Connecting the Dots: CVD, Inflammation, and Hormones

Doreen Saltiel, MD JD FACC

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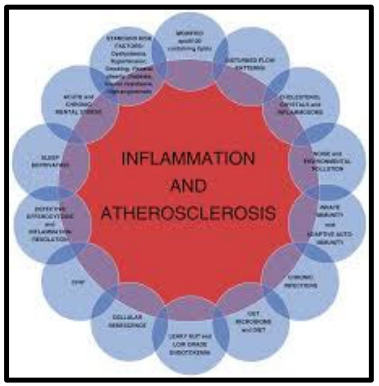

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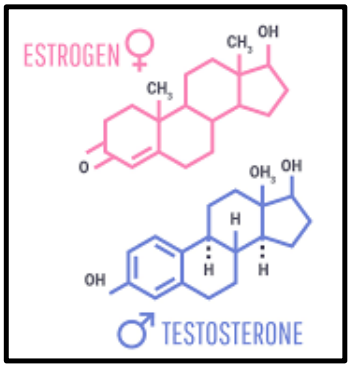

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Disclaimer

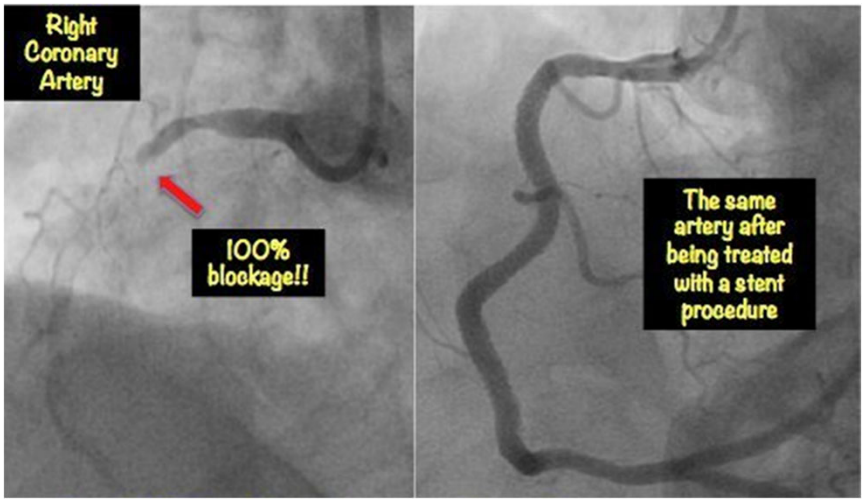
This lecture and the cited scientific literature, when referring to women/females, are referring to individuals born biological females; when referring to men/males, this lecture is referring to individuals born biological males.

Objectives

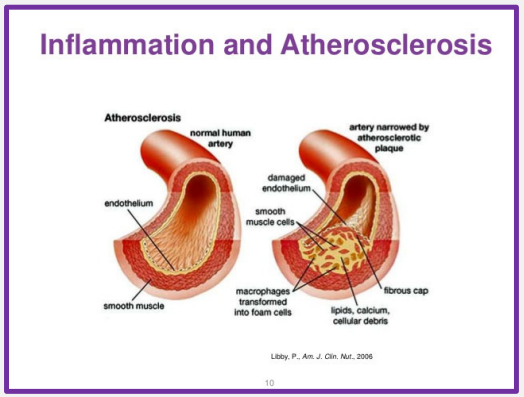
- **At the end of this presentation, attendees should have a better understanding of, and gain insights into:**
  - Cardiovascular disease as a chronic inflammatory disease
  - Traditional risk factors as inflammatory triggers
  - The role of sex hormones in cardiovascular disease

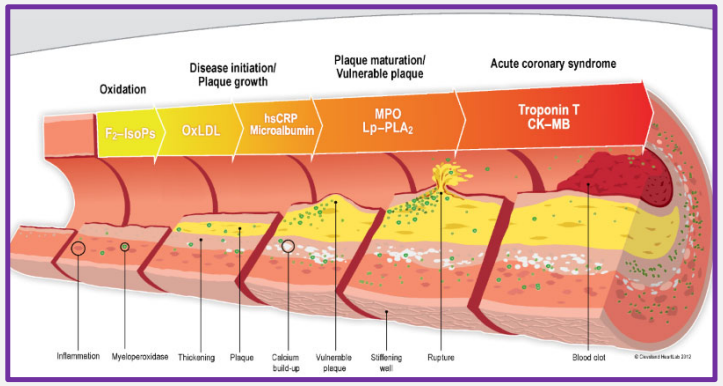
Goals

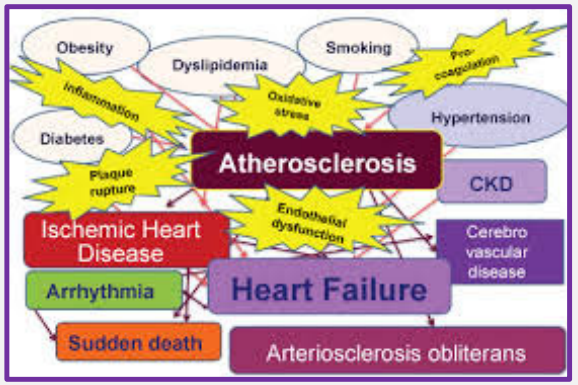
- **Prevent cardiovascular (CV) events in those at risk and in those with subclinical cardiovascular disease: primary prevention**
- **Prevent secondary events in those who have had a prior event: secondary prevention**



What Do We Know?







CVD: What Do We Know?

- CVD is the #1 killer in both men and women
- CVD is not a lipid storage disease
- **CVD is a chronic inflammatory disease**
- Inflammation is the key driver during all stages of the atherosclerotic process, from initiation through progression, and ultimately leading to thrombotic complications: MI, CVA, ischemic limb, DVT, PE
- Risk factors matter and are inflammatory triggers, impacting CVD risk

Libby P. Circulation. 2002; 105(9): 1135-1143.
Libby P. J Am Coll Cardiol. 2005; 46(7): 1225-1228.
Libby P, et al. Nat Rev Dis Primers. 2019; 51(1): 56.
Moriya J. J Cardiol. 2019; 73(1): 22-27.
Hedin U, et al. J Vasc Surg. 2019; 69(3): 944-951.

CVD: How Does It Happen?

- The first step in the atherosclerotic process is glycocalyx degradation (EGCX), which leads to endothelial dysfunction
- EGCX degradation and endothelial dysfunction ⇨ to “leaky blood vessels”
- Leaky blood vessels ⇨ to LPS translocation ⇨ to LPS-induced LDL oxidation ⇨ oxidative stress, immune activation ⇨ foam cell formation and end organ damage

Risk Factors Matter

- Dyslipidemia ⇨ inflammation ⇨ EGCX degradation and endothelial dysfunction ⇨ vascular permeability and ASCVD
  - Vascular inflammation occurs simultaneously with arterial wall lipid accumulation, lipid oxidation occurs, inflammatory cells accumulate, and atherosclerotic lesions develop
  - Monocytes enter, proliferate, become macrophages, which uptake LDL and become foam cells, eventually developing fibrous cap, etc.
  - Functioning HDL protects against atherosclerosis via reverse transport mechanism and transporting antioxidant enzymes into the intima, which break down ox-lipids, neutralizing pro-inflammatory effects

Libby P, et al. Circulation. 2002; 105(9): 135-1143.
Hurtubise J, et al. Curr Atheroscler Rep. 2016; 18(12): 82.
Libby P, et al. Nat Rev Dis Primers. 2019; 5(1): 56.

Risk Factors Matter

- HTN ⇨ inflammation ⇨ EGCX degradation and endothelial dysfunction ⇨ vascular permeability and ASCVD
  - Inflammation mediates HTN and HTN mediates inflammation
  - Angiotensin 2 can cause intimal inflammation by increasing endothelial cell (EC) ROS, and proinflammatory cytokines
  - Activation of the RAS increases ROS, ox-LDL receptor expression, increased adhesion molecules, chemotactic factors, and proinflammatory cytokines

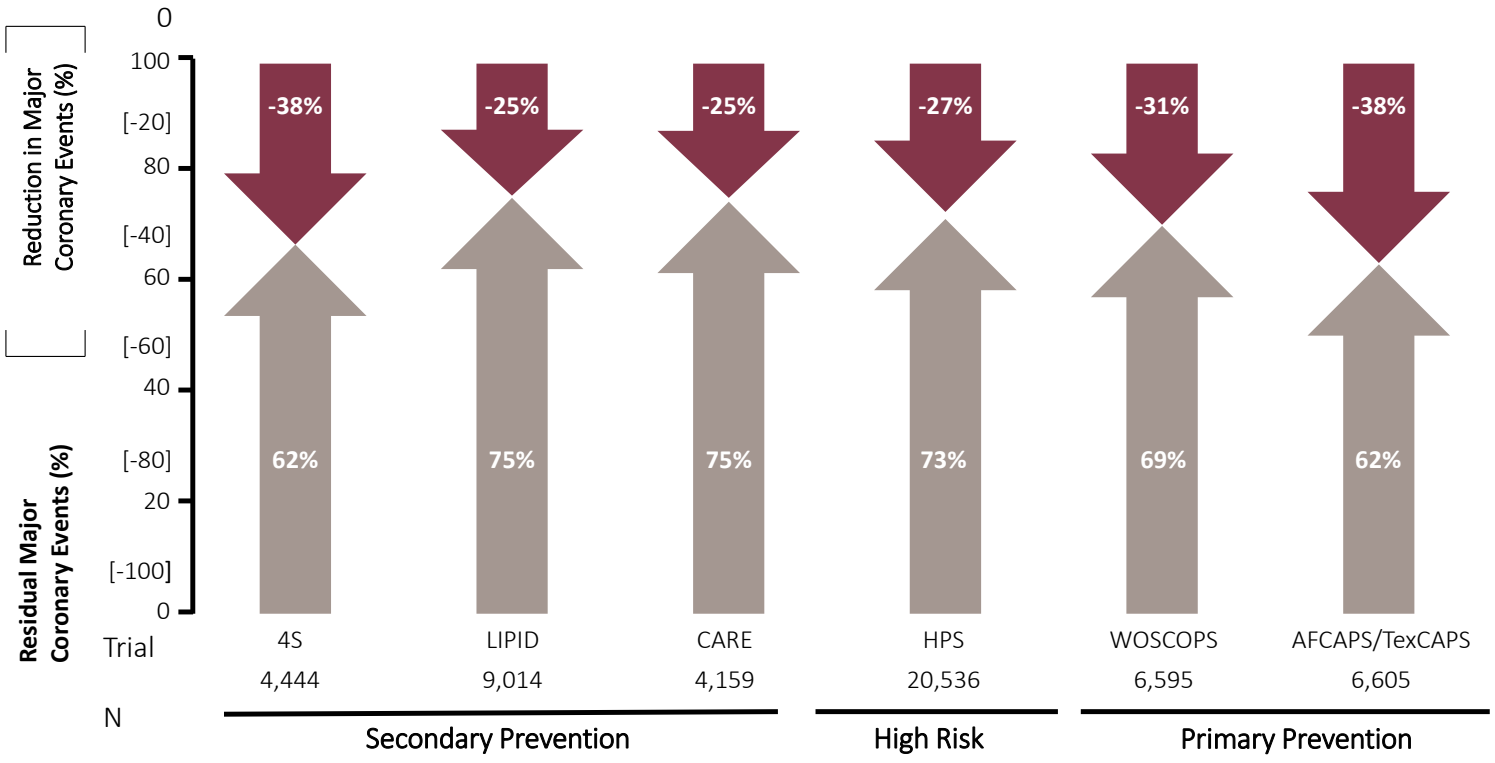
Libby P, et al. Circulation. 2002; 105(9): 135-1143.
Hurtubise J, et al. Curr Atheroscler Rep. 2016; 18(12): 82.
Libby P, et al. Nat Rev Dis Primers. 2019; 5(1): 56.

