- Lower UpH reinforces oxidative stress via albumin reabsorption (tyrosine kinase 2)
- Also correlated to intimal medial Thickness (IMT)
- Independent correlations to UpH and progression UA serum, Ankle brachial plexus index, EGFR, heart rate and number of RASI's taken.

Souma T, Abe M, Moriguchi T, et al. Luminal alkalinization attenuates proteinuria-induced oxidative damage in proximal tubular cells. J Am Soc Nephrol 2011;22:635–48.

Basic Concept pH and Progression of Renovascular Disease

Lower Urinary pH Is Useful for Predicting

Renovascular Disorder Onset in Patients With

Diabetes 300 patient over 10 years tracked

- UpH negative correlation with deoxyguanosine
- UpH a useful and predictive marker for onset of renovascular disease in people with diabetes
- Aids precipitation of uric acid
- Also common issue in CKD and Obesity

Susumu Ogawa; Kazuhiro Nako; Masashi Okamura; Sadayoshi Ito BMJ Open Diabetes Res Care. 2015;3(1)

Katsiki N, Papanas N, Fonseca VA, et al. Uric acid and diabetes: Is there a link? Curr Pharm Des 2013;19:4930–7.

Conclusion

- Over a decade improvements in BG, Lipids and BP occurred with drug therapy
- Number of drugs went up significantly in the trial
- NO improvements were seen in obesity, acidosis, fatty liver, hyperuricemia Nor could advancement of renovasuclar disease be halted
- Lower UpH also directly correlates the need for RASI's.
- Study follows findings in previous non-diabetic population.

How Do You Alkalinize?

1. Change the diet (green drinks, lower meats and increase veggies)
2. Minerals especially magnesium and potassium
3. Vegetable broth (collect peelings from all but nightshades)
4. Rescue dosing with bicarb if needed or can use vitamin C ascorbate that is buffered.

Renal Mast Cell Degranulation

- Currently only correlated with Diabetic Nephropathy
- Changes in mast cell number, degranulation noted
- Renal mast cells produce TGF-B1, TNF alpha
- Histamine production is underappreciated as a point of focus to renal damage

Zheng JM, Yao GH, Cheng Z, Wang R, Liu ZH. Pathogenic role of mast cells in the development of diabetic nephropathy: a study of patients at different stages of the disease. Diabetologia. 2011

Comprehensive Vitamin D Test

- Measures level of 25-hydroxy vitamin D
- Includes:
  - Vitamin D2 (ergocalciferol) and D3 (cholecalciferol)
- Importance:
  - bone health, heart function, blood sugar control, immune balance, sex hormone balance, breast and prostate health, mood (including depression), GI health and cancer.

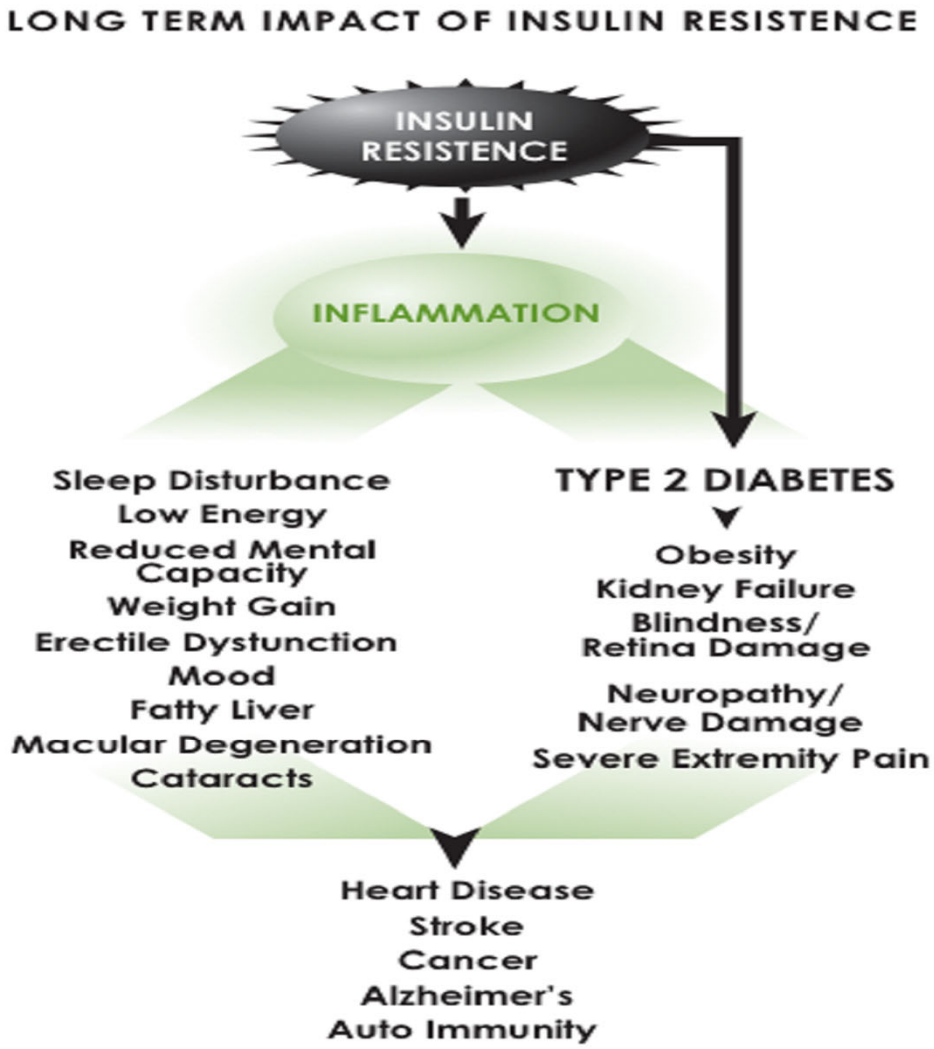
Comprehensive Vitamin D Test

- Vitamin D plays role in over 4000 reactions in the body
- Obesity is associated with low vitamin D
- VDR gene snp can influence insulin receptor function
- If taking Vit D, and no effect on lab, add vitamin A(retinal palmitate) to improve absorption of D.

Muscogiuri G, Sorice GP, Prioletta A, et al. 25-Hydroxyvitamin D Concentration Correlates With Insulin-Sensitivity and BMI in Obesity. Obesity (Silver Spring). 2010. 18(10):1906-10. PMID: 20150902.

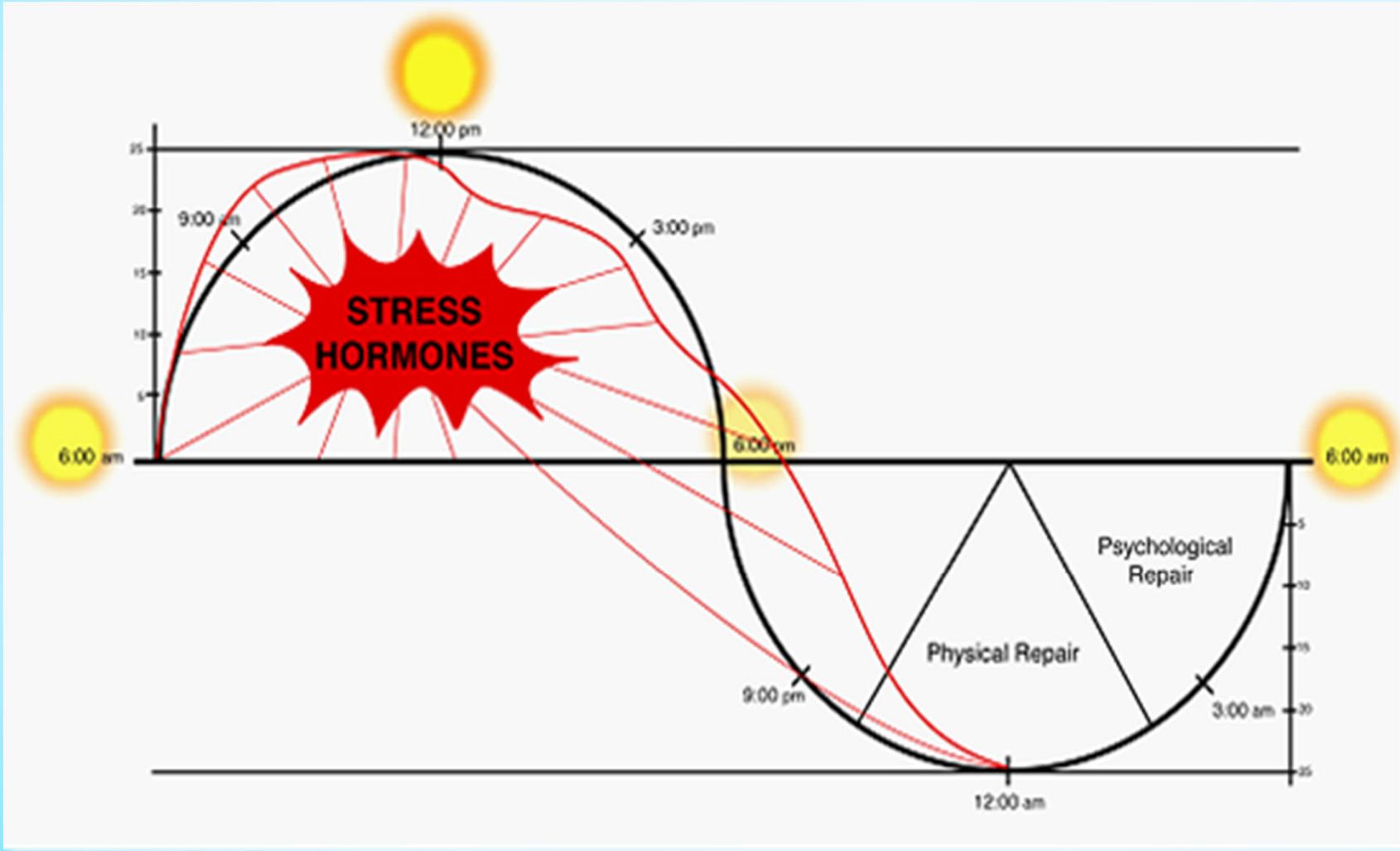
Comprehensive Vitamin D Test

- Optimal vitamin D level = 50-80 ng/ml
- Trending low = 20-49.9
  - 5,000 IU daily
  - Recheck in 90 days
- Alert Low = <20
  - 5,000-10,000 IU daily
  - Recheck in 90 days
- Alert high = >100
  - Can lead to toxicity including calcium deposits in soft tissue



