Module II
Advanced Cardiology

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Disclosure

MARK HOUSTON MD MS MSc has indicated that he has no conflicts of interest or disclosures

Status of FDA devices used for the material being presented
NA/Non-Clinical

Status of off-label use of devices, drugs or other materials that constitute the subject of this presentation
NA/Non-Clinical

Precision and Personalized Cardiovascular Medicine for the Clinician

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Learning Objectives

1. Review, understand and develop a logical approach to personalized and precision cardiovascular medicine.
2. Review and apply cardiovascular genomics, nutrigenomics and gene expression testing.
3. Review, understand and apply non invasive vascular testing such as EndoPAT, CAPWA (computerized arterial pulse wave analysis) and CAC (coronary artery calcium score), carotid duplex, autonomic function testing, ECHO and stress ECHO, CPET, MCG, retinal scans, watchPat.
4. Understand, review and apply cardiovascular risk scoring systems.
5. Review, understand and apply advanced cardiovascular lab testing for CV risk .

Precision and Personalized Medicine Definitions

- Precision Medicine: medical care designed to optimize efficiency or therapeutic benefit for particular groups of patients, especially by using genetic or molecular profiling.

- Personalized Medicine is a *medical* model that separates people into different groups—with *medical* decisions, practices, interventions and/or products being tailored to the individual patient based on their predicted response or risk of disease.

Precision and Personalized Testing and Approach

- Cardiovascular genetics and nutrigenomics
- Metabolomics
- Cardiovascular gene expression testing (CGET)(CORUS)
- PULS CV testing
- Arterial Compliance Testing (CAPWA)
- Endothelial Function Testing (EndoPAT)
- Cardiopulmonary Exercise Testing (CPET)
- WatchPat for Obstructive Sleep Apnea (OSA)
- Coronary Artery Calcium Scoring (CAC) and CTA
- ECHO and stress ECHO as indicated
- Carotid Doppler and intimal medial thickness (IMT)
- Retinal scan and OPA (Ocular Pulse Amplitude)
- Ankle Brachial index: rest and exercise
- 24 hour Ambulatory BP (ABM) monitor for hypertensive and non hypertensive patients
- HRV: Heart rate variability and HRRT(heart rate recovery time)
- MCG (magnetocardiography)
- Autonomic Function Testing with ABI, SNS/PNS balance, arterial tone, sudomotor function
- Body impedance analysis (BIA) and BW, BMI, WC, WHR, neck circumference, LMM, Body Fat (total and regional)
- CHD risk scoring analysis : COSHEC, Rasmussen and CHAN2T3
- Gut Microbiome Testing
- Advanced Cardiovascular Lab Testing

Cardiovascular Genetic and Nutrigenomic Testing

Cardiovascular Genetic and Nutrigenomic Testing

1. 9p21 (GG/CC): CHD, MI, ASCVD, DM, IR AAA, thrombosis, plaque rupture , inflammation and intracranial aneurysms.
2. 6p21.4: CHD,MI, DVT.
3. 4q25: Atrial Fibrillation, long QT and PR intervals.
4. ACE I/D (DD Allele): HBP,LVH,CRF, MAU, nephroangiogenesis, carotid IMT,MI and CHD.
5. COMT : Catecholamines, CHD,MI, HBP, ASA and vitamin E responses
6. 1q25 (GLUL): CHD in DM, enterocytes and ED.
7. APO E : Dyslipidemia, CHD, MI, nitric oxide, statin response.
8. MTHFR: Methylation (1298C and 677T): hypertension, CHD, MI, CVA, thrombosis, homocysteine, ED.
9. CYP 1A2 : Caffeine ,HBP,MI, aortic stiffness, PWV, AI, tachycardia, arrhythmias, vascular inflammation, cateholamines.
10. Corin : Hypertension, CHF, volume overload, sodium sensitive, CVD, CRF, pre-clampsia, ANP and BNP.
11. CYP 11 B2 (TT allele): HBP , aldosterone and response to spironolactone.