Risk Factors Matter

- IR/DM ⇨ inflammation ⇨ EGCX degradation and endothelial dysfunction ⇨ vascular permeability and ASCVD
  - Hyperglycemia and the production of advanced glycation end products (AGE) increases EC proinflammatory cytokines and proinflammatory pathways
  - DM increases ROS and oxidative stress
- Obesity ⇨ inflammation ⇨ EGCX degradation and endothelial dysfunction ⇨ vascular permeability and ASCVD
  - Obesity predisposes to IR/DM and contributes to atherogenic dyslipidemia
  - Adipose tissue is proinflammatory, synthesizing proinflammatory cytokines, promoting inflammation, and potentiating atherogenesis

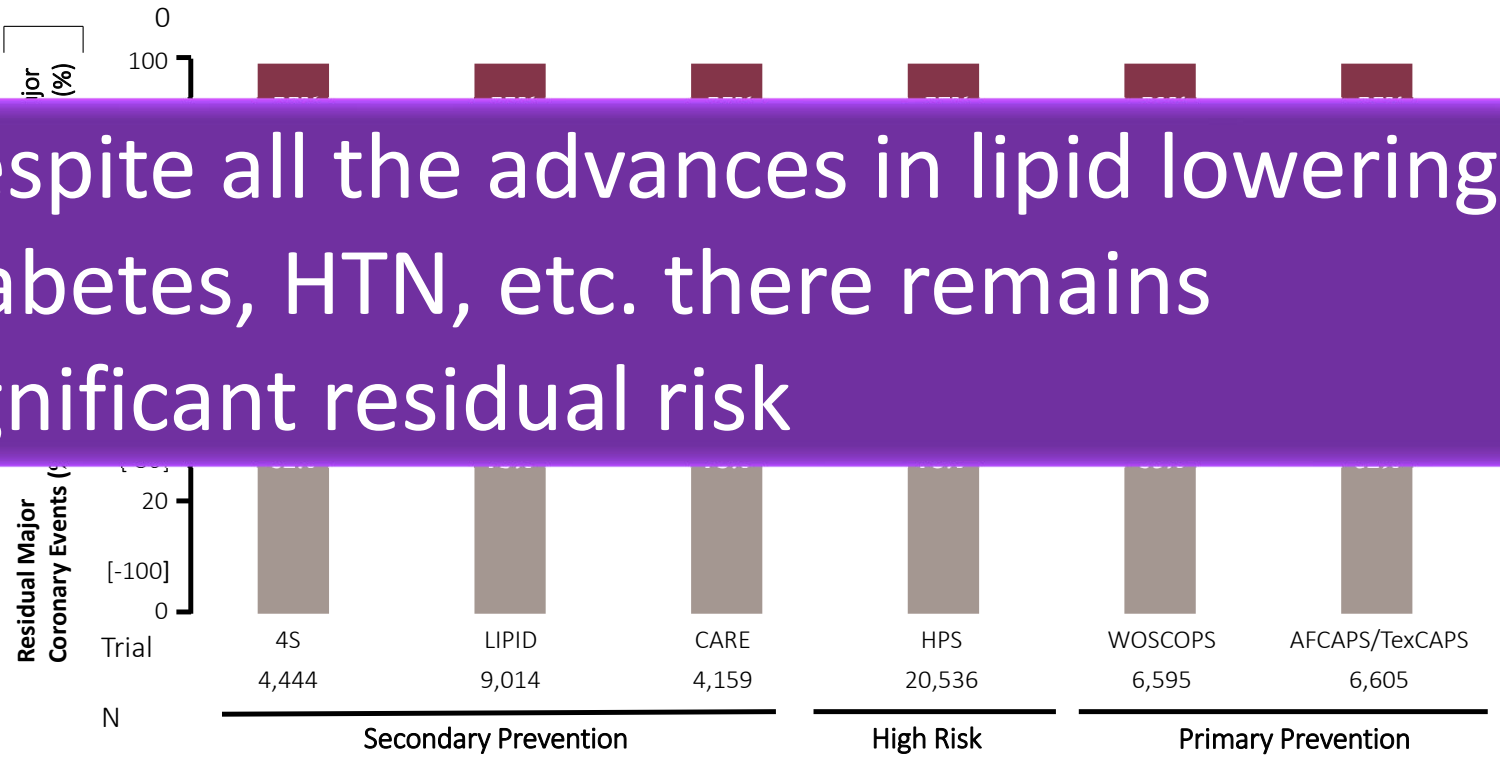
Libby P, et al. Circulation. 2002; 105(9): 135-1143.
Rocha VZ, Libby P. Nat Rev Cardiol. 2009; 6(6): 399-409.
Ormazabal V, et al. Cardiovasc Diabetol. 2018; 17(1): 122.
Di Pino A, DeFronzo RA. Endocr Rev. 2019; 40(6): 1447-1467.

Residual Risk Persists Despite LDL Lowering Treatment



Adapted from: Libby P. J Am Coll Cardiol. 2005; 46(7): 1225-1228.

Residual Risk Persists Despite LDL Lowering Treatment



Despite all the advances in lipid lowering, diabetes, HTN, etc. there remains significant residual risk

Adapted from: Libby P. J Am Coll Cardiol. 2005; 46(7): 1225-1228.

Question?

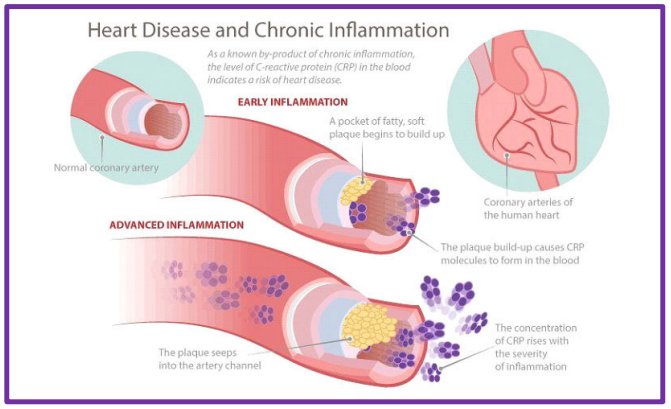
Are we chasing the wrong targets?

Question?

Are we chasing the wrong targets?

Yes!

Inflammation: One Right Target



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Decreasing Inflammation

- JUPITER Trial and hs-CRP, a primary prevention trial
  - Large, multinational, long-term, double-blind, placebo-controlled trial
  - Assessed whether rosuvastatin 20mg/d should be given to “apparently healthy” men (> 50 y/o) and women (> 60 y/o, ~ 40%); 41% MetS
    - 17,802 primary prevention patients; LDL < 130mg/dL and hs-CRP ≥ 2.0 (mean 4.3)
  - Results: 44% decrease in CV events, despite no change in lipids
    - Consistent in all subgroups evaluated, including men and women, and minority populations
    - The absolute risk increased with increasing hs-CRP and
    - The absolute event risk reduction that was associated with statin therapy was also greatest in those with the highest baseline hs-CRP
  - **Conclusion: regardless of LDL-C, patients with elevated hs-CRP will benefit from statin therapy with reduced CV event rates**

Mora S, et al. Am J Cardiol 2006; 97(2A): 33A-41A.
Watson KE. Rev Cardiovasc Med. 2009; 10(2): 91-96.
Ridker PM, et al. Am J Cardiol. 2010; 106(2): 204-209.

Decreasing Inflammation

- Cantos, a secondary prevention trial
  - A randomized, double-blind, prospective, placebo-controlled trial designed to assess 3 SQ canakinumab (IL-1β inhibitor) doses, 50mg, 150mg, 300mg, Q 3 months in patients with a previous MI and hs-CRP ≥ 2.0 despite optimum medical therapy
    - 10,061 patients, mean age 61, most s/p revascularization
    - Primary end point was: [1] non-fatal MI, non-fatal stroke, or CV death, [2] new onset type 2 DM, [3] death from any cause, and [4] composite endpoint
  - Results: compared to placebo
    - 3.7 years: primary end point findings documented a modest but significant:
      - 40% relative risk reduction (RRR) in IL-6 and hs-CRP
      - 15% RRR in MI, stroke, or CV death incidence
    - Placebo group, on optimal therapy with LDL-C ~ 80mg/dL had a 25% 5-year event rate
  - **Conclusion: The IL-1β inhibitor significantly decreased CV event rates, independent of lipid lowering. However, infections and deaths were too high to justify its use**

Ridker PM. J Am Coll Cardiol. 2018; 72(25): 3320-3331.
Ridker PM, et al. Eur Heart J. 2018; 39(38): 3499-3505.
Hassan M. Glob Cardiol Sci Pract. 2018; 2018(1): 2.

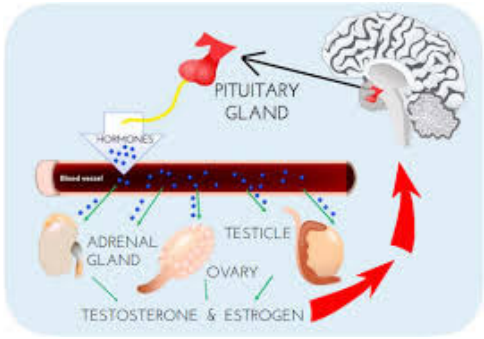
Key Points

- CVD is a chronic inflammatory disease and decreasing inflammation in the absence of lipid lowering, decreases CVD event rates
- RFs matter, are inflammatory triggers that if not addressed, increase CV adverse outcomes
- Decreasing the inflammatory burden will decrease vascular events

Clinical Pearls

- Clinicians should take a 2-pronged approach:
  - Risk factor prevention and treatment
  - Preventing and treating inflammation

The Role of Functional Medicine and the DUTCH TEST



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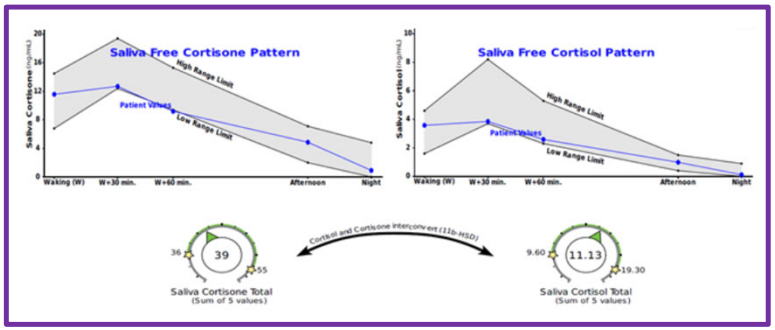
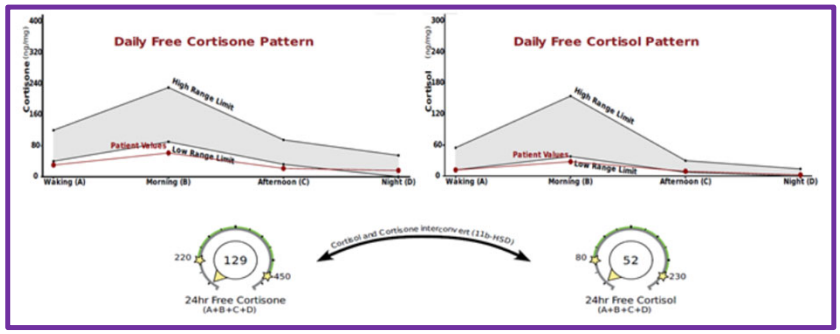
The HPA Axis (T1): One Right Target

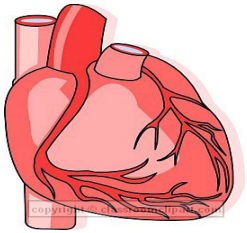


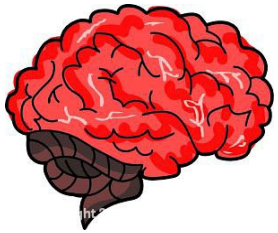


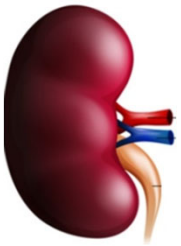
Cortisol Drives Inflammation and CVD

Inflammation
Oxidative stress

EGCX and endothelial dysfunction





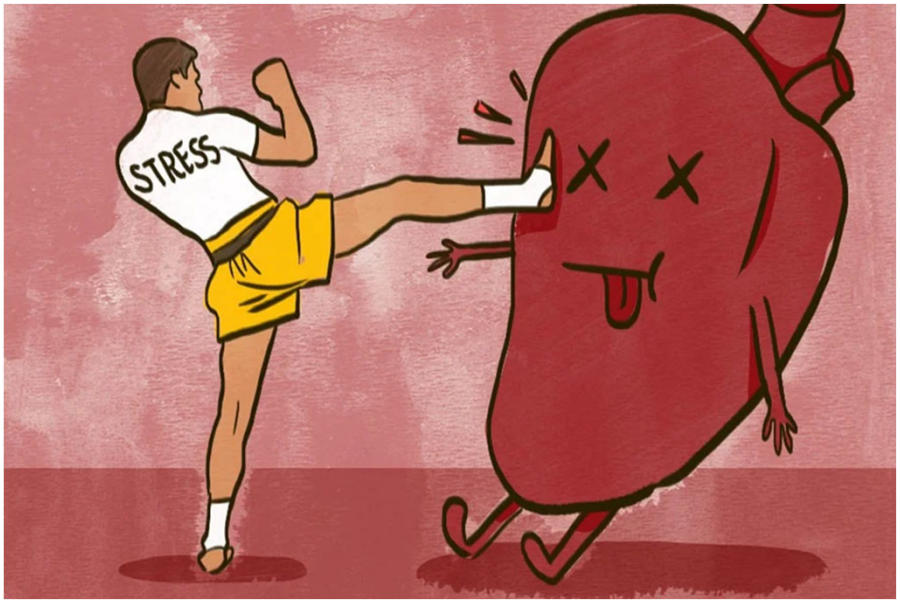


Questions?