Zeyda M, Stulnig TM. Obesity, inflammation, and insulin resistance--a mini-review. Gerontology. 2009;55(4):379-86. Epub 2009 Apr 8. Review.

Normal Diurnal Hormone Release



Cortisol impacts Insulin

- Cortisol excess induces ↑ TNF-α levels
- TNF-α blocks the action of insulin
  - in vivo and in vitro
- TNF-α results in serine phosphorylation of insulin receptor substrate 1, decreasing the tyrosine kinase activity of the insulin receptor

Hotamisligil GS, Peraldi P, IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. Science 1996]
[Hotamisligil GS, Shargill NS, Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 1993]

Cortisol = Sugar ↑

- Cortisol stimulates mass glucose mobilization
  - Liver gluconeogensis
    - Blocks protein synthesis to push amino acids into sugar
  - Fatty acid liberation → glucose

- Stimulates visceral fat storage if excess glucose not utilized → weight gain

Diurnal Cortisol and Physical Performance in Aging Males

- 2011 study - Middle aged men (45-59) measured AM and night cortisol
- Tracked twenty years later (65-83)
- Outcome measures walking speed and balance time morning serum and 4 salivary samples for two consecutive days
- Worst Performance in people with poor morning cortisol response & less nocturnal decline.

Outcome: HPA axis dysregulation is associated with worse physical performance later in life.

Gardner et al. Diurinal cortisol ptterns are associated with physical performance in Caerphilly Prospective Study Int J epidemiol 2011 dec;40(6):1693-702.

Diurnal Cortisol and Fasting Glucose

- 2020 six-year study n= 512

  A) change in diurnal cortisol curve features with ΔFG; n= 512

  B) prior annual percent change in FG with diurnal cortisol curve features; n= 1275

  C) baseline cortisol curve features with ΔFG over 6 years n = 700

- Study participants were in the 2020 Multi-Ethnic Study of Atherosclerosis (MESA)

Das JP, et al. The longitudinal association of changes in diurnal cortisol features with fasting glucose: MESA. Psychoneuroendocrinology. 2020;104698.

Diurnal Cortisol and Fasting Glucose

- Each annual % change increase in wake-up cortisol, total area under the curve (AUC), and overall decline slope was associated with a significant increase in FG over 6 years in all models
- A 1% prior annual increase in FG was associated with a 2.8 % lower bedtime cortisol among participants with NFG at baseline.
- A 1 % flatter overall decline slope was associated with a 0.19 % increase in subsequent annual % change in FG over 6 years among participants with diabetes.

Das JP, et al. The longitudinal association of changes in diurnal cortisol features with fasting glucose: MESA. Psychoneuroendocrinology. 2020;104698.

Diurnal Cortisol and Fasting Glucose

Authors Conclusion:

- Among participants with diabetes there was a positive association of change in wake-up cortisol, total AUC and overall decline slope with change in FG
- Baseline overall decline slope was positively associated with change in FG among the baseline diabetes group
- **Results suggest a detrimental role of cortisol contributing to glycemia among individuals with diabetes**

Das JP, et al. The longitudinal association of changes in diurnal cortisol features with fasting glucose: MESA. Psychoneuroendocrinology. 2020;104698.

Cortisol and Weight Gain

- Chronic stress directly related to weight gain
- Imbalances in hypothalamic-pituitary-adrenal (HPA) axis
- Leads to:
  - Increased cortisol output
  - Insulin resistance
  - Inflammation
  - Sleep problems
  - Hormonal imbalances
  - Weight gain
- Increases visceral “belly” fat
- “Fad” diets reported to increase cortisol levels

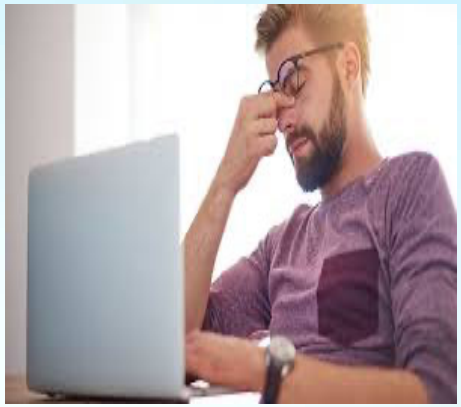
Dam Offspring Study population. Depress Anxiety. 2010. 27(9):846-51. PMID: 20112247.
Andrews RC, Herlihy O, Livingstone DEW, et al. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance.The Journal of Clinical Endocrinology. 2002;87(12):5587-5593.

Cortisol Level: Triggers

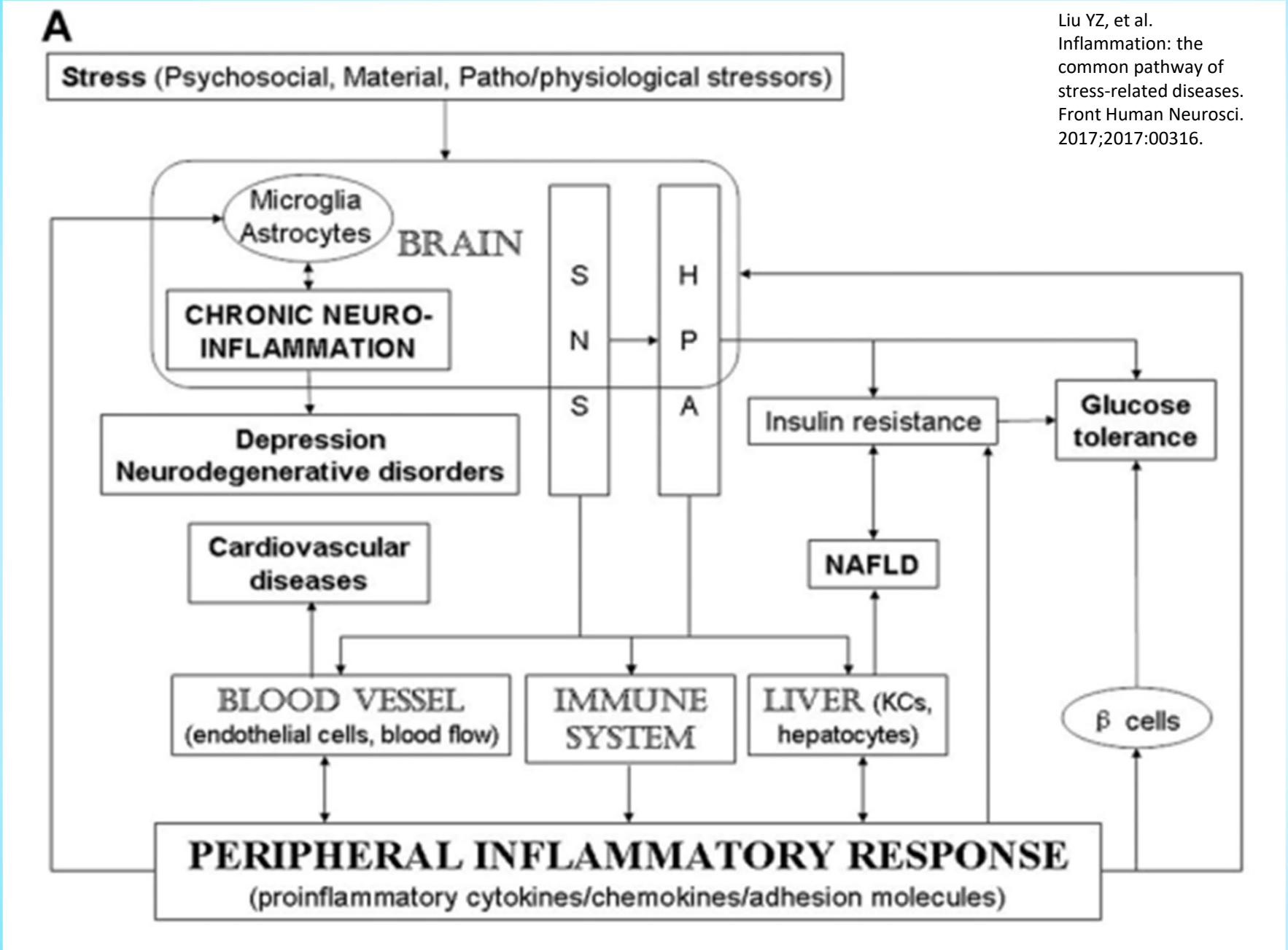
- Wired and Tired
- Tired and flat
- Poor sleep
- Poor performance
- Weight gain around the abdomen
- Mind racing
- Immune problems
  - Allergies and Asthma
  - Inflamed Joints
  - Poor exercise recovery

Stress - Metaflammation

- Stress activates Inflammation via
  - Overactivated Immunity
  - Increased Sympathetic tone
  - Reduced glucocorticoid responsiveness
- Stress – inflammation related diseases include:
  - Stress/Inflammation Metabolic – glucose/insulin, NAFLD
  - Stress/Inflammation CVD
  - Stress/Inflammation Depression
  - Stress/Inflammation Neurodegenerative diseases
  - Stress/Inflammation Cancer



Liu YZ, et al. Inflammation: the common pathway of stress-related diseases. Front Human Neurosci. 2017;2017:00316.