Cardiovascular Genetic and Nutrigenomic Testing

1. GSHPx : CHD, MI, hypertension, LVH, CHF, Glutathione, ALA 6 alleles, selenium.
2. ADR B2: HBP, PRA, inflammation and DASH diet with ACEI, ARB or DRI.
3. APO A1 : Lipids, HDL, CHD, MI obesity.
4. APO A2 : Lipids, HDL, CHD, M I, obesity.
5. APC C 3 : Dyslipidemia, CHD,MI, dysfunctional HDL, inflammation, DM.
6. CYP 4 A11: Hypertension, ENaC and sodium, volume overload, CHD and Amiloride.
7. CYP 4F 2 : Hypertension, ENaC and sodium, volume overload, CHD and Amiloride.
8. AGTR1 (ATR1AA): HBP, ARBs and potassium.
9. NOS 3: Nitric oxide, hypertension, MI, CHD,CVA, thrombosis, ED, oxidative stress, inflammation.
10. SCARB1: Lipids, dysfunctional HDL with high HDL, CHD, MI.

Genetics of CHD

Clin Cardiol 2012;35(9):536-540
Curr Opin Lipidol 2013;24:410, Am J Hum Genet. 2010; 9;86(4):592-5.

Over 60% of the CHD risk variants mediate risk independent of traditional known risk factors with unknown mechanisms or pathways. 40% overlap with genomic loci with classical risk factors.

The three most frequently found loci are:

1. 9p21.3 (epigenetic gene regulation- non coding RNA to induce CHD.
2. 6p24.1. (CHD in Chinese and Singapore and DVT)
3. 1p13.3 (affects LDL-C).

Reduction and prevention of CHD is not likely to improve without using genetic markers and gene expression testing to identify these pathways.

Genomics of Cardiovascular Disease

NEJM 2011;365:2098-109

- Most are polygenic: SNPs, haplotypes, CYP-450.
- There are 30 to 60 loci associated with MI and CHD, but only a minority of loci mediate effects on CHD through the known top 5 risk factors.
- The best yield of genome wide association studies (GWAS) are pathways that underlie causes of CVD/CHD such as inflammation, oxidative stress and immunologic vascular dysfunction.

ROLE OF APO-E 4 ALLELE

American Journal of Nutrition 2002; 75:191-212

More CHD, CVD , MI, Alzheimer's and dementia

Increased CVD risk with smoking and alcohol intake (E4/E4 and E4/E3.)

Unable to activate or repair vascular endothelium (APO ER2 receptor) to produce nitric oxide (NO).

Increased cholesterol absorption , higher serum LDL delayed clearance.

Best reduction of LDL with dietary restriction CHO, low fat diets and omega 3 FA .

Less response to statins.

ROLE OF APO E 2 ALLELE

American Journal of Nutrition 2002; 75:191-212

Increased CHD in men.

Decreased Alzheimer's and dementia.

Delayed chylomicron remnant clearance.

Increased TG and remnant lipoprotein.

Lower LDL.

More response to statins.

Good response of TG to omega 3 FA.

Genomics of Cardiovascular Disease
9p21 Genetic Variant

NEJM 2011;365:2098-109;JAMA 2010;303:648

- Two SNPs: Alleles are rs10757278 and rs 1333049.
- One copy is 1.25 to 1.5 increase risk for CHD /MI and 2 copies is 1.56 to 2.0x increase in risk for CHD/MI. (gg and cc homozygotes) (normal non carriers are aa and gg) respectively.
- 33 gene enhancers are located in 9p21 region that are implicated in inflammatory pathways, plaque rupture, thrombosis, AAA, ASCVD, CHD, MI, DM, IR and cancer. On short arm or chromosome 9
- Interaction of 9p21 with STAT 1, CDKN2A/B and interferon gamma and increases MTAP polyamine pathways and increases the genetic susceptibility to CHD, MI and response to inflammatory signaling in vascular cells.