- **What does the literature tell us about cortisol and CVD?**
- **How do we measure this risk?**

Cortisol and CVD

- **INTERHEART Study (2004)**
- **CARDIA Study (2006)**
- **WHITEHALL II Study (2011)**
- **InCHIANTI Study (2010)**



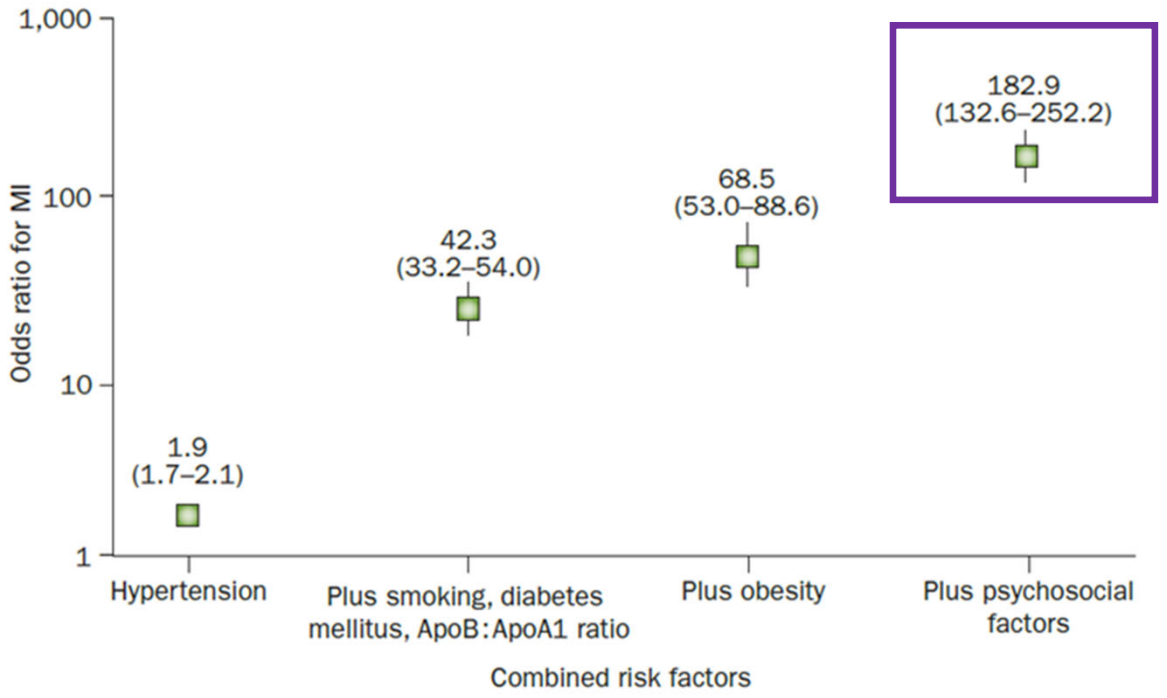
INTERHEART STUDY: [1] Yusuf S, et al. Lancet. 2004; 364(9438): 937-952. [2] Fioranelli M, et al. Front Immunol. 2018; 9: 2031.
CARDIA STUDY: Mathews K, et al. Psychosom Med. 2006; 68(5): 657-661.
WHITEHALL II STUDY: Kumari M, et al. J Clin Endocrinol Metab. 2011; 96(5): 1478-1485
InCHIANTI STUDY: Vogelzangs N, et al. J Clin Endocrinol Metab. 2010; 95(11): 4959-4964.

Cortisol and CVD

- INTERHEART case-controlled study (2004)
  - Largest study to assess long-term stress and CAD; 4 year study
  - Study: 15,152 MI patients, 14,820 controls from 52 countries world-wide between 1999-2003; stress documented by questionnaire
  - 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 stressors are significantly related to AMI risk in all populations**

Yusuf S, et al. Lancet. 2004; 364(9438): 937-952.
Fioranelli M, et al. Front Immunol. 2018; 9: 2031.

Cortisol and CVD



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

Salivary Cortisol and CaC: CARDIA Epidemiologic Study (2006)

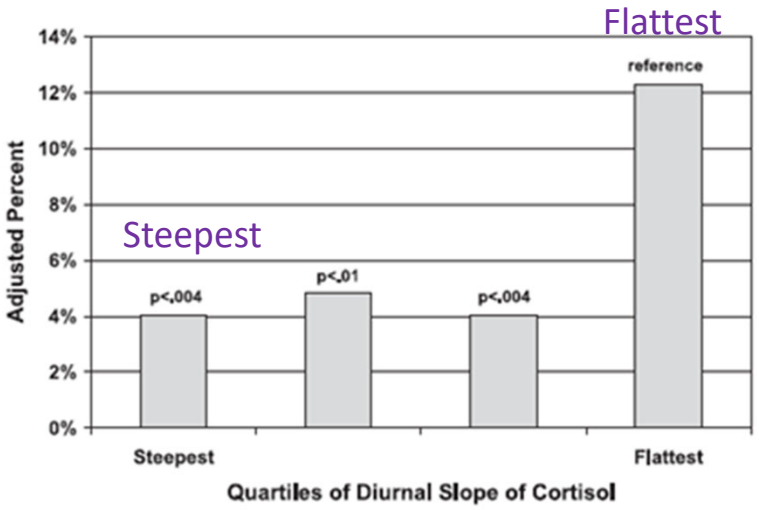


Figure 1. Probability of detectable coronary artery calcification by quartiles of diurnal slope of salivary cortisol adjusted for sex, race, treatment for diabetes, and age. *p* values refer to tests for whether the quartile group differs from the reference group.

- **First study** linking cortisol patterns to CAD; used saliva testing
- **Study:** 718 young participants (avg age 40); 15 year follow-up
- **Objective:** to determine if CaC was associated with average daily cortisol levels and the diurnal slope
- **Results:** A flat diurnal cortisol curve associated with CaC
  - Flatter slope was the result of elevated afternoon, evening, bedtime levels
  - The flattest cortisol slopes was SS associated with CaC
  - When compared to the group with the steepest slope, the group with the flattest slope were 3 and one-third more likely to have CaC
- **Conclusion: HPA axis dysfunction may affect CAD risk**

Mathews K, et al. Psychosom Med. 2006; 68(5): 657-661.

Salivary Cortisol and CVD Mortality: Whitehall II Prospective Cohort Study (2011)

Association of Diurnal Patterns in Salivary Cortisol with All-Cause and Cardiovascular Mortality: Findings from the Whitehall II Study

Meena Kumari, Martin Shipley, Mai Stafford, and Mika Kivimaki

Department of Epidemiology and Public Health, University College London, London WC1E 6BT, United Kingdom

TABLE 3. HR of all-cause, cardiovascular, and noncardiovascular mortality among 4047 participants of the Whitehall II study from phase 7 (2002–2004) through to January 2010 by z-scores of measures of cortisol

	All-cause mortality	Noncardiovascular deaths	Cardiovascular deaths
Waking cortisol	0.94 (0.80–1.12)	0.93 (0.77–1.13)	0.95 (0.67–1.36)
CAR	0.94 (0.80–1.12)	0.90 (0.74–1.10)	1.12 (0.79–1.57)
Slope across the day	1.30 (1.09–1.55)	1.17 (0.96–1.43)	1.87 (1.32–2.64)
Bedtime cortisol	1.33 (1.11–1.59)	1.17 (0.96–1.44)	1.98 (1.39–2.81)

- **First study** to document that daily salivary diurnal cortisol patterns are predictive of subsequent CV mortality in men and women
- **Study**: 4047 men and women, average age 61, mean FU 6.1 years
- **Objective:** to examine the association between cortisol patterns, CV and non-CV mortality
- **Results:** A flattened cortisol curve was SS associated with increased CV mortality; elevated PM cortisol was an independent predictor of subsequent CV mortality
  - No association between waking cortisol, CAR, and mortality
- **Conclusion: A flattened cortisol curve and elevated PM cortisol levels are robust CV mortality predictors in middle-aged adults**

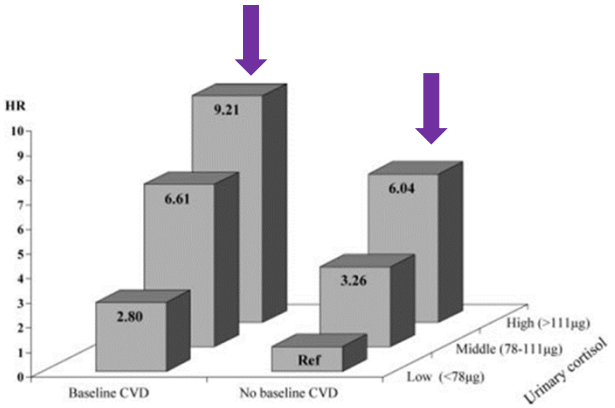
Kumari M, et al. J Clin Endocrinol Metab. 2011; 96(5): 1478-1485.

Urinary Cortisol and CVD Mortality: InCHIANTI, a Prospective Cohort Study (2010)

Urinary Cortisol and Six-Year Risk of All-Cause and Cardiovascular Mortality

Nicole Vogelzangs, Aartjan T. F. Beekman, Yuri Milaneschi, Stefania Bandinelli, Luigi Ferrucci, and Brenda W. J. H. Penninx

Department of Psychiatry and EMGO Institute for Health and Care Research (N.V., A.T.F.B., B.W.J.H.P.), VU University Medical Center, 1081 HL Amsterdam, The Netherlands; Clinical Research Branch (Y.M., L.F.), National Institute on Aging, Baltimore, Maryland 21225; Tuscany Health Regional Agency (Y.M.), 50125 Florence, Italy; and Geriatric Rehabilitation (S.B.), Azienda Sanitaria Firenze, 50122 Florence, Italy



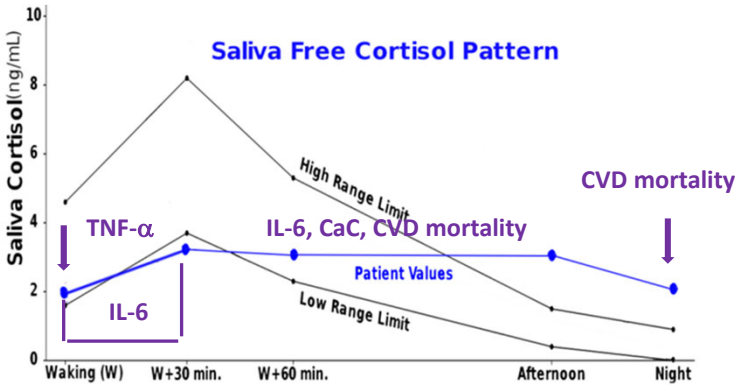
- **First urine study** to document that 24-hour urinary free cortisol (UFC) levels predict CV mortality
- **Study:** 862 older individuals, mean age 74, 55% women; 6-year study; samples at baseline
  - UFC divided into 3 terciles: low < 78µg; moderate: 78-111µg; high: > 111µg
- **Objective:** To determine whether 24-hour UFC levels predict all-cause and CV mortality
- **Results:** UFC strongly predicts CV mortality, not non-CV mortality in persons with and without baseline CVD
  - Risk increased with increasing UFC levels
  - Those in the highest tercile had a 5x increased CVD mortality risk over 6-years
    - No baseline CVD: 6x increased risk of dying from CVD
    - Baseline CVD: 9.2x increased risk of dying from CVD
- **Conclusion: UFC is a strong CVD mortality predictor in persons with and without baseline CVD**

Vogelzangs N, et al. J Clin Endocrinol Metab. 2010; 95(11): 4959-4964.

MESA Stress Study: A Longitudinal Prospective Study (2012)

Associations of salivary cortisol levels with inflammatory markers: The Multi-Ethnic Study of Atherosclerosis

A.S. DeSantis[a,*], A.V. DiezRoux[a], A. Hajat[a], A.E. Aiello[a], S.H. Golden[b], N.S. Jenny[c], T.E. Seeman[d], S. Shea[e]



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

- **Study:** Multi Ethnic Study Atherosclerosis; 869 adults; cortisol curves x 3 days
- **Objective:** Assess associations between cortisol patterns and inflammatory biomarkers
  - Was there a relationship between diurnal cortisol curves (waking, CAR, slope) and IL-6, IL-10, 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:**
  - Higher IL-6: SS associated with lower CAR, flatter slope, greater area under the curve; No SS association with waking cortisol or HS cortisol
  - Higher TNF-α: SS associated with lower waking cortisol; Non-significant association with flatter curve
  - Higher IL-10: Non-significant flatter slope
- **Conclusion: HPA axis may mediate associations between stress and inflammation**

Key Points

- Cortisol is an acute and chronic stress marker
- Chronic stress is significantly associated with MI risk
- Cortisol is a strong predictor of CVD risk, events, and mortality
  - Salivary flattened diurnal cortisol pattern with high PM cortisol
    - Associated with increased CAC deposition
    - Cause specific association with 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

CVD Risk

- **What does the literature tell us about cortisol and CVD?**
  - **Chronic stress and HPA axis dysfunction increase inflammation, CVD risk, CVD events, and CVD mortality**
- **How do we measure this risk?**
  - **Saliva + CAR**
  - **Urine + metabolites**

How Do We Translate This Into Clinical Practice?

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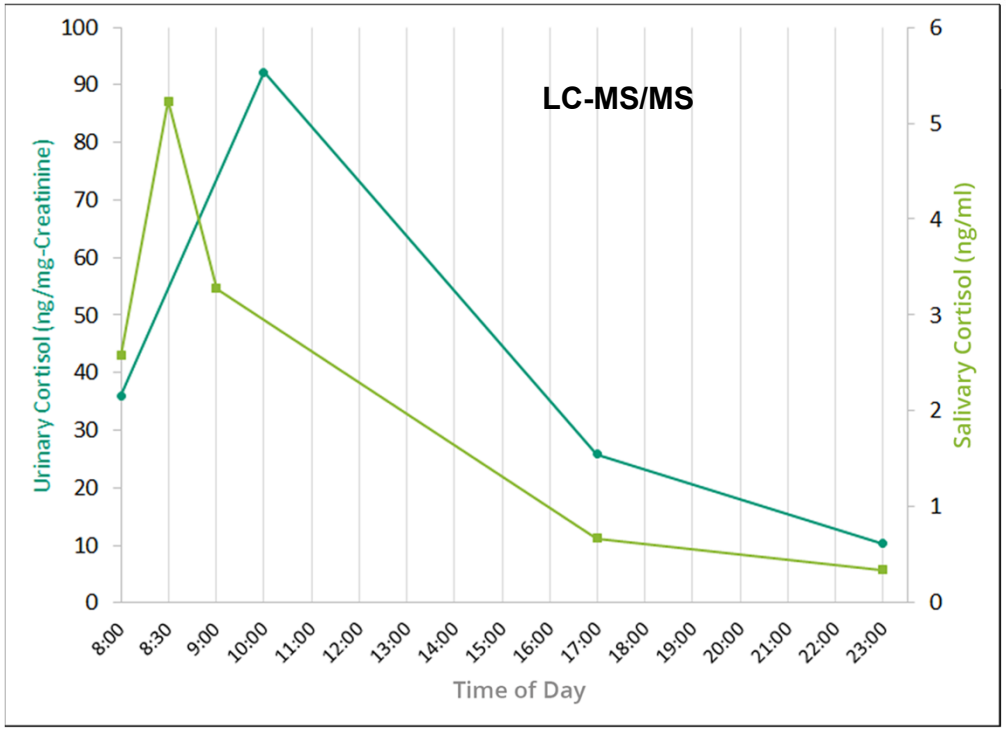
Saliva: The Gold-Standard for HPA Axis Testing

Before choosing a test other than saliva testing, the test should be validated against the gold-standard or studied and documented to improve clinical outcomes!