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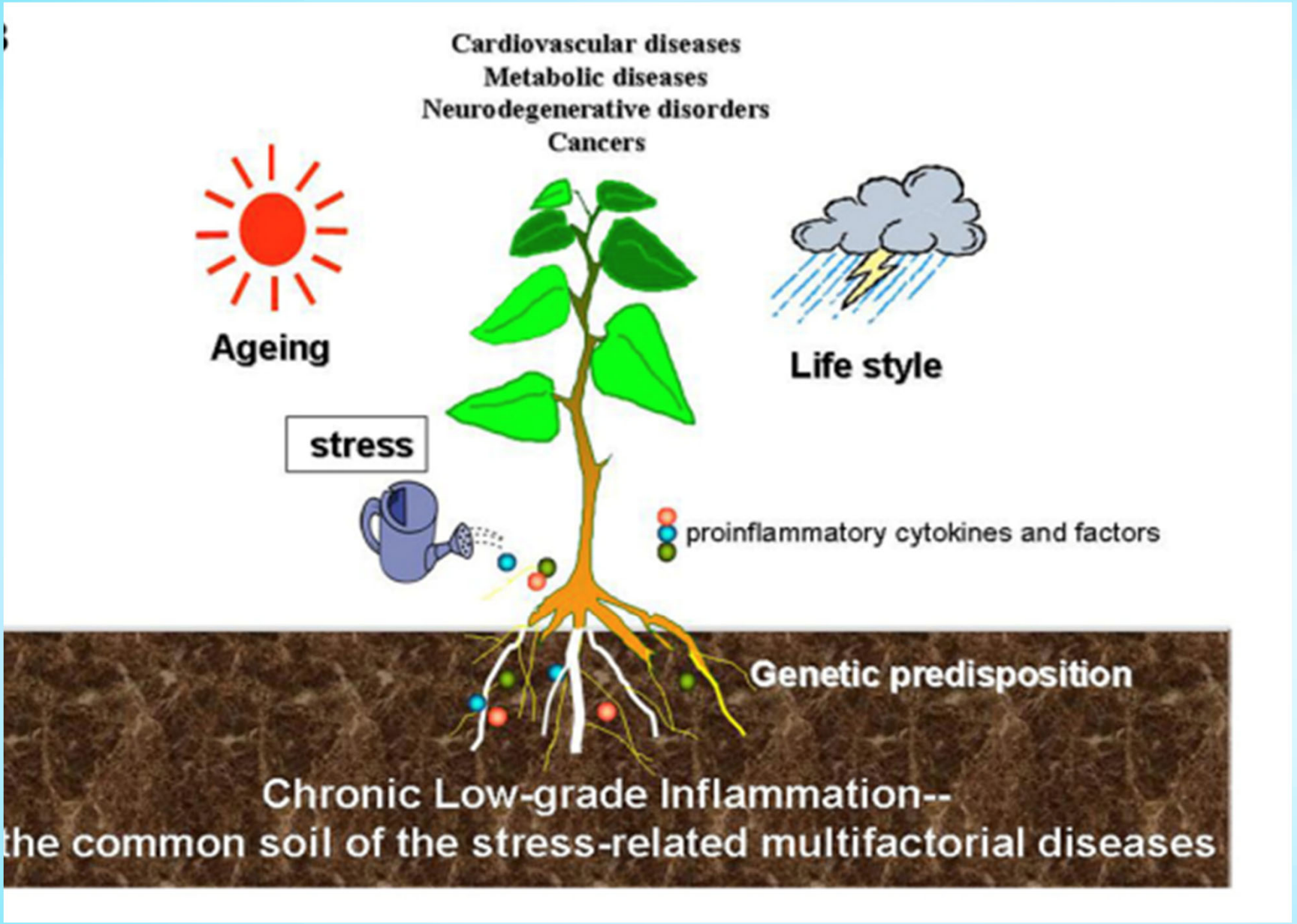

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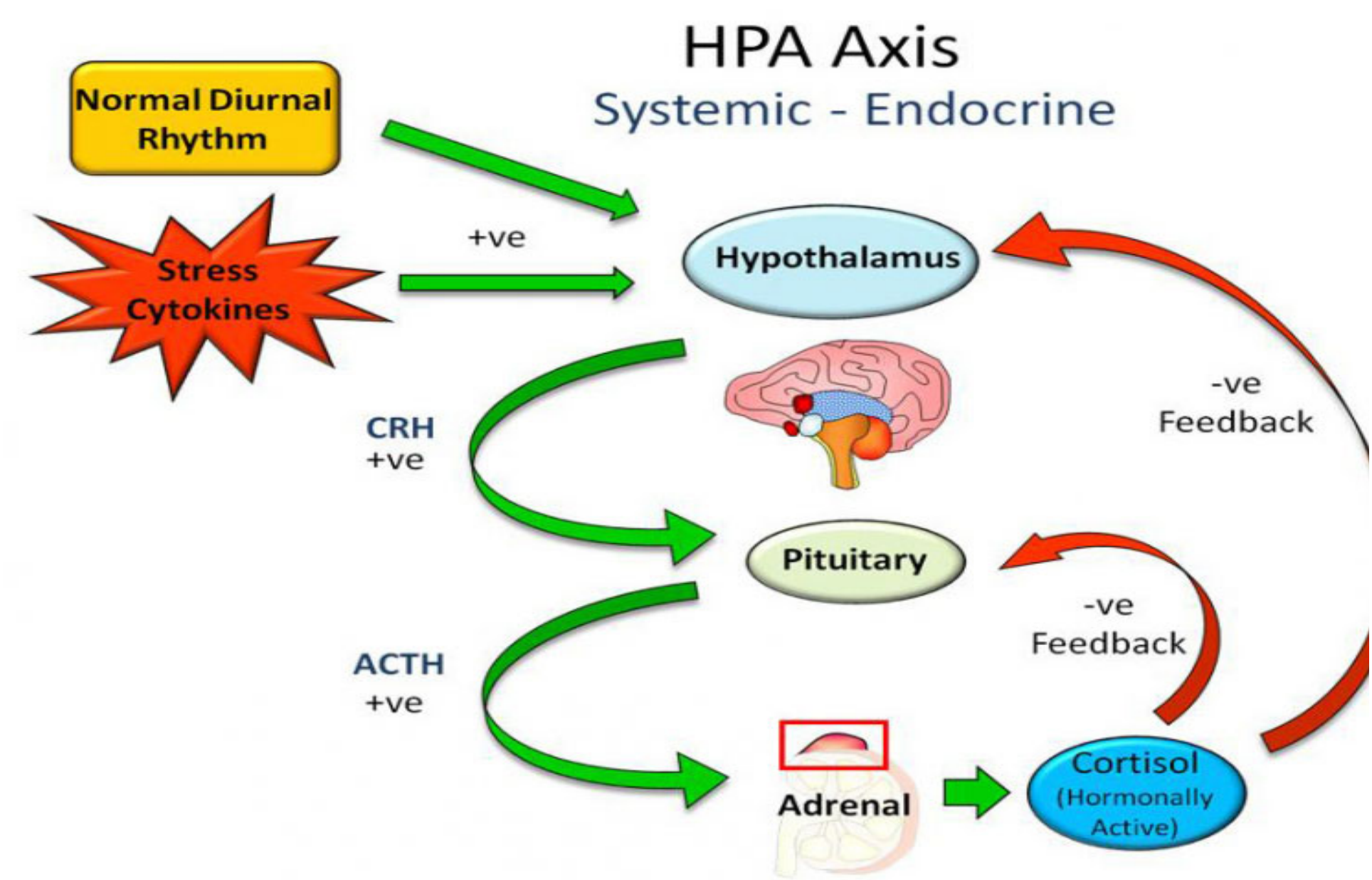

HPA Axis
Systemic - Endocrine
Normal Diurnal Rhythm
Stress Cytokines
+ve
Hypothalamus
-ve Feedback
CRH +ve
Pituitary
-ve Feedback
ACTH +ve
Adrenal
Cortisol (Hormonally Active)