MTHFR (methylenetetrahydrofolate reductase)

Eur J Med Genet. 2014 Nov 4: S1769-7212

- A1298C SNP ($Glu^{429}Ala$):affects the conversion of MTHF to BH4 (tetrahydrobiopterin), an important cofactor in the production of neurotransmitters, synthesis of nitric oxide, and detoxification of ammonia, hepatic methylation pathways, endothelial dysfunction, hypertension, CVA, thrombosis, CVD, CHD, MI. Also some neurological diseases and cancer.

- C677T SNP ($Ala^{222}Val$): Dementia, depression, hyper-homocysteinemia.

COMT polymorphisms and CVD

Arterioscler Thromb Vasc Biol.2014;34(9):2160

- COMT valine/valine has highest enzymatic activity. COMT methionine/methionine has the lowest enzymatic activity.
- Homocysteine converted to S-adenosylhomocysteine (SAH) is a competitive inhibitor of COMT.
- rs4680 (val 158 met) SNP encodes valine (G) to methionine (A) at amino acid 158 in the membrane form and 108 in the secreted from of the enzyme. The met/met allele has 3-4 x lower enzyme activity. Higher dopamine and catecholamine levels. Has a 34% higher risk of hypertension, CHD and myocardial infarction.

COMT polymorphisms and CVD

Arterioscler Thromb Vasc Biol.2014;34(9):2160

- rs4818 is a C to G transversion in the same exon as rs4680 causes differential stability of COMT mRNA secondary structure with similar outcomes as rs4680. C/C responds favorably to ASA and Vitamin E. G/C is non-significant and GG has increased risk.
- rs4680 valine/valine allele (GG) protects against CHD with RR of.66 (p=0.0007) but is abolished by ASA giving RRR of 1.85 (p=0.033). The methionine/methionine allele (AA) has lower CVD rates of .60 on ASA (p=0.23).

COMT polymorphisms and CVD

Arterioscler Thromb Vasc Biol.2014;34(9):2160

- rs 4680: Vitamin E increased CHD in valine/valine allele by RR 1.5 (non-significant at p= 0.18) compared to methionine/methionine allele with RR of .53 (p=0.006) with Vitamin E.
- The met/met had .80 RR CVD with both ASA and Vitamin E.
- Conclusions

1. rs 4680 allele: Give ASA or Vitamin E to met/met (A/A) but neither to val/met(G/A) or val/val (G/G).
2. rs 4818 allele: Give ASA or Vitamin E to C/C(met/met) but neither to G/C (val/met) or GG (val/val).

OXIDATIVE STRESS, GLUTATHIONE, GLUTATHIONE PEROXIDASE, HYPERTENSION AND CHD

Circulation 2004; 109:544-549 Coronary Artery Disease 2003; 14:149-153). (NEJM 2003; 349:1605-13).

- Increased Glutathione Peroxidase (GSH-Px) decreases BP, MI, LVH CHF.
- GSH-Px confers more cell, tissue and organ protection than SOD (superoxide dismutase) or catalase, or the combination of both.
- 5 GSH-Px (#1-#4 are selenium-dependent, #5 is not) is located in mitochondria and cytosol, which neutralize lipid peroxides and hydrogen peroxide, preventing formation of hydoxyl radical and other ROS. GSH-Px has greater affinity for hydrogen peroxide than catalase.
- Peroxiredoxins (Prx) (#1-6) scavenge hydrogen peroxide as well. Prx #3 is in the mitochondria and protects cardiac muscle.
- Highest quartile of RBC GSH-Px had 71% lower risk of MI compared to lowest quartile ($p<.001$).
- GSH-Px is major CHD risk factor *(NEJM 2003; 349:1605-13)*.
- Abnormal genotypes also exist which increases risk (ALA-6 alleles). A characteristic polyalanine sequence polymorphism in exon 1 of hgpx1 produces three alleles with five, six or seven alanine (ALA) repeats in this sequence*(Coronary Artery Disease 2003; 14:149-153)*.

Genetics and CVD: Glutamic Acid Metabolism and DM

JAMA 2013;310: 821

- GLUL gene on chromosome 1q25 increases CHD in DM by reducing expression of glutamine synthase which converts glutamic acid to glutamine.
- Important in GSH (glutathione), cell proliferation and signaling, inhibition of apoptosis, insulin and glucose metabolism, incretin, enterocyte health, and endothelial cell metabolism.