A Commercially Available Validated Urine Test



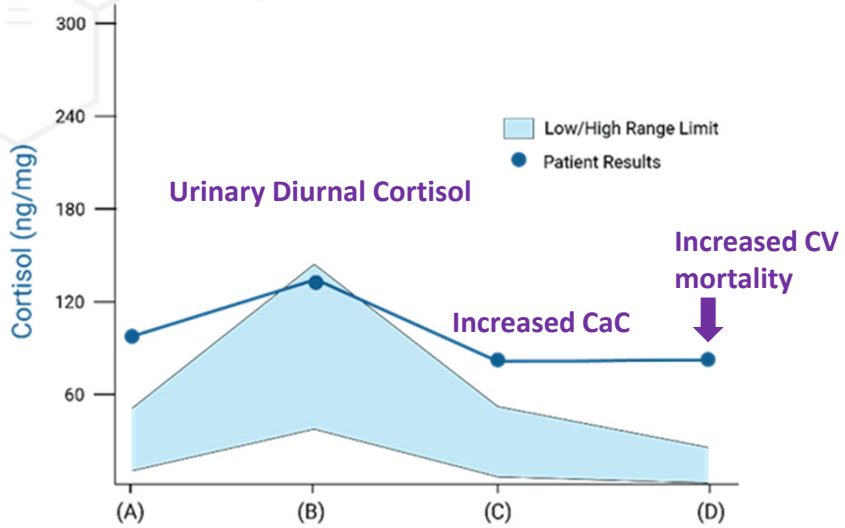
Dried urine and salivary profiling for complete assessment of cortisol and cortisol metabolites

Mark Newman [a], Desmond A. Curran [a], Bryan P. Mayfield [a,b,*]

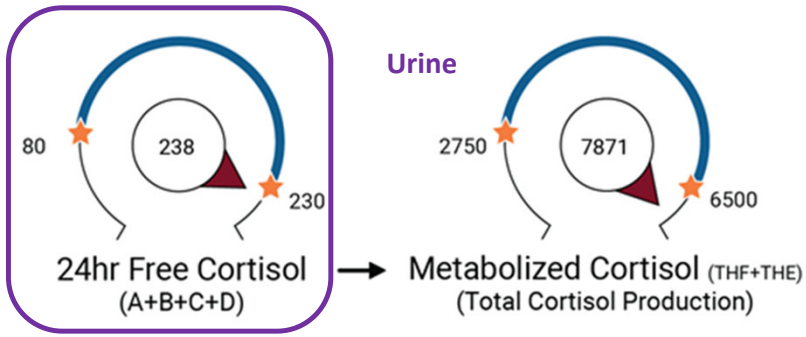
- **Study Objectives**
  - Determine the utility of dried urine to measure cortisol and cortisol metabolites
  - Is the 4-spot dried urine representative of 24-hour liquid urine?
  - Can the diurnal pattern be observed in urine?
- **Study group**
  - 68 individuals with both saliva and urine diurnal cortisol measurements on the same day
- **Conclusion**
  - **4-spot dried urine a viable alternative to liquid urine for measuring cortisol and metabolites**
  - **4-spot dried urine is good surrogate for the salivary diurnal pattern**

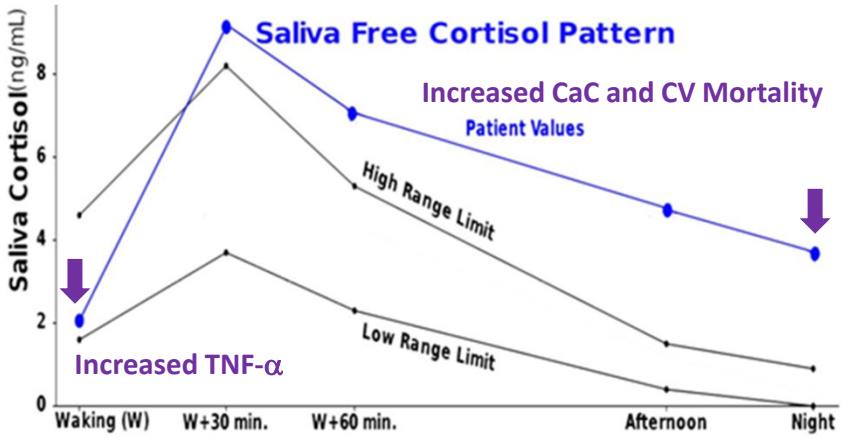
Newman M, et al. J Clin Transl Endocrinol. 2020; 100243.

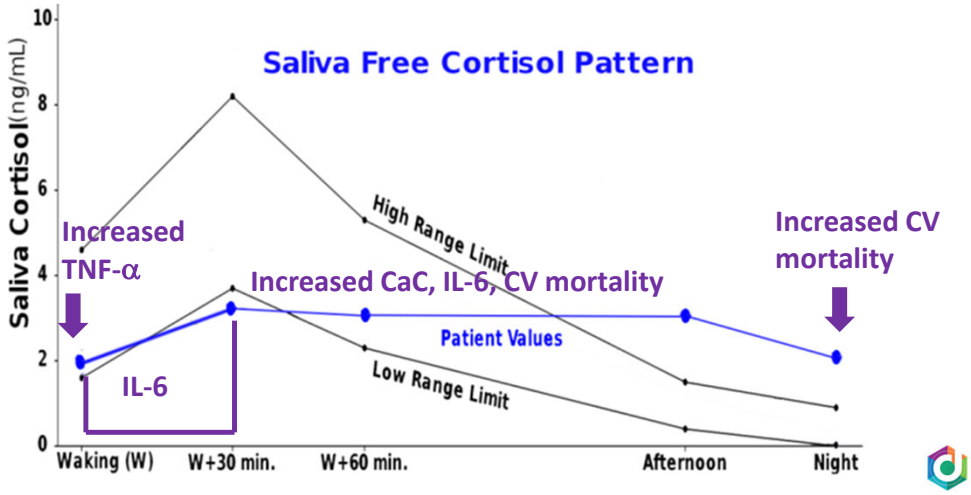
Daily Free Cortisol Pattern



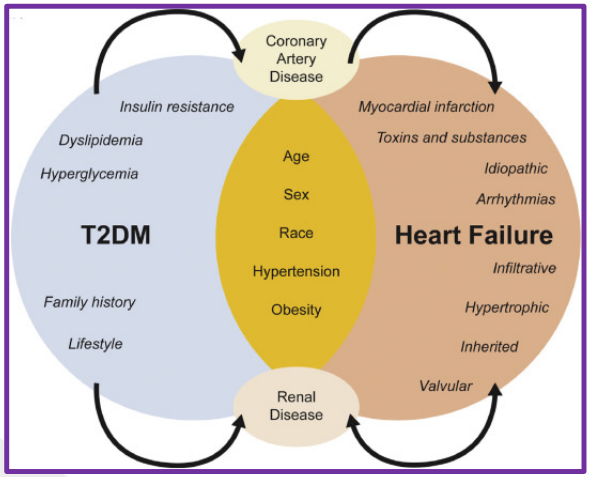
Increased CV mortality







Blood Sugar Regulation: Another Right Target



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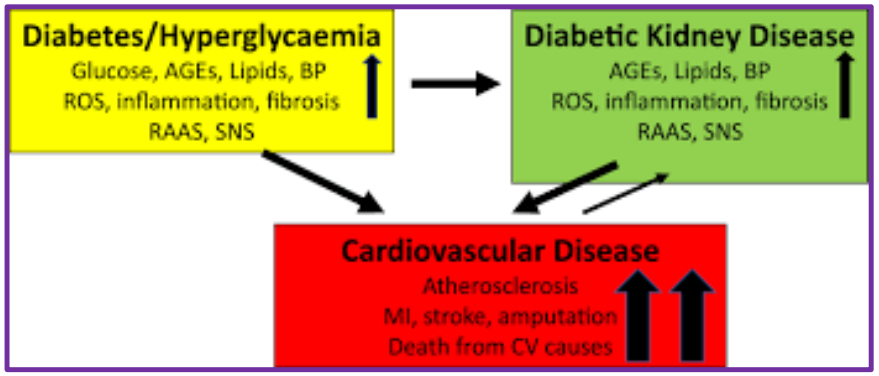


seminarsinnephrology.org

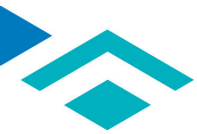
The Endothelial Glycocalyx (EGCX): What Is It and What Does It Do?

- What is the EGCX
  - It is a microscopically thin, negatively charged, gel-like, sugar mesh coating that “blankets” the vascular endothelium’s entire luminal side, providing a nonadherent shield
  - EGCX is made up of glycoproteins, proteoglycans, and glycosaminoglycans
  - It’s thickness varies, depending on the blood vessel and its location
- Healthy EGCX: First Line of Defense
  - Regulates EC functions such as:
    - Barrier and filtration functions, limiting leukocyte access and adhesion to ECs
    - Active cell-cell communication, and
    - Vascular tone

Kolarova H, et al. Mediators Inflamm. 2014; 2014: 694312.

The Endothelial Glycocalyx (EGCX): What Is It and What Does It Do?

- Healthy EGCX: First Line of Defense
  - It is exquisitely sensitive to shear stress and blood flow patterns
    - High shear stress: uniform laminar flow in straight vessel segments, EGCX is robust, healthy, and thus protects the endothelium, with upregulation eNOS and NO
    - Low shear stress: complicated blood vessel segments, bifurcations, branches, and curvatures where blood flow is non-laminar, reversed, oscillatory, and turbulent, the EGCX is thin, and predisposes to atherosclerosis
  - It provides anti-inflammatory and anti-thrombotic effects by docking major proteins made by endothelial cells such as:
    - Anticoagulation: Antithrombin III and its cofactors: heparin cofactor II, thrombomodulin
    - Antioxidant: Superoxide Dismutase (SOD): which decreases ox-stress, quenches ROS, and maintains NO availability



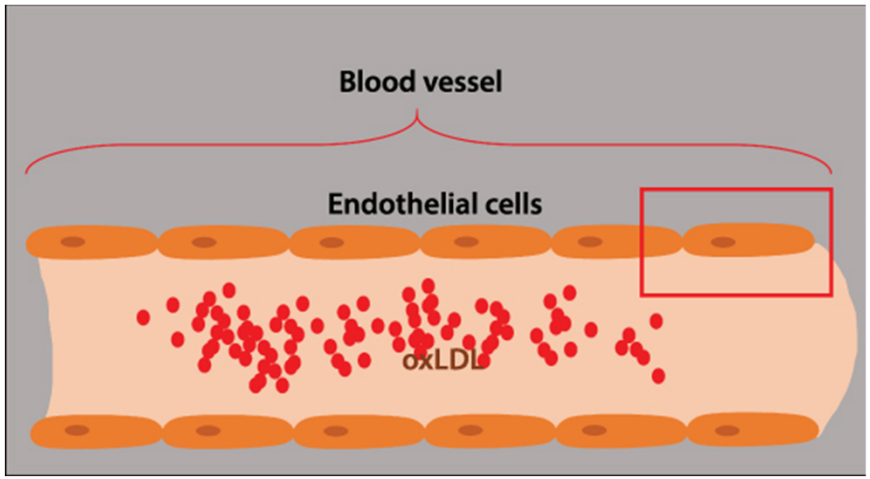
Kolarova H, et al. Mediators Inflamm. 2014; 2014: 694312.