Serum Cortisol Ranges

- 8 am serum cortisol range 4.3 – 22.4 mcg/dL
  - Alert LOW = <4.3
  - Trending Low = 4.4-9.9
  - OPTIMAL = 10-17
  - Trending high = 17.1-22.4
  - Alert High = >22.4
- Trending or alert Low
    - Licorice if BP stable
    - + Adrenal concentrate is no anxiety and energy improvement needed
    - + Adrenal cortex is anxiety present
- Trending or alert High
    - Adaptogens
    - Adaptogens + 5-HTP if cravings
    - Adaptogens + Magnolia/Phellodendron combination if anxiety
    - Also L-theanine if anxious, drowsy in daytime

Thyroid Panel

- Includes
  - Free T4 (thyroxine)
  - Free T3 (triiodothyronine)
  - TSH (thyroid stimulating hormone)
  - TPO (thyroid peroxidase antibody)
  - ThyAB (thyroid antibodies)
  - rT3 (reverse T3) - optional
- Importance: Thyroid produces energy and balances metabolism
- Important in weight loss – thermogenesis
- Evaluation for thyroid autoimmunity

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

Energy and Metabolism: Triggers

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

Cortisol/HPA - Supportive Nutrients

- Herbs
  - Rhodiola – adaptogen; 2011 meta-analysis supported positive effects on physical and mental performance
  - 500mg daily std. to 5 % rosavins

Hung SK, Perry R, Ernst E. The effectiveness and efficacy of Rhodiola rosea L.: a systematic review of randomized clinical trials. Phytomedicine. 2011;18(4):235-44.

  - Eurycoma (Tongkat ali) – Malaysian adaptogenic herb supports testosterone/cortisol ratio; support bone health
  - 400mg daily 100:1 extract

Talbott SM, Talbott JA, George A, et al. Effect of Tongkat Ali on stress hormones and psychological mood state in moderately stressed subjects. J Int Soc Sports Nutr. 2013;10(1):28.

Cortisol - Supportive Nutrients

- Herbs
  - Magnolia/Phellodendron combination
    - Anxiety
    - Supports cortisol and DHEA
    - Stress-related eating issues
    - 250mg TID

GLUCOSE

- Fasting blood glucose and 2 hour post prandial (glucose tolerance test or GTT)
- Insulin and glucose management critical in weight loss
- Chronic stress, sleep disorders high risk for developing insulin resistance and type 2 diabetes

GLUCOSE TARGET RANGES

- Fasting blood glucose
  - Alert Low = <65
  - Trending Low = 65-72
  - OPTIMAL = 73-89
  - Trending high = 90-110
  - Alert High = >110
- Postprandial
  - Target 1hr < 140
  - Target 2 hr< 90

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% increase 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.

Insulin Levels

- Range = 2 – 19.6 microIU / ml
- Optimal = 0-7
- Trending hi = 7.1-19.6
- 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 = < 5.2
  - Trending high = 5.3-5.7
  - Alert high = >5.7

HOMA-IR

- Homeostasis model assessment of insulin resistance
- 1st described in 1985
- Formula to detect insulin resistance before pre-diabetes or T2D occurs
- Index score for mg/dL= (fasting insulin)x(Fasting glucose) / 405 mg/dL
- If mmol/L used = (fasting insulin)x(Fasting glucose) / 22.5 mmol/L

Cobb J, et al. A novel fasting blood test for insulin resistance and prediabetes. J Diabetes Sci Technol. 2013;7(1):100-110.

HOMA-IR Study - MetS Correlation

- 2013 Cross sectional study

- N= 691 healthy adolescents (10-17 yrs)
  - 295 Normal BMI
  - 205 overweight BMI
  - 199 obese BMI

- RESULTS
  - HOMA-IR scores increased progressively from normal to obese BMI
  - HOMA-IR also increased from sexual maturity rating in both sexes

Singh Y, et al. A study of insulin resistance by HOMA-IR and its cutt off value to identify metabolic syndrom in Urban Indian Adolescents. J Clin Res Pediatr Endocrinol. 2013;5(4):245-51.

HOMA-IR Study - MetS Correlatic

- HOMA index value of 2.5
  - Maximum sensitivity and specificity in diagnosing MetS
  - Both genders per ATPIII (Adult Tx Panel) and IDF (Int Diabetes Federation)
  - Sensitivity of > 70% for MetS
  - Specificity of > 60% for MetS

- Common reference range : 0.7 – 2 mg/dL

Singh Y, et al. A study of insulin resistance by HOMA-IR and its cutt off value to identify metabolic syndrom in Urban Indian Adolescents. J Clin Res Pediatr Endocrinol. 2013;5(4):245-51.

GGT

- Gamma glutamyl transferase
- Enzyme found mainly in liver
- Also in kidneys, pancreas, spleen , heart
- High levels can indicate fatty liver
- Also pancreas problems, alcohol abuse

GGT

- Ranges gender specific
- Male
  - Range = 3-95 U/L
  - Optimal = 18-75
- Female
  - Range = 3-70
  - Optimal = 15-55
- Note alcohol use or medications can increase GGT levels

RBC Magnesium

- Low mag levels directly correlated with increased HOMA-IR
- Higher risk for cardiovascular diseases
- RBC mag Ranges: (4-6.4 mg/dL)
  - Alert Low = <5
  - Trending Low = 5-5.6
  - OPTIMAL = 5.7-6.2
  - Trending high = 6.3-6.4
  - Alert High = >6.2

Laires MJ, et al. Magnesium insulin resistance and body composition in healthy postmenopausal women. J A coll Nutr. 2004;23(5):510S-513S.

Dietary Magnesium

- 2013 dietary interventional study
- N=234 MetS patients, non-diabetic
- Assessed dietary mag intake at baseline, 6 months and 12 months
- REULTS: Mag intake inversely associated with IR biomarkers
- Mag Intake :
  - HOMA-IR index 71% lower in highest Mag intake quartile vs lowest
  - Over time individuals meeting Mag RDA = HOMA-IR or 0.37
- Authors conclude: meeting Mag RDA decreases IR risk

Wang J, et al. Dietary magnesium intake improves insulin resistance among non-diabetic individua with MetS participating in a dietary trial. Nutrients. 2013;5(10):3910-19.

Magnesium Supplementation

- 2004 double blind placebo controlled
- N=63 T2D with low serum mag (≤0.74 mmol/L)
- Oral magnesium soln. $MgCl_2$ _ 2.5 gm or placebo daily x 16 weeks
- Mag supplementation :
  - Significantly higher serum mag (0.74 mmol/L post tx vs 0.65 pre)
  - Lower HOMA-IR index (3.8 post tx vs 5 pre tx)
  - Lower Fasting glucose (144 mg/dL vs 180 pre)
  - Lower HgA1c (8% post vs 10.1% pre)

Rodriquez-Moran M, et al. Oral magnesium supplementation improves insulin sensitivity and metabolic control in T2D subjects. Deiabets Care. 2003;26(4):1147-52.

Magnesium

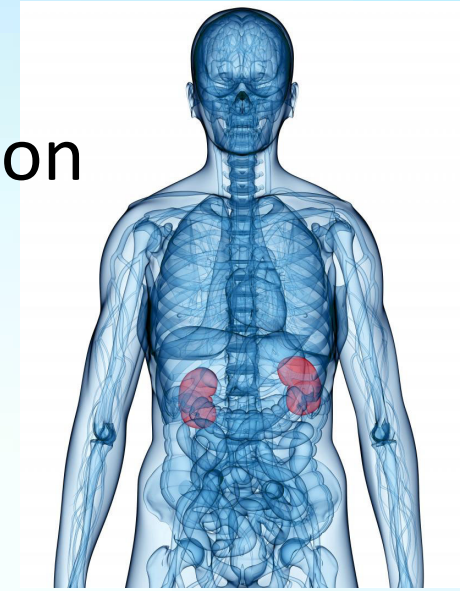
Metabolic imbalances associated with trending low or alert low magnesium levels :

- 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

Magnesium

- Dose 7.5-10mg/kilo: magnesium glycinate, taurate, malate

Cystatin C



- Along with GFR indicator of kidney function
- Non-glycosylated, low molecular weight (13,250 kD) cysteine proteinase inhibitor
- Produced by all nucleated cells
- Found in all body fluids
- Formed at a constant rate and freely filtered by the kidneys
- Serum concentration is inversely correlated with the glomerular filtration rate (GFR)

Cystatin C

- Also produced (elevated levels) when
  - Age
  - Diabetes and insulin signaling issues
  - Metaflammation
  - Obesity
- Cystatin C associated with IR and biomarkers of inflammation independently of renal function
- Link between Cystatin C levels and CVD in T2D
- Lab range = 0.55 - 1.15 mg/L

Uruska A, et al. Does serum cystatin C level reflet insulin resistance in patients with type 1 diabetes? Clin Biochem. 2014;47(13-14):1235-38.
Jeon EJ, et al. Cystatin C as a predictor for diabetes according to glycosylated hemoglobin levels in Korean Patients. Diabetes Metab J. 2016'40(1):32-34.

RBC Chromium

- Increased chromium excretion in urine reported in high sugar diet
- Important in blood glucose and insulin regulation
- Supports thyroid conversion of T4 to T3
- RBC chromium more accurate representation of chromium levels than serum

Vincent J. The biochemistry of chromium.
Journal of Nutrition. 2000;130:715-718.