Polymorphisms of anti-oxidant enzymes, blood pressure and risk of hypertension

Journal of Hypertension 2011;29:492

- Chronic oxidative stress and decreased oxidative defense increases the risk for hypertension.
- Increase in pro-oxidant enzymes like NADPH oxidase and xanthine-oxidase.
- Reduction in cytoplasmic anti-oxidant systems.
- Polymorphisms for:
  - CYBA for NADPH oxidase
  - Xanthine-oxidase gene
  - SOD 3: c.172G>A
  - Catalase: c.-20C>T
  - GPx1: c.*891C>T
  - TXN (thioredoxin): c.-793T >C (NFkb, AP-1, Ref-1, SP -1) and DNA binding to transcription factors.

AGTR1 polymorphisms and autoantibodies to the AT1R

J Am Soc Hypertens.2014 ;8(1):21-7

- AT1R-AA (AT1R autoantibodies) are involved in the immunopathogenesis of hypertension.
- Exhibit agonist action to increase BP just like A-II binding to the AT1R.
- AGTR1 phenotype also determines response to ARB.
- ARB are the most effective agent compared to ACEI in those with ATR-AA.
- CC, AC and AA alleles present for AGT1R1. AA and AC alleles have best BP response to ARB. Furthermore, haplotypes (GCC) and (AAC) had impacts on BP.

Plasma Levels of Matrix Metalloproteinases (MMP) and Their Inhibitors (TIMP) in Hypertension, CV risk and Biomarkers of CV Remodeling

J of Hypertension 2011;30:3

- MMP-2, MMP-9 and TIMP-1 have a role as biomarkers of cardiovascular remodeling in hypertension
- Improve risk stratification.
- Identify LVH, LV remodeling, systolic and diastolic dysfunction, CHF.
- MMP are enzymes involved in remodeling of the extracellular matrix (ECM). They digest structural or fibrillar collagen types I and II, gelatinases that digest denatured collagen , gelatin, laminin, fibronectin and protoglycans etc.

Corin and Salt Sensitivity and Intravascular Volume

Curr Opin Nephrol Hypertension 2013;22:713

Curr Hypertens Rep.2014;16(2):415

Am J Hypertens.2015 Feb 7. pii: hpv002. [Epub ahead of print

- Corin is a serine protease that is the key enzyme in the biosynthesis of ANP (atrial natriuretic peptide) and BNP (brain natriuretic peptide), which regulate salt and water balance, intravascular volume and blood pressure.
- Congestive heart failure (CHF) patients have reduced corin.
- Corin SNPs T555/Q568P is more common in blacks and may account for the increase in salt sensitive hypertension and CVD.
- Corin is expressed in the kidney and uterus and thus has a role in renal insufficiency and preclampsia.

Genetic Variant CYP4A11 and Hypertension
Salt Sensitivity, ENaC and Amiloride

JASH 2014;8:475; Hypertension 2008;51:1393-1398

- In blacks, with resistant volume-dependent hypertension who are homozygous for the C allele at rs3890011 of CYP4A11 are associated with blood pressure that is resistant to spironolactone but responds to amiloride. (renal epithelial sodium channel inhibitor-ENaC)
- CYP4 A11 oxidizes AA (arachidonic acid) to 20 HETE (hydroxyeicosatetraenoic acid) which induces renal vasoconstriction that increases BP, SVR and ED but induces renal natriuresis via renal tubule. Also CYP4F2 AA/GA allele.
- HETE levels decrease with the CC allele blocking natriuresis.
- Increased activity of ENaC by loss of 20 HETE induces salt sensitive/volume overload resistant hypertension.
- Have increased urinary aldosterone/potassium ratio.
- Amiloride 5 to 10 mg per day will lower blood pressure.

Genetic variants in the renin-angiogensin-aldosterone system and blood pressure responses to potassium intake

J of Hypertension 2011;29:1719

- Numerous SNPs in the RAAS system play an important role in determining an individual's BP responses to dietary potassium intake. These improve BP response to dietary potassium intake.