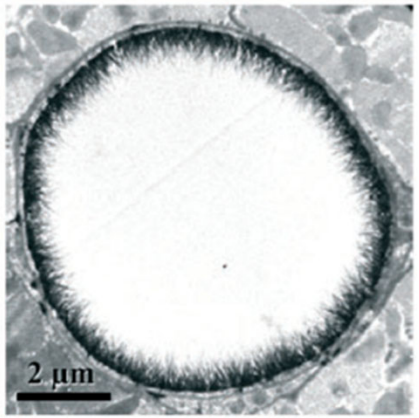
EGCX Constituents and Their Functions

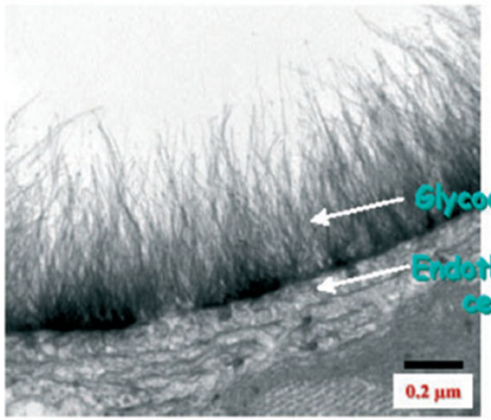
Major GCX constituent families	Well-known family members	Functions
Glycosaminoglycans (GAGs) and Sialoglycoproteins	Heparan Sulfate	GCX function is determined based on concentration and organization of GAGs.
	Chondroitin Sulfate Hyaluronic Acid	GCX thickness and protrusion into the vascular lumen is derived from the lengthy (hundreds to thousands) disaccharide units that make up the GAGs.
	Sialic Acid	The strong negative charges carried by the disaccharide units further extend the GCX.
Proteoglycans	Glypicans Syndecans	These are backbone molecules that have attachment sites for tethering the GAGs. Proteoglycan family members play an important role in incorporating the extracellular GCX into the EC body. Glypicans are glycosylphosphatidylinositol anchored to the caveolae compartment of the cell membrane. Syndecans are transmembrane and connected to cytoskeleton.
Glycoproteins	Selectins	Glycoproteins reside near the GCX base and are adhesive when exposed.
	Integrins	E-Selectin and P-Selectin contribute to EC interactions with cells in the blood circulation, i.e. leukocytes and platelets.
	Immunoglobulin Superfamily	Integrins control interaction between ECs and surrounding extracellular matrix (i.e., collagen, fibronectin) as well as neighboring cells. Immunoglobulins act as ligands for integrins on leukocytes and platelets and contribute as mediators of adhesion to the endothelium.
Plasma Proteins	Albumin	Plasma proteins penetrate GCX pores (≤ 7 nm, when GCX is intact) and prevent GCX collapse. Albumin transports spingosine-1-phosphate (S1P), which binds to S1P receptors and, as a result, inactivates matrix degradation enzymes and subsequently protects against GCX shedding.

Adapted from: Mitra R, et al. Curr Atheroscler Rep. 2017; 19(12): 63.

Endothelial Cell







(a)

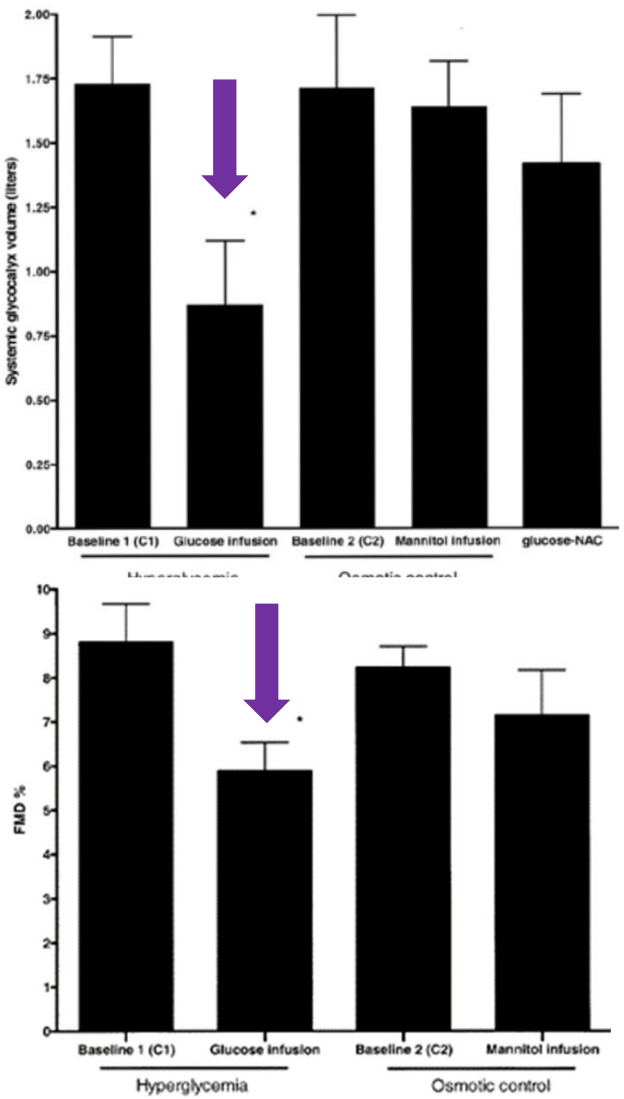
dovepress.com

Adapted from: Gouverneur M, et al. J Intern Med. 2006; 259(4): 393-400

Glucose Dysregulation and EGCX Degradation

- Hyperglycemia, IR, DM, MetS, and EGCX degradation
  - Hyperglycemia causes generalized EGCX thinning and impaired EGCX function
    - When glucose levels are increased for 20 minutes, shear stress-induced (NO) vasodilation is decreased or abolished
  - Hyperglycemia has been associated with EGCX proteoglycan degradation
- Diabetes and its complications
  - Acute, as well as prolonged, long-term hyperglycemia is associated with profound EGCX thinning, EGCX degradation, and vascular vulnerabilities
    - Leaky glomerular capillaries and albuminuria
    - Leaky blood vessels and impaired NO release, increased vascular permeability, with increased cellular adhesion and migration, and an imbalance of coagulation and antioxidant defenses culminating in an accelerated atherosclerotic risk

Gouverneur M, et al. J. Intern Med. 2006; 259(4): 393-400.
Noble MIM, et al. QJM. 2008; 101(7): 513-518.
Kolarova H, et al. Mediators Inflamm. 2014; 2014: 694312.
Dogne S, Flamion B. Am J Pathol. 2020; 190(4): 768-780.

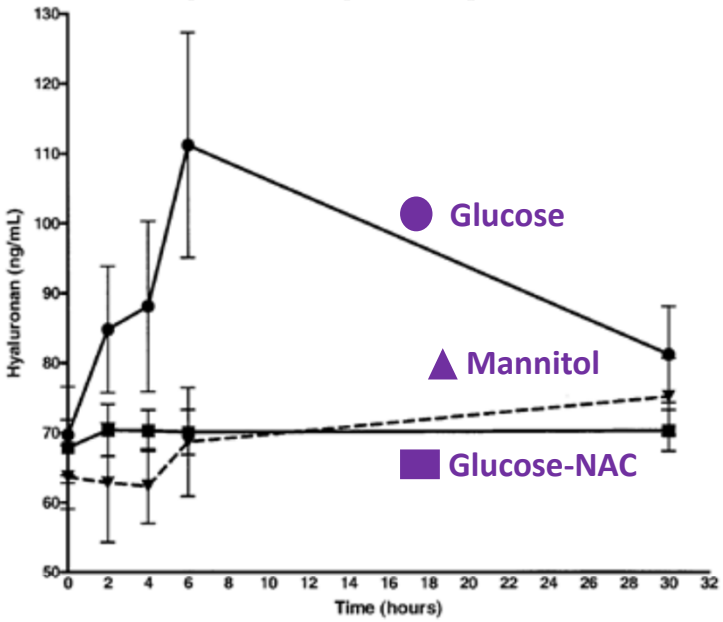


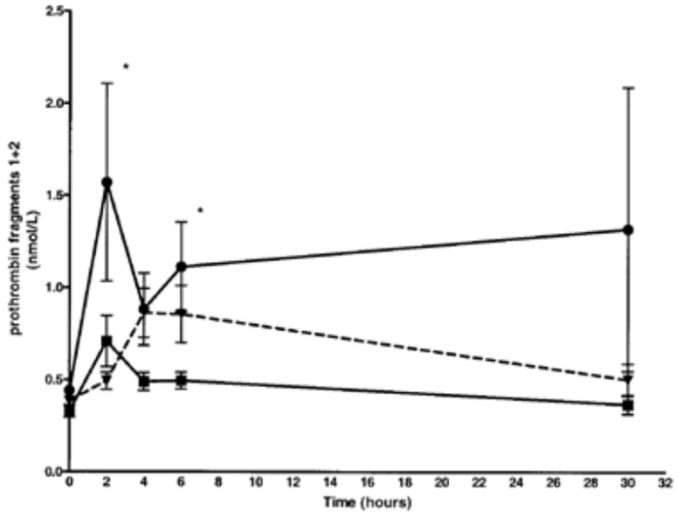
Hyperglycemia and EGCX Degradation

- **Objective:** To determine hyperglycemia's impact on the EGCX in healthy volunteers
- **Study:** Measured changes in systemic EGCX volume, endothelial function (FMD), hyaluronan, and coagulation parameters. In addition, they assessed the role of ROS on EGCX volume
- **Results:**
  - Hyperglycemia SS:
    - Decreased glycocalyx volume,
    - Decreased endothelial function,
    - Increased plasma hyaluronan levels (shedding),
    - Increased coagulation system activity

Nieuwdorp M, et al. Diabetes. 2006; 55(2): 480-486.

Hyperglycemia and EGCX Degradation





- **Results:**
  - Hyaluronan and thrombotic parameters (prothrombin, and D-dimer) were significantly increased
  - ROS plays a significant role in EGCX damage
- **Conclusion: Hyperglycemia-induced ROS upregulation leads to:**
  - **SS EGCX degradation and shedding**
  - **SS decrease in FMD (decreased NO availability)**
  - **SS increased prothrombotic potential**
  - **NAC, by decreasing ROS, mitigated hyperglycemia-induced EGCX degradation**

Nieuwdorp M, et al. Diabetes. 2006; 55(2): 480-486.

Key Points

- Cortisol is an acute and chronic stress marker
- Chronic stress is significantly associated with MI risk
- Cortisol is a strong predictor of CVD risk, events, and mortality
  - Salivary flattened diurnal cortisol pattern with high PM cortisol
    - Associated with increased CAC deposition
    - Cause specific association with 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
- Optimal glucose regulation is key to EGCX and endothelial function
- NAC, by decreasing ROS, mitigates hyperglycemia-induced EGCX degradation and endothelial dysfunction

Sex Hormones: One More Right Target

dailywellness.com

medicalnewstoday.com

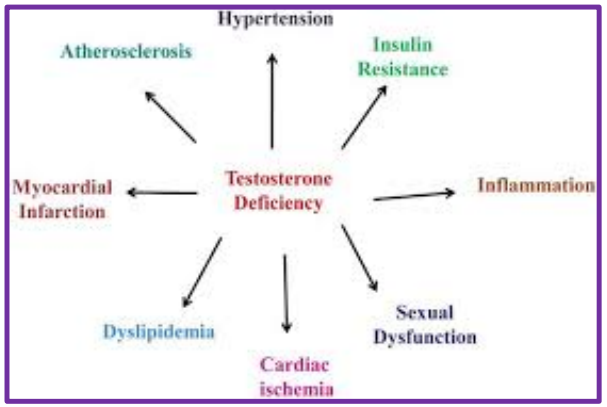
fitfatherproject.com

Hormones and the Immune System

- Aging and the immune system
  - Aging is associated with a chronic inflammatory state characterized by:
    - Increased proinflammatory cytokines: TNF-α, IL-6, and IL-1β
    - Increased free radical production and decreased redox potential
- E2, Pg, and T are immune modulators and anti-inflammatory hormones
  - E2's, Pg's, and T's anti-inflammatory actions include: inhibiting proinflammatory cytokines: IL-6, IL-1β, and TNF-α and stimulating anti-inflammatory cytokines: IL-4, IL-10
  - E2 receptors (ERs), Pg receptors (PRs) and T receptors (TRs) are expressed in: immune cells, endothelial cells, and vascular smooth muscle cells
- **Aging is associated with a decline in sex hormones, and both influence immune competence and disease susceptibility, i.e. CVD**

Gubbels Bupp MR. Cellular Immunol. 2015; 294(2): 102-110.
Gubbels Bupp MR, et al. Front Immunol. 2018; 9: 1269.
Mauvais-Jarvis F, et al. Endocrinology. 2020; 161(9): bqaa127.

Males, Testosterone, and CVD



intechopen.com

Testosterone in Men and the Endothelium

- General Information
  - T gradually declines ~ 1%/year in men after the third decade
- Testosterone's endothelial effects
  - Testosterone is an independent determinant of endothelial function
  - Testosterone deficiency (TD) leads to endothelial dysfunction (ED)
    - TD decreases NO production
    - TD increases ADMA expression (competitive inhibitor of eNOS)
      - Nitric oxide synthase is the enzyme responsible for converting arginine to NO
    - TD decreases endothelial progenitor cells (involved in endothelial repair)
  - TD increases proinflammatory cytokines (IL-6, IL-1β, TNF-α)
  - Testosterone replacement decreases proinflammatory cytokines and increases anti-inflammatory cytokines, i.e. IL-10

Hotta Y, et al. Sex Med Rev. 2019; 7(4): 661-668.
Moreau KL, et al. Biol Sex Differ. 2020; 11(1): 18.
Babcock MC, et al. J Clin Endocrinol Metab. 2021; dgab175.