RBC Chromium

- RBC Chromium Ranges (4-7 mg/dL)
  - Alert Low = < 2
  - Trending low = 2-3.9
  - OPTIMAL = 4-6.5
  - Trending high = 6.6-7
  - Alert high = >7
- Chromium supplementation in obesity with insulin resistance targeted at 800-1200mcg/day
- Dinicotinate diglycinate or Histidinate
- Glucose tolerance factor GTF chromium

8-OHdG (deoxyguanosine)

- Reliable marker for oxidative stress
- Correlated to DNA Damage
- Useful marker in cancer patients
- Range 0-54.1 ng/ml
- Optimal = 0-35
- Trending hi = 35.1-54.1
- Alert hi = >54.1

Khorjahni, etal Oxidative DNA damage responses to an acute session of hypertrophy-and strength –intensity resistance exercise, Annals of Biological Research 2012,3(12):54900-5493

CRP – C-reactive protein

- CRP major marker for metaflammation
- Correlated with
  - Sleep problems
  - Insulin resistance/blood sugar imbalances
  - Cardiovascular events
  - Metabolic syndrome
  - Weight gain/obesity
- Abdominal fat predictive of inflammation
- When a patient loses weight, CRP declines
- High-glycemic index diets associated with elevated CRP
- Chronic cortisol elevation increases inflammatory responses

Glycemic Load and C-Reactive Protein

- 244 apparently healthy women
- Determined BMI
- Glycemic load determined using semi-quantitative food frequency Q
- ***As glycemic load increased, CRP increased***
  - Q1 (lowest glycemic load): CRP = 1.9 mg/L
  - Q4 (highest glycemic load): CRP = 3.7 mg/L
- Women with BMI > 25:
  - Q1: CRP = 1.6 mg/L
  - Q4: CRP = 5.0 mg/L
- Ingestion of rapidly digested and absorbed CHO may exacerbate the inflammatory process

Liu S, et al. Am J Clin Nutr 2002 Mar;75(3):492-8.

CRP – C-reactive protein

- 2012 meta-analysis of 7 randomized clinical trials (n=32,918)
  - Increased dietary magnesium results in decreased levels of CRP

Dibaba DT, et al. Dietary magnesium intake is inversley associated with serum C-reactive protein levels: meta-analysis and systematic review. Eur J Clin Nutr. 2014;68(4):510-516.

hs-CRP Serum Values

- Range = <3
- <1 is optimal
- Trending hi = 1 – 3
- > 3 is hi

Leptin

- Reduces intracellular lipid levels in skeletal muscle, liver and beta cells, improves insulin receptor fx
- Genetic component for leptin receptor mutations (morbidly obese, hyperphagic, decrease sexual maturation)
- Leptin resistance & elevations lead to arterial thrombosis (platelet leptin receptor)
- Informs body of fat stores signal for satiety

Leptin Levels

- Depends on gender and BMI (body mass index, kg/m^2)
- If female and BMI 18-25
  - Range 4.7 – 23.7 ng/ml
  - Optimal = 6.1-17.7
  - Trending hi = 17.8-23.7
  - Hi > 23.7
- If female and BMI 25-30
  - Range 8- 38.9 ng/ml
  - Optimal = 10.6 - 30
  - Trending hi = 31.1 – 38.9
  - Hi = >38.9

Leptin Levels

- If male and BMI 18-25
  - Range= .03 – 13.4 ng/ml
  - Optimal = 1.6-10
  - Trending hi = 10.1-13.4
  - Hi > 13.4
- If male and BMI 25-30
  - Range 1.8-19.9
  - Optimal = 5.1-15.8
  - Trending hi = 16-19.9
  - Hi = >19.9

Adiponectin

- Increases insulin receptor sensitivity
- Elevates AMP kinase which ↑ glucose transport into skeletal muscle and burning of fat (fatty acid oxidation)
- Current top target for glucose control along with incretins (GIP, GLP-1).
- Antiathrogenic properties
- IL-6, TNFα inhibit adiponectin release
- High levels adiponectin ↓ risk of MI

Adiponectin

- Ranges based on gender and BMI (kg/m^2)
- Low and high numbers of concern
- Female BMI <25
  - Range 5-37 mcg/ml
- Female BMI 25-30
  - Range 5-28
- Female BMI >30
  - Range 4-22

Adiponectin

- Male BMI <25
  - Range 4-26 mcg/ml
- Male BMI 25-30
  - Range 4-20
- Male BMI >30
  - Range 2-20

LIPID profile

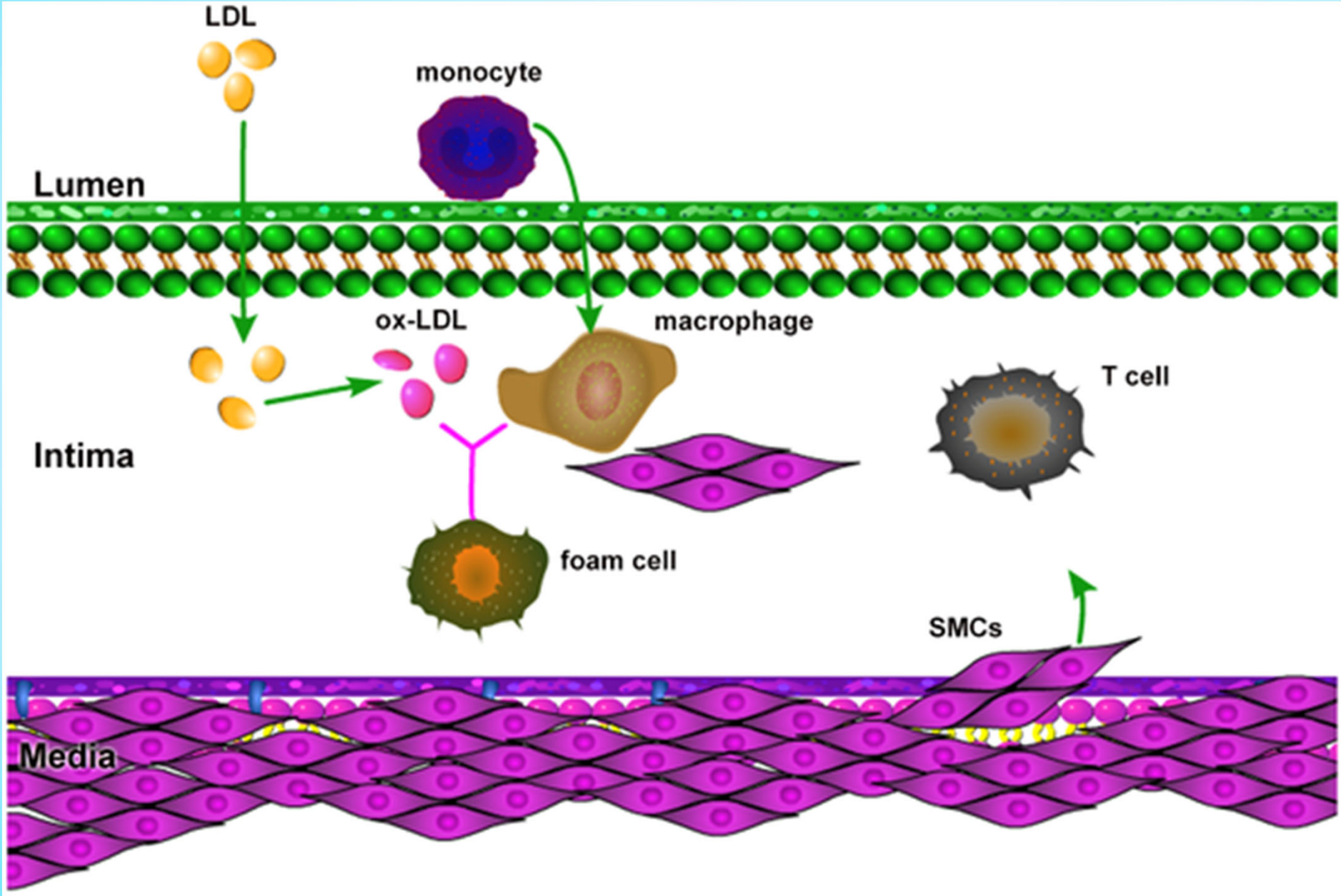
- Total Cholesterol
- LDL-C
- oxLDL
- LDL-P
- Particle size
- HDL
- HDL-P
- Triglycerides
- ApoB
- Lp(a)

LDL ox

- 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.

LDL ox


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

LDL-P

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

LDL-P Ranges

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

Lpa

- Lipoprotein(a)
- 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 - CV disease risk
  - < 75 nmol/L
  - 75-125 moderate risk
  - > 125 high risk
- T2D

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

ApoB

- Indicator of CAD
- Superior to LDL for marker of vascular disease
- Elevated even in presence of normal LDL
- Quest range < 90 mg/dL
- 90-119 moderate risk
- 120 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.

ApoE

- Gene polymorphisms associated with pathogenesis of Alzheimer's and T2D
- ApoE 3,4 4,4 alleles increases in both T2D and CAD
- Cognition decline follows T2D and CAD closely

Zhen J, et al. Association of ApoE Genetic Polymorphism and Type 2 Diabetes with Cognition in Non-Demented Aging Chinese Adults: A Community Based Cross-Sectional Study. 2018;9(3):346-57.