- Nuclear receptor subfamily 3 group C :NR3C2.
- Angiogensin II type receptor : AGTR1.
- Hydroxysteroid 11 beta dehydrogenase : HSD11B1 and B2.

Caffeine, Coffee, Hypertension, CHD and MI.

J of Hypertension 2009;27:1594;Am J Clin Nutr 2007;86:457
European J Clinical Nutrition 2007;61:796; Am J Clin Nutr 2011;94:1113
Current Opinion in Lipidology 2007;18:13;JAMA 2006;295:1135.

- Cytochrome P-450 - CYP1A2 genotype modifies the response to caffeinated coffee intake and the risk of hypertension, CVD, CHD and MI in a linear relationship. Caffeine is exclusively metabolized by CYP1A2 to paraxanthine, theobromine and theophylline.
- Chromosome 15q24.1. SNP is rs7762551 A to C. C SNP decreases enzymatic activity. Caffeine also blocks vasodilating adenosine receptors.
- Rapid metabolizers of caffeinated coffee IA/IA allele have lower BP and lower risk of MI. Hypertension .36 to .80 RR. SBP decreases 10/7 mm Hg. MI is 17%-52% reduction. About 40-45% of the population.
- Slow metabolizers of caffeine IF/IF or IA/IF allele have higher BP 8.1/5.7 mm Hg lasting > 3 hours after consumption. Have tachycardia, increased aortic stiffness, PWV, AI, SBP, PP, vascular inflammation and increased catecholamines. Increased hypertension 1.72 to 3.00 RR. Over age 59: MI 36% increase (2-3 cups/d) ; 64 % increase 4 cups or more/d. Under age 59: MI 24% (1 cup/d), 67%(2-cups/d) and 233%(4 or more cups/d). About 55-60% of population .
- Polyphenols, chlorogenic acid and dihydro-caffeic acid increase eNOS, NO, improve ED and lower BP 10/7 mm Hg at 140 mg / day (cocoa in coffee). Diterpenes in unfiltered coffee and caffeine increase risk of CHD.

Modulation of aldosterone levels by -344 C/T CYP11B2 polymorphism and spironolactone use in resistant hypertension.

J of Am Society of Hypertension 2014;8(3): 146

- Plasma aldosterone levels are significantly associated with -344 C/T CYP11B2 (chromosome 8) polymorphism and treatment with spironolactone in resistant hypertensive subjects.
- CYP 11B2 is the aldosterone synthase gene
- Higher urinary aldosterone levels occur in T carriers for -344C/T polymorphisms in CYP11B2.
- C allele reduces aldosterone synthase (ASyn) transcription.
- Aldosterone levels: lowest with CC (0.08 frequency), then CT (.53 frequency) and highest with TT (.39 frequency). TT has higher BP.
- TT allele binds to steroidogenic factor (SF-1) with lower affinity than the C allele which results increased expression of the enzyme (ASyn).
- 30% of hypertensive patients with resistant hypertension have elevated plasma aldosterone concentration and intravascular volume expansion.
- Aldosterone breakthrough on ARB and ACEI may be more common with the TT allele.

Meta-analysis of the association between the insertion/deletion polymorphism in ACE gene and coronary heart disease among the Chinese population.

J Renin Angiotensin Aldosterone Syst.2012 ;13(2):296-304.
***Kidney International* (1998) 53, 1743–1747**
Atherosclerosis.1998;139(1):153-9; *Circulation. 1995; 91: 2721-2724*

- Meta-analysis to assess the association of ACE/ID polymorphism and CHD susceptibility among the Chinese population. (DD insertion)

- Forty-six studies (5215 cases and 4782 controls) were identified. The results from the meta-analysis indicated statistically significant association between ACE I/D polymorphism and CHD risk under all genetic models

- **CONCLUSIONS:** The meta-analysis indicated a significant association between ACE I/D polymorphism and CHD susceptibility among the Chinese population. 1.19 to 2.40 greater risk of CHD with DD phenotype. In Caucasians ACE gene is also associated with CHD, MI, hypertension, increase carotid IMT, LVH, nephroangiogenesis, CRI, microalbuminuria.