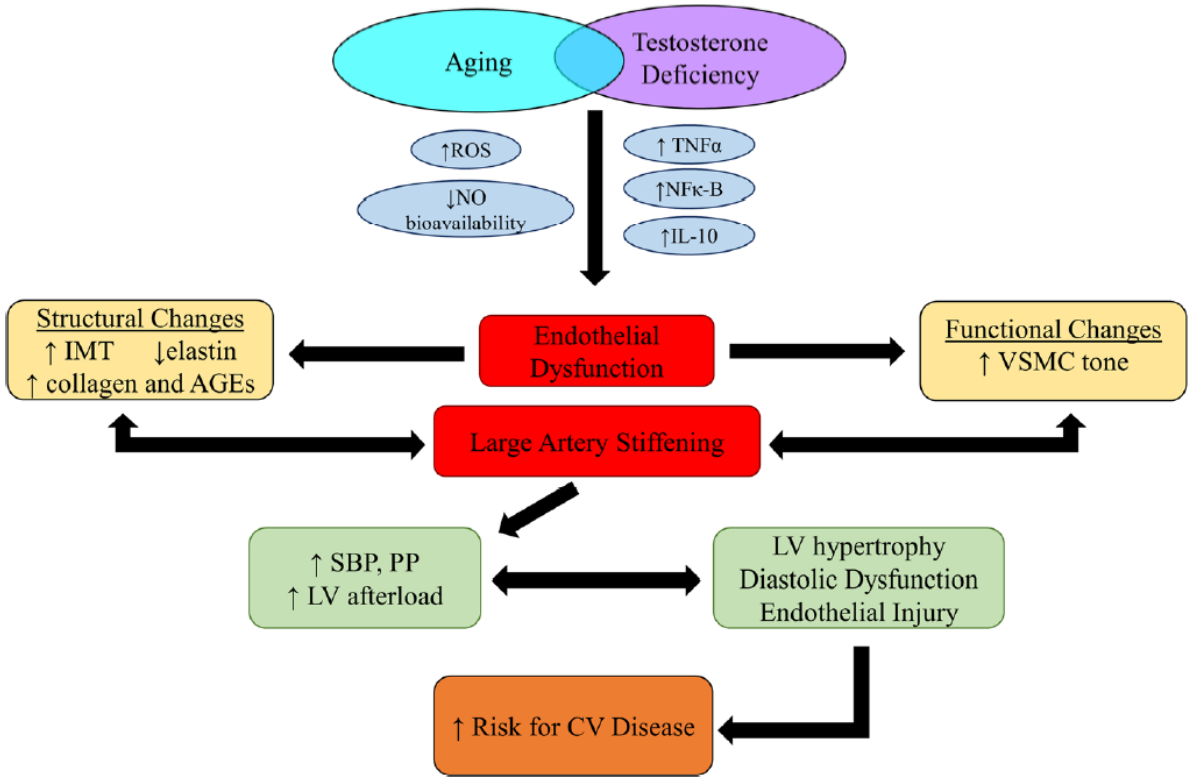
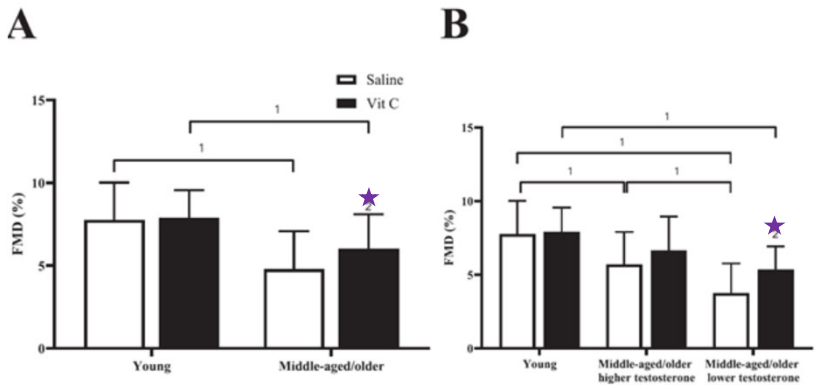


Fig. 1 Hypothesized mechanisms by which testosterone deficiency may contribute to vascular aging in women and men. AGEs, advanced glycation end products; CV, cardiovascular; IL-10, interleukin-10; IMT, intima-media thickness; LV, left ventricle; NFκ-B, nuclear factor κ-B; NO, nitric oxide; PP, pulse pressure; SBP, systolic blood pressure; ROS, reactive oxygen species; TNFα, tumor necrosis factor-α, VSMC, vascular smooth muscle cell

Moreau K, et al. Biol Sex Differ. 2020; 11(1): 18.

TD associated ED is Related to Inflammation and Oxidative Stress



- **Graph A: young vs all middle-aged/older men**
  - Young men: No improvement with Vitamin C
  - All middle-aged/older men: SS lower FMD with saline and Vit C
- **Graph B: Middle-aged/older higher TT vs Middle-aged/older lower TT**
  - Middle-aged/older higher TT: SS difference in FMD when compared to young that was no longer SS after Vitamin C
  - Middle-aged/older lower TT: SS lower FMD when compared to middle-aged/older with higher TT and young. Vit C improved FMD in low TT group, still lower than peers, but not SS different when compared with peers, but still SS lower than young

- **Objective:** Determine if middle-age/older men with low TT would have greater age-associated endothelial dysfunction, related to inflammation and oxidative stress
- **Study:** Cross-sectional study; 58 healthy men: 20 younger men, 20 middle-age/older higher TT, 20 middle-aged/older lower TT
  - Young men (20) TT: 500 ± 58ng/dL
  - Middle-aged/older higher TT (20): 512 ± 115ng/dL
  - Middle-aged older lower TT (18): 269 ± 48ng/dL
- **Results:**
  - Middle-aged older men with lower TT may have accelerated vascular aging as a result of age-associated ED compared with age-matched peers with higher TT, in part due to increased inflammation and oxidative stress
  - Results are independent of CVD risk factors or androgen deficiency symptoms
- **Conclusion: Physiologic TT levels (> 500 to < 1000ng/dL, goal > 500-800ng/dL) may attenuate the age-related decline in endothelial function, independent of symptoms and traditional RF, by decreasing inflammation and oxidative stress**

Babcock MC, et al. J Clin Endocrinol Metab. 2021; dgab715.

Question?

T improves endothelial function and TD leads to endothelial dysfunction, inflammation, and oxidative stress. So, why is there a black-box warning on all testosterone prescriptions re: possible increase in CVD?

Question?

So, why is there a black-box warning on all testosterone prescriptions re: CVD?

4 studies, whose accuracy, validity, and credibility are questioned by experts all over the world, as well as the FDA, prompted the mandated warning

Study	Design/Drugs	Results
Studies suggesting increased CV risk		
Basaria, 2010	• TOM Trial; 6-month RCT, older frail men • Maintain TT levels > 500 to < 1,000ng/dL • Not a CV Study • Primary objective: assess whether T gel increased muscle strength and physical function in elderly frail men	• T significantly improved leg press muscle strength, chest press strength, and stair climbing power • Increased "CV events" stopped study early • Most CV events were not clinically significant: palpitations, PVCs, NS EKG changes, pedal edema • 4 clinically significant events occurred in men with higher TT levels who were given higher than recommended T doses
Vigen, 2013	• Retrospective 3-year, VA observational study • Men with TD, undergoing coronary angiography • Compared those who received T prescription with those who did not	• Initial results: 3-years after angiography, T prescriptions were associated with increased CV events • However, data flawed and contaminated (10% women) • Reanalysis documented a 10.1% absolute event rate in T prescriptions vs 21.1% events in the non-T group
Finkle, 2014	• Retrospective, observational study of a health insurance database • Assessed nonfatal MI rates up to 90 days after a T prescription	• Compared post-prescription MI rates to pre-prescription MI rates, which are unrelated • No validation of actual events, only used ICD codes • No control group, important data points, i.e., TT levels, risk factors, etc. were unknown • T-related events were low and actually lower than that expected in the general population

Xu, 2013	• Metanalysis 27 RCTs
	• Assessed CV events and TTh
		• 2 studies made up 35% CV events
		  • Basaria (TOM) 2010 study; events of questionable clinical significance
		  • Copenhagen study involving high dose oral T resulting in supraphysiologic TT levels in men with cirrhosis; most common CV adverse event: esophageal variceal bleeding
		• When 2 studies removed: no SS difference in event rates between T-treated men and the placebo group

Study	Design/Drugs	Results
Studies suggesting decreased CV risk		
Basaria, 2015	• TEAAM Trial, 3-year RCT • Determined if increasing TT levels into the mid-normal range (500-900ng/dL) would affect CIMT or CaC • Same authors as TOM study	• No increase in CIMT or CaC in T-treated when compared to placebo
Snyder, 2016	• Testosterone Trials (T Trials) • 1-year RCTs with a 2nd year safety follow-up • 3 main studies with 4 additional studies	• Intervention trials: Major adverse cardiovascular events (MACE) rates were identical when comparing T vs placebo groups • Second year (safety data): T-treated men with fewer CV events, hospitalizations, or deaths than placebo
Budoff, 2017	• T Trial: CV study • 1-year RCT, 2nd year follow-up • 138 men at moderate to high risk for a CV event • Assessed noncalcified plaque volume, CaC	• T-treated men had higher non-calcified coronary plaque volume; unclear how translates clinically • No increase in CaC or calcified plaque when T-group compared with placebo • No difference in MACE between T-treated and placebo
Sharma, 2015	• Large, retrospective, observational study • Compared TTh resulting in normal TT levels (TTh-normal) vs TTh resulting in persistently low TT (TTh-low) vs no TTh (no-TTh) • Study objective was to evaluate TTh's association with all-cause mortality, MI, and stroke • Study duration 4.6-6.2 years	• Compared to no-TTh, TTh-normal levels had a 56% reduction in death, 24% reduction in MI, and a 35% reduction in stroke • Compared to TTh-low, TTh-normal had a 37% reduction in death, 18% reduction in MI, and a 30% reduction in stroke • Compared to no-TTh, TTh-low had a decreased mortality • Adverse events similar between the 2 groups
Anderson, 2016	• 3-year, retrospective observational study • Compared men with low serum TT levels who received TTh and either had low, normal, or high T levels • MACE: nonfatal MI, stroke, death	• Confirmed Sharma's 2015 study results • Men who achieved normal TT levels, 3-year MACE rates were significantly lower than men with low TT • MACE rates similar in men with normal TT and high TT • However, men in high TT-group trended toward increasing stroke rates
Wallis, 2016	• Retrospective, population-based, cohort observational study • Follow-up duration ~ 5.3 years in the TTh group and 5.1 years in the control group	• TTh was associated with decreased mortality • Longer the TTh, greater the risk reduction
Cheetham, 2017	• Retrospective, cohort study • Evaluated the association between TTh and CV outcomes • Composite outcome: AMI, coronary revascularization, unstable angina, stroke, transient ischemic attack (TIA), and sudden cardiac death (SCD) • Follow-up 3.4 years	• T treatment was associated with decreased CV adverse outcomes after a median follow-up of 3.4 years • The hazard ratio for adverse CV events was one-third lower in the TTh group when compared to the non-treated group

Question?

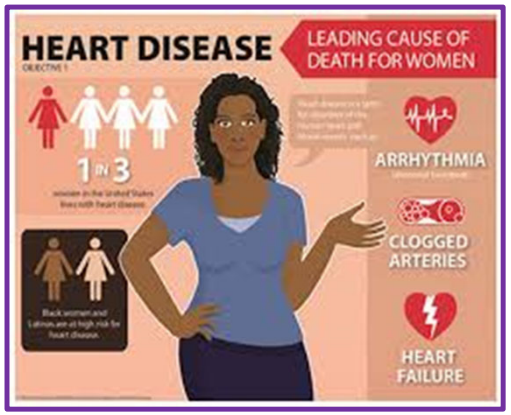
Should there be a black-box warning?

Probably Not!

Key Points: T in Men

- Serum is the gold-standard, there is no viable alternative
- Regardless of dose, serum TT levels > 500 to < 1,000ng/dL, goal > 500-800ng/dL improve clinical outcomes
- Serum TT levels > 200ng/dL with intact aromatization (T → E2) and E2 levels > 10-15pg/mL prevent marked and significant bone loss
- Maintain serum (LC-MS/MS) E2 levels between 20-40pg/mL, goal 30-35pg/mL to maximize clinical impact
- Serum TT levels generally higher with gels than creams
  - May need up to 2x the T cream dose to achieve similar serum TT levels and clinical outcomes as with T gels

Females, E2, and CVD



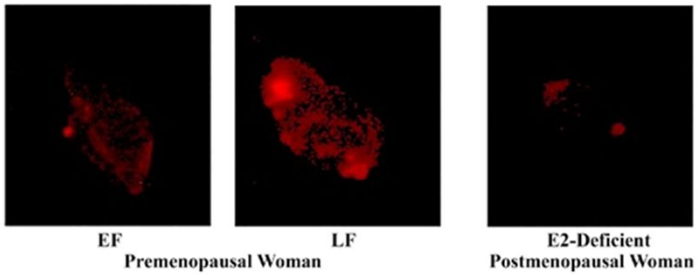
cardiachealth.ca

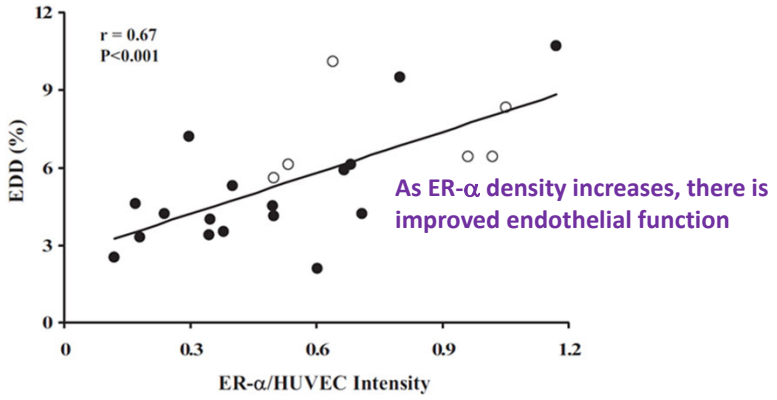
Estradiol and the Endothelium

- Estradiol's endothelial effects
  - E2 increases endothelial NO production → vasodilation
  - E2 decreases endothelin-1, a potent vasoconstrictor and pro-inflammatory peptide secreted by the endothelium
  - E2 has direct antioxidant effects: scavenging/inhibiting ROS
  - E2 increases mitochondrial antioxidant defense
- Estrogen receptor signaling: Use it or Lose it!
  - The endothelium has both ER-α and ER-β receptors; ER-α >> ER-β
  - ER-α is a key determinant in maintaining endothelial vascular function
    - ER-α binding increases eNOS and SOD
  - E2 modulates endothelial cell ER expression, which impacts ER signaling, sensitivity, and function

Somani YB, et al. Am J Physiol Heart Circ Physiol. 2019; 317(2): H395-H404.