Novel Markers

Asymmetric Dimethylarginine (ADMA)

- Methylated arginine binds to NOS
- Decrease in nitric oxide leading to kidney damage, predictive for progression to nephropathy
- Modulates coronary endothelial function
- Inverse relationship with GFR and predictive for progression

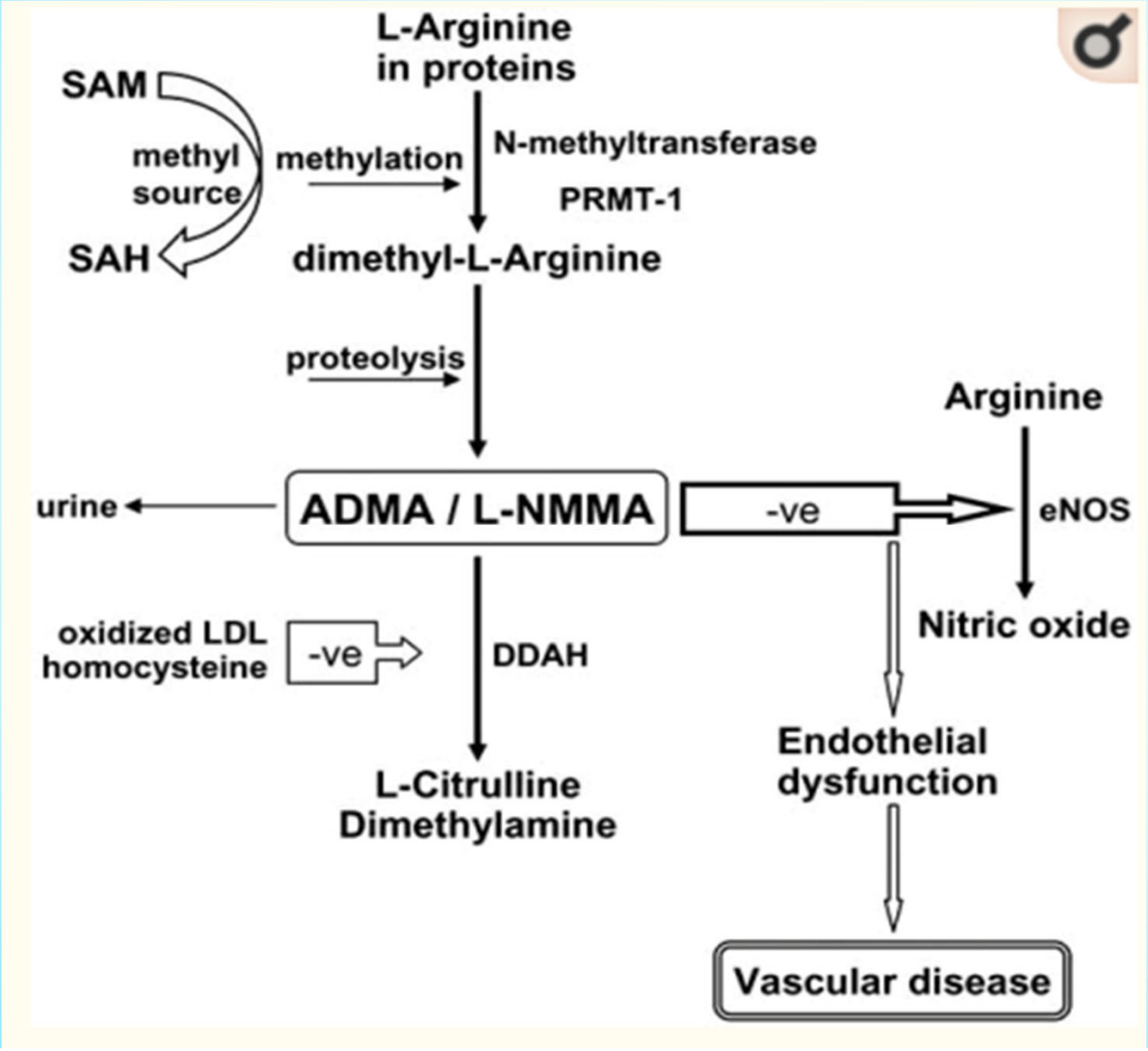
Hanai K, Babazono T, Nyumura I, Toya K, Tanaka N, Tanaka M, et al. Asymmetric dimethylarginine is closely associated with the development and progression of nephropathy in patients with type 2 diabetes. *Nephrol Dial Transplant. 2009 Jun. 24(6):1884-8.* [Medline].
Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol. 2005 Aug. 16(8):2449-55.* [Medline].

ADMA

- Optimal < 100 ng/mL
- Levels increased in T2D, obesity, MetS
- Associated with diabetic neuropathy
- CoQ10 supplementation reported to decrease ADMA in pts with T2D
  - 2015 Double blind placebo controlled study
  - 64 patients w/ T2D
  - 200mg/day x 12 wks
  - Significant improvement in ADMA, NOx, HbA1c, LDL-C

Hosseinzadeh-Attar M, et al. Reduction in asymmetric dimethylarginine plasma levels by coenzyme Q10 supplementation in patieents with type 2 diabetes mellitus. Minerva Endocrinol. 2015;40(4):259-66.

Overview of synthesis and metabolism of ADMA

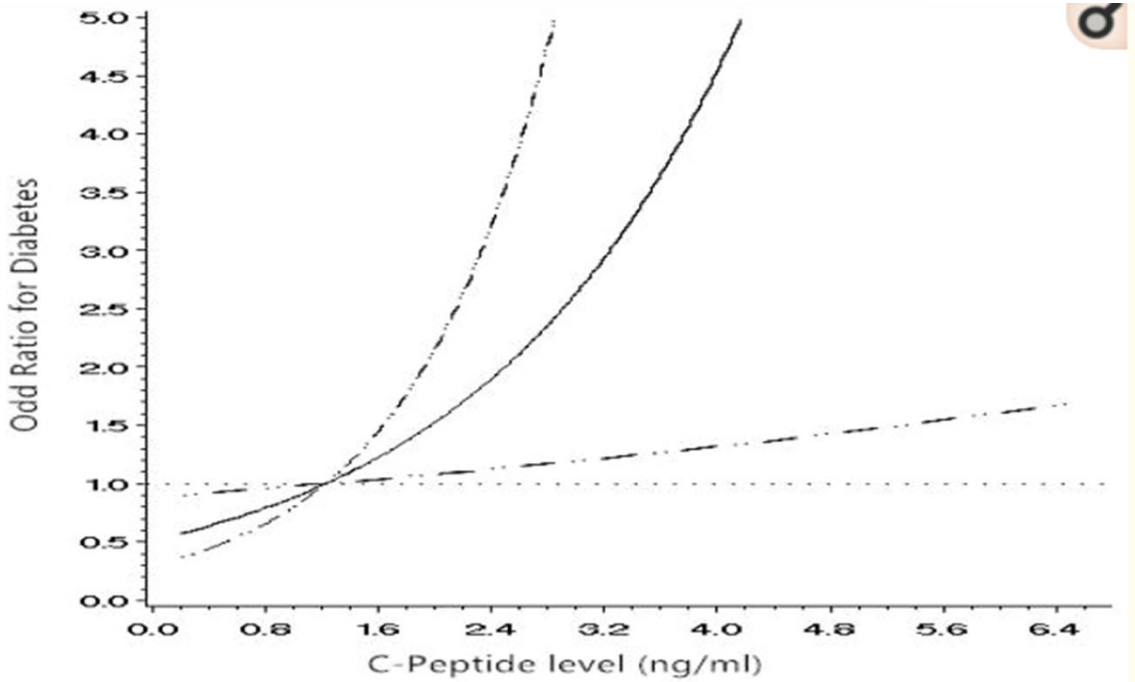


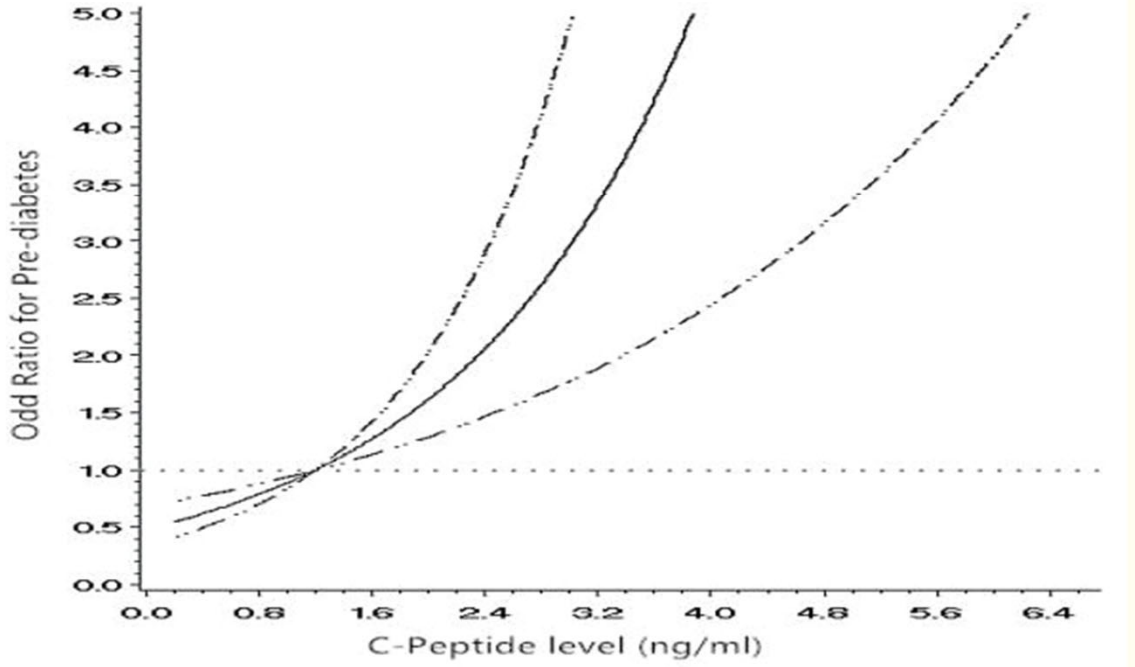
C-peptide

- By product of insulin formation
- Mainly excreted by kidney
- Optimal range : 0.5-3 ng/mL
- Elevated levels associated with increased risk of insulin resistance, pre-diabetes, T2D
- Helpful in characterizing at-risk Type 1 diabetics for hypoglycemia
  - Low levels in T1D indicate poor metabolic control