ER-α Endothelial Cell Expression: a Dynamic Process Dependent on E2 Status





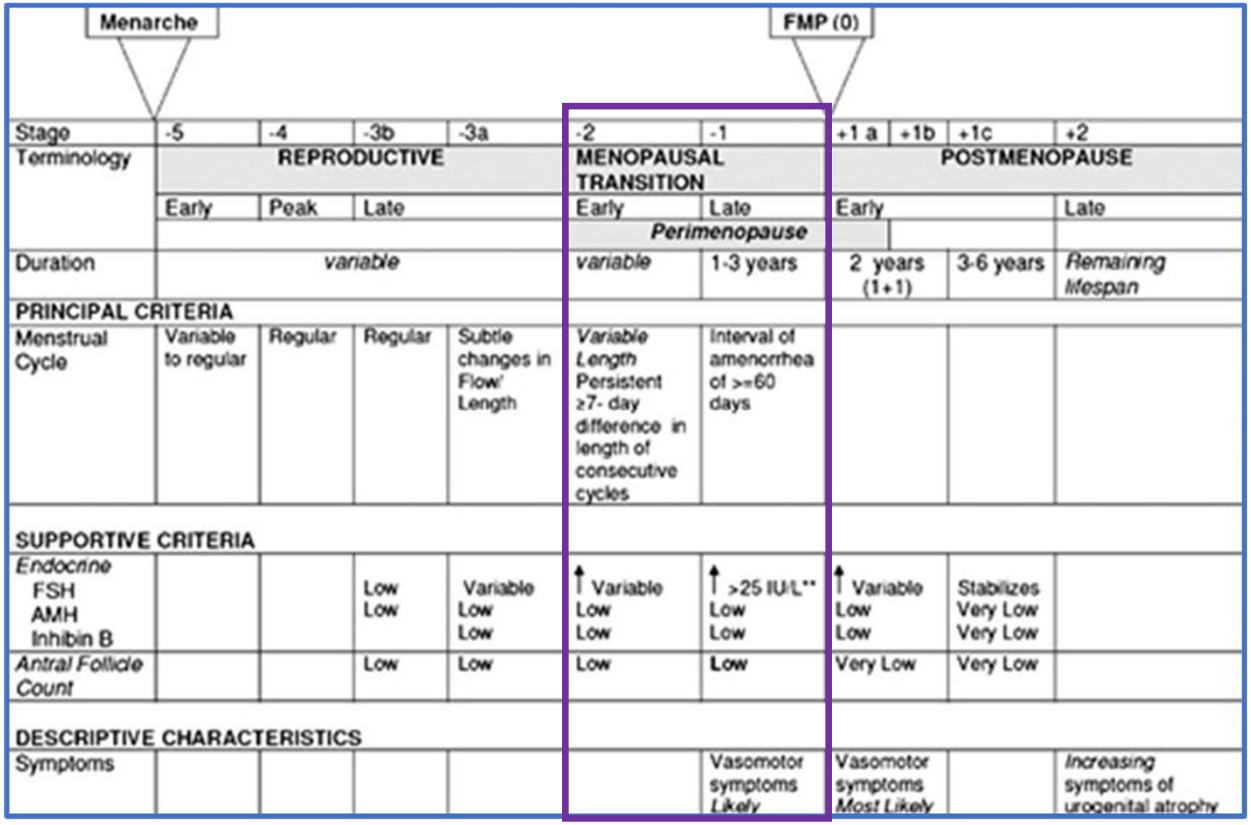
As ER-α density increases, there is improved endothelial function

Gavin KM, et al. J Clin Endocrinol Metab. 2009; 94(9): 3513-3520.

- **Objective:** Determine whether vascular endothelial cell ER-α expression is influenced by E2 status and related to endothelial cell function
- **Study:** Observational study, 16 healthy premenopausal and 17 PMP women were studied
- **Method:** Immunofluorescent staining of peripheral venous endothelial cells and brachial artery flow-mediated vasodilation was performed
- **Results:**
  - Serum E2 levels
    - Premenopausal EF: 36 ± 7pg/mL; LF: 83 ± 17pg/mL
    - PMP: 30 ± 6pg/mL
  - ER-α expression
    - EF: 30% less than LF (SS, P < .0001)
    - PMP: 33% less than LF (SS, (P < .0001)
  - ER-α expression positively associated with serum E2 levels, eNOS expression and activation
  - Endothelial-dependent vasodilation
    - 30% less in PMP women
    - Positively related to endothelial ER-α expression
    - Not related to CVD risk factors
- **Conclusion: Serum E2 may regulate ER-α expression, which influences endothelial function by modulating eNOS**

Question: Do the hormone changes that occur during the menopause transition accelerate vascular aging and contribute to endothelial dysfunction?

The Menopause Transition: an Overlooked Target



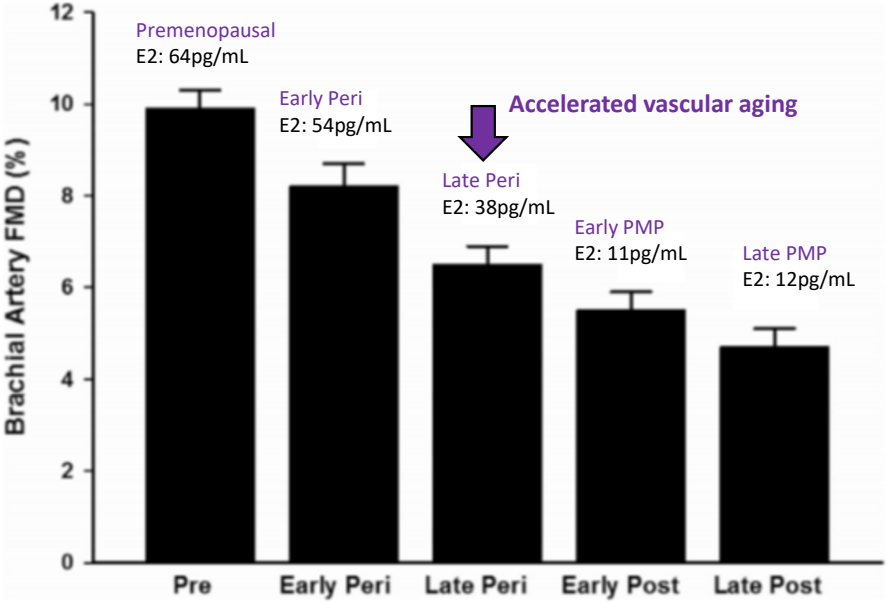
	Menarche					FMP (0)				
Stage	-5	-4	-3b	-3a	-2	-1	+1 a	+1b	+1c	+2
Terminology	REPRODUCTIVE				MENOPAUSAL TRANSITION		POSTMENOPAUSE			
	Early	Peak	Late		Early	Late	Early			Late
					Perimenopause					
Duration	*variable*				*variable*	1-3 years	2 years (1+1)	3-6 years	*Remaining lifespan*	
PRINCIPAL CRITERIA										
Menstrual Cycle	Variable to regular	Regular	Regular	Subtle changes in Flow/ Length	*Variable Length* Persistent ≥7- day difference in length of consecutive cycles	Interval of amenorrhea of >=60 days				
SUPPORTIVE CRITERIA										
Endocrine FSH AMH Inhibin B			Low Low	Variable Low Low	↑ Variable Low Low	↑ >25 IU/L** Low Low	↑ Variable Low Low	Stabilizes Very Low Very Low		
Antral Follicle Count			Low	Low	Low	Low	Very Low	Very Low		
DESCRIPTIVE CHARACTERISTICS										
Symptoms						Vasomotor symptoms *Likely*	Vasomotor symptoms *Most Likely*		*Increasing* symptoms of urogenital atrophy	

Harlow SD, et al. Menopause. 2012; 19(4): 387-395.

The Menopause Transition: an Overlooked Target

Endothelial Function Is Impaired across the Stages of the Menopause Transition in Healthy Women

Kerrie L. Moreau, Kerry L. Hildreth, Amie L. Meditz, Kevin D. Deane, and Wendy M. Kohrt



Early peri: 17% decrease vs Late peri: 34% decrease

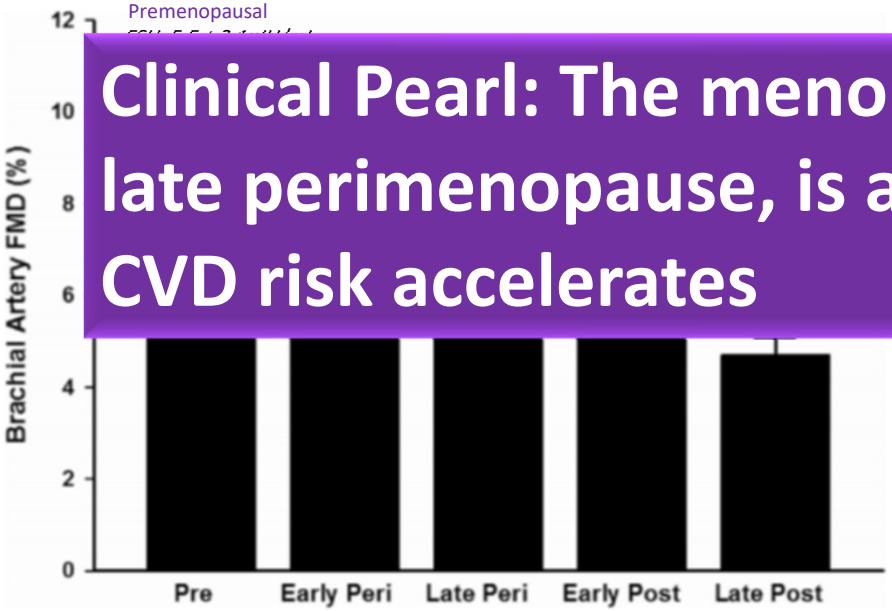
Moreau KL, et al. J Clin Endocrinol Metab. 2012; 97(12): 4692-4700

- **Objective:** To determine whether the menopause transition affected endothelial function, as measured by brachial artery flow-mediated vasodilation (FMD)
- **Study:** Cross-sectional observational study involving 132 healthy women; not on hormone therapy/contraception for ≥ 6 months
  - Early peri: > 2 cycles, length ≥ 7d, **late peri: amenorrhea ≥ 2 months, but ≤ 12 months,** early PMP: ≤ 5 years, late PMP: > 5 years (STRAW criteria)
- **Results:**
  - The menopause transition was associated with endothelial dysfunction, independent of traditional RF
  - When compared to premenopausal women:
    - FMD was SS lower in early peri ($P = 0.03$) late peri ($P < 0.001$), and early and late PMP ($P < 0.001$)
  - Early peri hormone levels may be sufficient to provide some endothelial protection
  - Late peri associated with a rapid decline in endothelial function that worsens with prolonged E2 deficiency
    - SS lower FMD than either pre- or early peri women, but was NOT SS different than PMP women
  - PMP women, with prolonged E2 deficiency had the lowest FMD
  - Lower FMD strongly associated with higher FSH and lower E2 levels
- **Conclusion: Menopause transition associated with a significant decline in endothelial function**

The Menopause Transition: an Overlooked Target

Endothelial Function Is Impaired across the Stages of the Menopause Transition in Healthy Women

Kerrie L. Moreau, Kerry L. Hildreth, Amie L. Meditz, Kevin D. Deane, and Wendy M. Kohrt



Early peri: 17% decrease vs Late peri: 34% decrease

Clinical Pearl: The menopause transition, especially late perimenopause, is a "critical period" during which CVD risk accelerates

- **Objective:** To determine whether the menopause transition affected endothelial function, as measured by brachial artery flow-mediated vasodilation (FMD), a validated prognostic marker for CVD events
- **Study:** Cross-sectional observational study involving 132 healthy women; not on
  - Early peri hormone levels may be sufficient to provide some endothelial protection
  - Late peri associated with a rapid decline in endothelial function that worsens with prolonged E2 deficiency
    - SS lower FMD than either pre- or early peri women, but was NOT SS different than PMP women
  - PMP women, with prolonged E2 deficiency had the lowest FMD
  - Lower FMD strongly associated with higher FSH and lower E2 levels
- **Conclusion: Menopause transition associated with a significant decline in endothelial function**

Moreau KL, et al. J Clin Endocrinol Metab. 2012; 97(12): 4692-4700

Question: Do the hormone changes that occur during the menopause transition accelerate vascular aging and contribute to endothelial dysfunction?

Yes!

What about menopause, E2, and CVD?