Kuhtreiber WM, et al. Low levels of C-peptide have clinical significance for established type 1 diabetes. Diabet Met. 2015;32(1):1346-53.

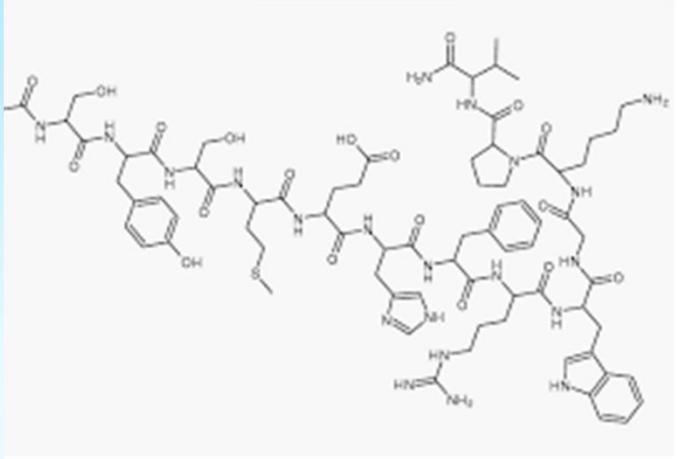
Risk of Pre-Diabetes and T2D using C-Peptide





Yin P, et al. J Diabetes complications. 2017;31(12):1658-1662.

Alpha MSH



- Melanocyte stimulating Hormones

- Tridecapeptide – released by pituitary

- Exists as α alpha, β beta, γ gamma

- Alpha MSH = 13 amino acids
  - Identical to ACTH 1-13 in humans

- Derived from pro-opiomelanocorticotropin (POMC)
  - Occurs with Leptin activation of POMC pathway
  - POMC = Precursor protein containing ACTH, beta MSH and gamma MSH

Loser K, et al. The neuropeptide alpha melanocyte stimulating hormone is critically involved in the development of cytotoxic CD8+ T cells in mice and humans. PLoS One. 2010:5(2):e8958.

Alpha MSH

- Regulatory neuropeptide
- Binds to melanocortin receptors (MC-1R – MC-5R)
- Role in skin darkening
  - Receptors found on malignant melanoma cells
- Helps control inflammatory pathways
  - More MC-R1 binding
  - Involved in Th1/Th2 balance - autoimmune
  - Regulation of proinflammatory cytokines
  - Expression of antioxidative enzymes
  - Nitric oxide regulation
  - Apoptosis

Ghanem GE, et al. Evidence for alpha melanocyte stimulating hormone receptors on human malignant melanoma cells. Int J Cancer. 1988;41
Hadley ME, et al. Biological actions of melanocyte-stimulating hormone. Ciba Foud Symp. 1981;81:244-62.

Alpha-MSH

- Weight regulation ; appetite control, energy expenditure
- Promotes muscle glucose uptake
- Stimulates aldosterone
- Learning/Memory
- Fever suppression – endotoxin related
- Peripheral nerve regeneration
- Sexual behavior

Enriori PJ, et al. Alpha-melanocyte stimulating hormone promotes muscle glucose uptake via melanocortin 5 receptors. Mol Metab. 2016;5(1):807-22.

Alpha MSH

- Serum Alpha MSH ↑ :
  - Fever due to endotoxin
  - HIV infection
  - Obesity w/ Insulin Resistance
  - Addison's
  - Pregnancy/ OC use

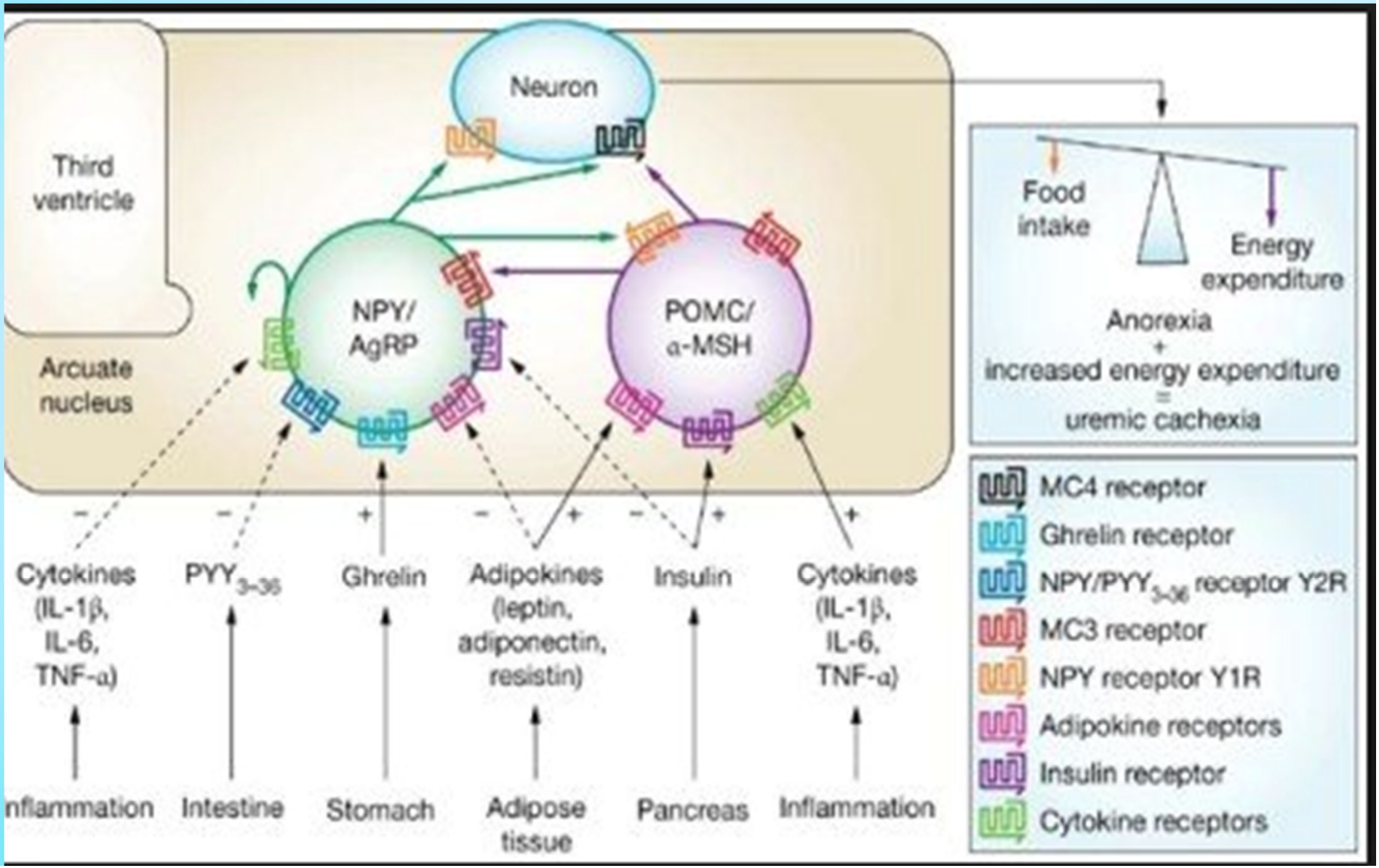
- Low levels:
  - Increased inflammation
  - Increased appetite; weight gain
  - Levels in mold are low in 95%
  - Hypopigmentation
  - Thirst/frequent urination
  - Sexual dysfunction

Alpha-MSH

- Levels increased in obesity
- Small study n = 15 obese n = 15 non-obese
- Results - Plasma levels of alpha MSH correlate
  - With increased BMI
  - Increased insulin levels
  - Visceral fat area
- Authors Concluded:
  - Elevated alpha MSH correlates with insulin Resistance in obese subjects

Katsuki A, et al. Elevated plasma levels of alpha melanocyte stimulating hormone (alpha MSH) are correlated with insulin resistance in obese men. Int J Obes Relat Metab Disord. 2000;24(10):1260-4.