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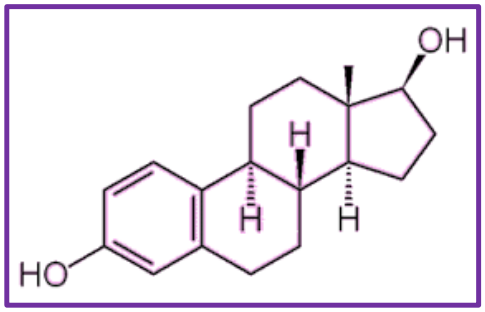

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Study	Study Drugs/Doses	Results
Studies documenting mixed results		
WHI	• CEE-alone (0.625mg/d) • Placebo	• PMP women age 50-59: 40% decreased MI risk and all-cause mortality • PMP women age 60-69: neutral effects on CV outcomes • PMP women 70-79: trend towards increased CV events
ELITE	• No uterus: O-E2 1mg/d • Yes, uterus: O-E2 1mg/d + VMP gel 45mg/d, days 1-10 • Placebo	• Two study groups: early (< 6 years PMP) and late (≥ 10 years PMP) with subclinical atherosclerosis (ASCVD) • PMP early group: o-E2 slowed CIMT progression compared to placebo, but only at 5-year follow-up • PMP late group: no difference in CIMT progression compared to placebo
ELITE post trial analysis	• No uterus: O-E2 1mg/d • Yes, uterus: O-E2 1mg/d + VMP gel 45mg/d, days 1-10 • Placebo	• On treatment serum E2 levels were differentially associated with CIMT progression according to timing of MHT initiation • Early PMP group: the higher the treatment serum E2 level, the slower the CIMT progression rate (serum E2: 48.2 ± 35.4pg/mL) • Late PMP group: with higher serum E2 levels, CIMT progression rate was increased (serum E2: 40.2 ± 23.6pg/mL)
Studies documenting no CV benefit or harm		
KEEPS	• PREMARIN 0.45mg/d + PROMETRIUM 200mg/d x 12d • CLIMARA 0.05mg/d + PROMETRIUM 200mg/d x 12d • Placebo	• Naturally PMP women within 3 years of menopause, none with subclinical ASCVD • Neither PREMARIN nor CLIMARA affected the rate of CIMT progression after 4 years • PREMARIN: trend toward reduced CaC accumulation • Serum E2 on CLIMARA: mean 44pg/mL, average: ~ 40pg/mL
Studies documenting CV benefits		
DOPS	• No uterus: O-E2 2mg/d • Yes uterus: O-E2 2mg/d x 12d; O-E2 2mg + 1mg NORETHISTERONE ACETATE x 10d; o-E2 1mg/d x 6 days • Placebo	• Recently PMP, treated 16 years • All treatment groups had a significantly lower coronary heart disease risk at both 10- and 16-years of follow-up • At 10 years, PMP women receiving O-E2 had a significantly reduced CV event risk such as heart failure and MI
FINNISH	• O-E2 1-2mg/d • TD E2 patches 0.025mg-0.1mg/d • TD E2 gels 1-2mg/d • Progestins used in PMP women with a uterus • Never users	• In all E2 users, CAD-related death risk was reduced by up to 54% in a time-dependent manner • The longer a woman was prescribed and used an E2-based MHT, the greater the risk reduction • All risk reductions were comparable in PMP women initiating E2 < age 60 and in women initiating therapy ≥ 60 years or older

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Key Points: E2 and CVD Risk

- Estradiol decreases CV morbidity and mortality
  - E2's CVD mortality reduction is positively related to E2 exposure time
  - TD E2 patches (0.025mg/d) and gels (1-2mg/d), and o-E2 (1-2mg/d) have been associated with decreased CV mortality
  - Treat women as early as possible, okay to initiate therapy later, and treatment may be continued for > 10 years, as long as initial and ongoing risk stratification
  - Adding OMP 200 or 100mg/d or VMP 100 or 45mg/d does not increase CVD risk, may provide CVD benefits, while protecting the endometrium and improving BMD
  - Adding TTh to TD E2 and OMP/VMP improves endothelial function to a greater degree than E2 and Pg

Key Points: E2 and CVD Risk

- Estradiol decreases CV morbidity and mortality
  - Recently menopausal women, depending on CVD risk (those with subclinical ASCVD), may require higher serum E2 levels closer to the low luteal range (40-60pg/mL or validated dried-urine levels of ~ 1.8-2.0ng/mg) for CIMT reduction
  - Older PMP women or women further from menopause onset probably do best with serum E2 levels just outside the PMP range (20 to < 40pg/mL, or validated dried-urine levels of 0.7 to ~ 1.3 to 1.5ng/mg), regardless of CVD risk
  - Time since menopause and age > 60 should cause pause, not prevent MHT initiation or continuation
  - Ongoing risk stratification and follow-up testing is a must for all women

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    Time since menopause and age > 60 should cause pause, not prevent MHT initiation or continuation
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Clinical Pearls: When counseling patients, remind them that currently estradiol is NOT indicated to prevent adverse cardiovascular outcomes; however, the data suggests that it will improve CVD outcomes and the earlier you initiate therapy the greater the benefits.

Question?

Why isn’t E2 indicated to prevent adverse CV outcomes?

Question?

Why isn’t E2 indicated to prevent adverse CV outcomes?

The guidelines got it wrong. They based their recommendations on synthetic hormones!

MHT Guidelines

Menopause: The Journal of The North American Menopause Society
Vol. 24, No. 7, pp. 728-753
DOI: 10.1097/GME.0000000000000921

Position Statement

The 2017 hormone therapy position statement of The North American

"For women who initiate [M]HT more than 10 or 20 years from menopause onset or are aged 60 years or older, the benefit-risk ratio appears less favorable because of the greater absolute risk of coronary artery disease, stroke, venous thromboembolism, and dementia."

depending on type, dose, duration of use, route of administration, timing of initiation, and whether a progestogen is used. Treatment should be individualized to identify the most appropriate HT type, dose, formulation, route of administration, and duration of use, using the best available evidence to maximize benefits and minimize risks, with periodic reevaluation of the benefits and risks of continuing or discontinuing HT.

For women aged younger than 60 years or who are within 10 years of menopause onset and have no contraindications, the benefit-risk ratio is most favorable for treatment of bothersome VMS and for those at elevated risk for bone loss or fracture. For women who initiate HT more than 10 or 20 years from menopause onset or are aged 60 years or older, the benefit-risk ratio appears less favorable because of the greater absolute risks of coronary heart disease, stroke, venous thromboembolism, and dementia. Longer durations of therapy should be for documented indications such as persistent VMS or bone loss, with shared decision making and periodic reevaluation. For bothersome GSM symptoms not relieved with over-the-counter therapies and without indications for use of systemic HT, low-dose vaginal estrogen therapy or other therapies are recommended.

Pinkerton JV, et al. Menopause. 2017; 24(7): 728-753.
Pinkerton JV. Clin Obstet Gynecol. 2018; 61(3): 447-453.

MHT Guidelines

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2012 Hormone Therapy Position Statement of The North American Menopause Society and identifies future research needs. An Advisory Panel of clinicians and researchers expert in the field of women's health and menopause was

- **The statement above is based on the WHI data, which are no longer relevant! In fact, guidelines remind us that OMP/VMP is the TOC, and TD E2 may be preferred, especially in certain high risk populations.**
- **Prior to initiating MHT in all women, especially in older women, risk stratification (endometrial, breast, bone, CVD, and cognition) is a must, as is ongoing surveillance. Individualize care!**

Pinkerton JV, et al. Menopause. 2017; 24(7): 728-753.
Pinkerton JV. Clin Obstet Gynecol. 2018; 61(3): 447-453.

E2 Practice Points

- Initiate MHT as close to menopause as possible, continue as long as appropriate with ongoing risk stratification, surveillance, and FU testing
- A TD E2 0.025mg/d patch + OMP 200 or 100mg or VMP 100 or 45mg
  - Protects the endometrium, improves VMS, VVA, and BMD
  - Decreases BC mortality
  - Improves cardiovascular outcomes, and
  - May or may not improve cognitive performance
- For those PMP women, who cannot tolerate a patch, TD E2 gel products are reasonable options, however, not FDA-approved for BMD
- Compounded products probably work, but have no outcome data
- Test, don't guess, include metabolomics, which are necessary for a successful hormone practice

Final Thoughts

- Cardiovascular disease is a chronic inflammatory disease, not a lipid storage disease
- It is not enough to treat and optimize all traditional CV risk factors
  - Dyslipidemia, HTN, glucose dysregulation, obesity, tobacco, etc.
- To optimally mitigate CVD risk, we should address **all** inflammatory triggers to include
  - The HPA axis
  - The GUT: dysbiosis, permeable gut, TMAO
  - Detoxification
- Hormones matter, are inflammatory modulators, and hormone deficiencies are associated with increased CV risk and adverse CV outcomes; test, don't guess

Next Week

Case Studies

- Approach to the patient
  - History
  - Laboratory testing
  - Treatments
- Algorithm for CV risk stratification

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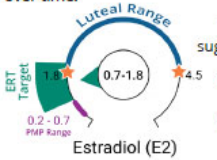
✔ DUTCH	✔ DUTCH	✔ DUTCH	✔ DUTCH	✖ DUTCH	? DUTCH	? DUTCH
The DUTCH Test® provides useful feedback when using OMP in women with PMP sleep disturbances. 5a (more active) and 5b metabolites are measured to individualize OMP dosing. OMP's sleep effects are via its 5a metabolites, predominately allopregnanolone binding to the GABA receptor. No lab test reflects OMP's effect on the endometrium.	Values between the top of the postmenopausal range and the lower limit of the premenopausal range correlate with patient clinical improvement (bone density, hot flash relief, etc.). Doses that push levels to the middle of the premenopausal range and beyond may be excessive. DUTCH is preferred over serum because in addition to metabolites, dried urine averages out the daily up and down E2 patterns. This is particularly helpful with gels and creams that may have serum values that change rapidly over time.  The aggregate clinical data suggests that a serum (LC-MS/MS) E2 level of ~20-40pg/mL improves clinical outcomes (VMS, VVA, BMD). This approximates a DUTCH value of ~ 0.7-1.8ng/mg.		The DUTCH Test® is unique in that it removes potential contamination, and monitoring is helpful with E2 and T. Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.	Pg is measured indirectly in urine by measuring pregnanediols. These metabolites may be underrepresented when Pg is taken vaginally. Serum Pg seems to increase to a higher degree than urine metabolites with vaginal Pg application.	Levels generally parallel changes in serum and clinical outcomes (increased lean body mass, erythrocytosis, etc. in men). Epi-testosterone (Epi-T) values can be used to assess gonadal suppression due to TRT (Epi-T levels in men decrease as TRT increases and are <10ng/mg with complete suppression).	Injections and pellets increase levels, as expected, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the dosing of hormones as well as metabolic patterns.
			Vaginal E2, Pg, and T are systemically absorbed. If placed in the top 1/3 of the vagina, a higher dose will get to the uterus (uterine 1st pass effect), which may be helpful for Pg, but not E2.		Urine testosterone does not correlate as reliably to T serum values, compared to E2 and other tests. Urine testing is best suited as a complimentary test to serum for T and should not be used solely for TRT decisions.	
✖ SERUM	✔ SERUM	? SERUM	✔ SERUM	? SERUM	✔ SERUM	✔ SERUM
Results go up and down quickly. If taken at bedtime, levels return to baseline within a few hours. Results can also be inaccurate due to progesterone metabolites cross-reacting with immunoassay tests.	Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion. Most recommendations are to push serum E2 levels to 20-40pg/mL for clinical impact.	The only published data for E2 creams shows serum results move up and down within a few hours, so serum testing can easily underestimate clinical impact. DUTCH results average out the daily up and down pattern and may be a better option.	Serum results rise quite dramatically with what may seem like modest doses due to the high uptake of hormones across the mucosal membrane. However, values may rise and fall quickly, so be careful with the interpretation of both low and high results.	Serum values increase with dosing and likely represent systemic exposure to Pg. However, the uterine first-pass effect loads the uterus with high levels of Pg (which may be desirable) and serum does not reflect uterine hormone levels.	A great deal of published research shows that serum levels reflect clinical changes in both men and women taking TD T. Be aware of potential up and down patterns throughout the day, but serum is the best tool for monitoring doses of TD T in both men and women.	Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion. Test injections halfway between doses or right before a dose.
✖ SALIVA	The literature does not support salivary testing's use for monitoring TD hormone creams. The saliva data is limited and, in fact, there are no saliva testing outcome studies using TD creams, injections, estradiol patches, oral estradiol, or vaginal hormones. While salivary testing is the gold standard for free cortisol measurement, avoiding its use for monitoring HRT is advised. For situations where saliva testing may parallel the clinical impact, DUTCH or serum testing are better options (see above).					
✖ Oral Estradiol, Estradiol Pellets, or Sublingual Hormones	Though not recommended, if you choose to use either oral estradiol or estradiol pellets, serum testing can monitor both, whereas urine should only be used with pellet therapy. Sublingual hormones may be used in some situations but lab monitoring is not helpful in optimizing doses.					
✖ Transdermal Progesterone	In PMP women, the evidence does not support TD Pg's use to protect the endometrium. When prescribed, laboratory monitoring is not helpful for TD Pg dosing.					

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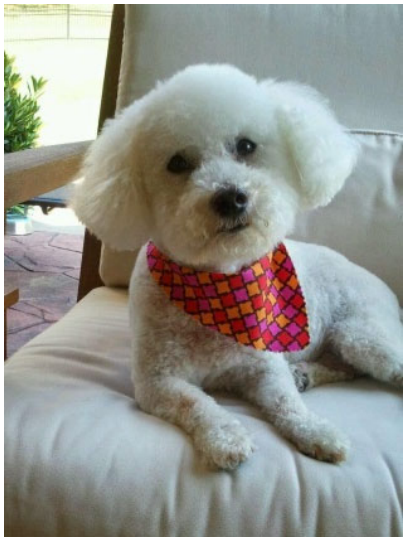

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