Alpha MSH Weight Loss/Appetite



MSH

- Range
  - Quest = 31-85 pg/ml
  - Lab Corp = 0-40 pg/ml

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.

Neopterin

- Marker of cellular immune system activation
- Marker of increased oxidative stress
- Produced by GTP-cyclohydrolase i in human macrophages and dendritic cells upon stimulation by Interferon-gamma
- Indicative of pro-inflammatory immune status
- Blood = < 2.5ng/ml cut off
- 2.5 and > = chronic immune activation

Mangge H, et al. Antioxidants, inflammation and cardiovascular disease. World J Cardiol. 2014;6(6):462-77.

Neopterin

- Increased levels reported in :
  - Oxidative stress
  - Viral and bacterial infections
    - Including Borrelia (Lyme) and H pylori
  - Predictor of adverse outcomes in HIV
  - Autoimmune conditions
  - Malignant tumors
  - Allograft rejection episode
  - Depression

Mangge H, et al. Antioxidants, inflammation and cardiovascular disease. World J Cardiol. 2014;6(6):462-77.

Neopterin

- 2007 study
- Long term prognostic value of neopterin as a novel marker of monocyte activation in patietns w/ acute coronary syndrome
- Neopterin levels measures on 3946 patients for 7 days
- Also at 4 months in 3369 patients after ACS in the Pravastatin or Atorvastatin Evaluation Infection Therapy–Thrombolysis In Myocardial Infarction (PROVE IT–TIMI 22) trial
- Assessed the relationship between plasma neopterin levels and the risk of death and death or acute coronary events (nonfatal myocardial infarction or unstable angina) over 2 years.

Ray KK, et al. A novel marker of monocytes activation in patients with acute coronary syndrome. Circ. 2007;115(24):3071-78.

Neopterin

- RESULTS:
  - Seven days after an ACS event, neopterin levels ≥12.11 nmol/L associated with :
    - Increased risk of death
    - Increased risk of death or acute coronary events
  - Neopterin levels ≥12.11 nmol/L at 4 months remained a predictor of death alone and of death or acute coronary events
- Conclusion
  - Increased monocyte activation detected by an elevated plasma neopterin level identifies patients at long-term risk of death or recurrent acute coronary events after ACS.

Ray KK, et al. A novel marker of monocytes activation in patients with acute coronary syndrome. Circ. 2007;115(24):3071-78.

Midlife systemic inflammatory markers are associated with late-life brain volume

The ARIC study

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ABSTRACT

Objective: To clarify the temporal relationship between systemic inflammation and neurodegeneration, we examined whether a higher level of circulating inflammatory markers during midlife was associated with smaller brain volumes in late life using a large biracial prospective cohort study.

Methods: Plasma levels of systemic inflammatory markers (fibrinogen, albumin, white blood cell count, von Willebrand factor, and Factor VIII) were assessed at baseline in 1,633 participants (mean age 53 [5] years, 60% female, 27% African American) enrolled in the Atherosclerosis Risk in Communities Study. Using all 5 inflammatory markers, an inflammation composite score was created for each participant. We assessed episodic memory and regional brain volumes, using 3T MRI, 24 years later.

Results: Each SD increase in midlife inflammation composite score was associated with 1,788 mm^3 greater ventricular ($p = 0.013$), 110 mm^3 smaller hippocampal ($p = 0.013$), 519 mm^3 smaller occipital ($p = 0.009$), and 532 mm^3 smaller Alzheimer disease signature region

Inflammation Brain Volume

- 2017 prospective cohort study. N= 1633 patients
- Assessed systemic inflammatory markers = fibrinogen, albumin, WBC, von Willebrand factor and Factor VIII
- inflammation composite score was created for each participant
- 24 years later, SD increase in midlife inflammation composite score associated with:
  - Lower regional brain volume
  - Reduced episodic memory

Walker KA, et al. Midlife systemic inflammatory markers are associated with late-life brain volume: The ARIC study.

Inflammation Brain Volume

- Subjects with elevations in a larger number of 5 inflammatory markers during midlife
  - Reported to have lower regional brain volumes
  - Reduced episodic memory in late life
- For several brain regions including the hippocampus,
  - Effect of a 1 SD increase in midlife inflammation composite score
  - Comparable to that of possessing a single *APOE* ε4 allele during late life

Walker KA, et al. Midlife systemic inflammatory markers are associated with late-life brain volume: The ARIC study.

Inflammation Brain Volume

- Compared to participants with no elevated (4th quartile) midlife inflammatory markers
  - Participants with elevations in 3 or more markers
  - on average, 5% smaller hippocampal region volumes
- **Authors conclude:**
  - Systemic inflammation is a major contributor to neurodegeneration and cognitive aging

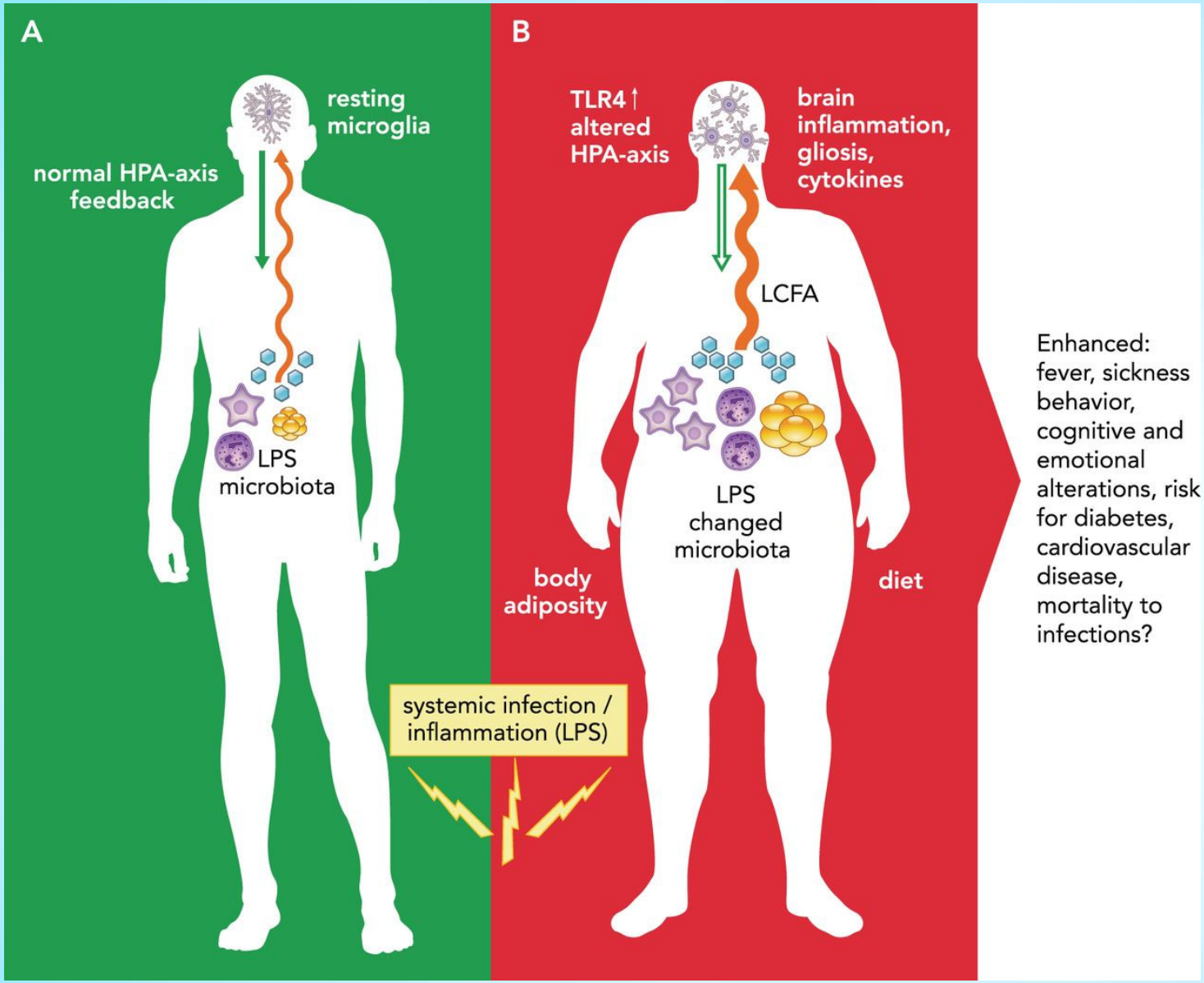
Walker KA, et al. Midlife systemic inflammatory markers are associated with late-life brain volume: The ARIC study.

LPS and Diabetes

- Presence of LPS lipopolysaccharide is closely associated with metaflammation, IR/T2D, obesity

- Toll-like receptor (TLR)4 upregulation

Liang H, et al. Effect of lipopolysaccharide on inflammation and insulin action in human muscle. PLoS One. 2013;8(5)::e63983.


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?

Review - Key Points to Check

- UpH
- Salivary pH
- SpH X 2 + UpH ÷ 3> 6.8
- Proteinuria
- RBC Magnesium >5.6
- Serum potassium >4.5
- Deoxyguanaosine urine or blood
- Uric acid (blood)
- eGFR >80 or adjust detoxification as instructed
- Creatinine within normal limits pay attention to trending high in relation to other values