The genes of atherosclerosis and cardiovascular diseases

Klin Med (Mosk).2011;89(3):14-8

- It was shown that DD genotypes of ACE gene, H+/+ of LPL gene and APO E3 and E4 are associated with an enhanced probability of myocardial infarction (MI) in CHD patients and can be regarded as high risk markers.
- The DD genotype is associated with an increased risk of recurrent MI, life-threatening post-IM complications and severe cardiac insufficiency.
- E2 allele of the Apo E gene and H allele of the LPL gene occur much more frequently in CHD patients aged above 90 years (long livers) than in younger subjects; hence, their value as markers of stable ischemic disease

DASH I and II Diets (Dietary Approaches to Stop Hypertension): Response and Genetics

Am J Clin Nutrition. 2010;92:444
J Hum Hypertension 2012;26:664

- DASH diet increases PRA (plasma renin activity) and aldosterone levels in response to decreases in BP which can counteract the BP lowering effect of diet.
- Association of this response with the G46A polymorphism of B2-AR (beta 2 adrenergic receptor).
- The A allele of G46 A had greater BP reduction and blunted PRA and aldosterone levels. AA genotype had best response and GG genotype had no response.
- Adding ARB, ACEI or DRI improves BP response to DASH diet in GG genotype due to reflex increase in PRA.

Dyslipidemia
SCARB1 (rs4238001)
Rodriquez-Oquendo PLOS ONE May 2015

- Variant of SCARBI changes a hepatic receptor protein from glycine to serine
- Increased blood levels of HDL due to inability of HDL to attach to hepatic receptor for break down, disposal and recirculation. The HDL is not protective (dysfunctional)
- Increased risk of CHD by 49% in black males and 24% higher in white males.
- Frequency: 3 % Chinese, 8% in blacks and 12 % in Latinos and Whites.

Evaluation of established coronary heart disease on the basis of HDL and non-HDL NMR lipid profiling.

J Proteome Res. 2010 ;9(2):897-91

- NMR lipid profiles in 47 patients with triple vessel disease and 41 patients with normal coronary arteries both documented angiographically. The presence of CHD was predicted with a sensitivity and specificity of 52% and 75% for HDL model and 78% and 80% for non-HDL, respectively.
- The lipid constituents of HDL lipoproteins which contributed to the separation between the two groups were the saturated fatty acids, cholesterol, total omega-3 fatty acids, degree of unsaturation, diallylic protons from polyunsaturated fatty acids, linoleic acid and, to a lesser extent, the number of fatty acids, triglycerides, unsaturated fatty acids and phosphatidylcholine.
- For non-HDL, lipoproteins were the saturated fatty acids, number of fatty acids, cholesterol, unsaturated fatty acids and phosphatidylcholine.

Cardiovascular Metabolomics

Metabolomic profiles predict CVD

Am Heart J 2012;163:844;
Evid Based Complement Alternat Med. 2014;2014:281829
PLOS Genet. 2104;Dec 10(12)

- **Dicarboxylacylcarnitines**
- **Medium chain acylcarnitines**
- **Fatty acids**

- **All of these metabolic measurements will predict CVD beyond any degree that is possible using readily available clinical characteristics and other CHD risk factors.**

Metabolomics signature improves the prediction of cardiovascular events in elderly subjects.

Atherosclerosis.2014 ;232(2):260-4.

- Targeted-mass-spectrometry-based profiling of 49 metabolites in CVD subjects.
- 3.5 year followup, 17 MACEs occurred (5 cardiovascular deaths, 1 nonfatal myocardial infarction, 7 nonfatal strokes and 4 peripheral artery surgeries).
- Metabolite factor 1, composed of medium-and long-chain acylcarnitines,(MCAC and LCAC) and factor 7 (alanine) were independently associated with MACEs, after adjustment for clinical CV covariates [HR = 1.77, p = 0.016) and HR = 2.18 , p = 0.014), respectively].
- Only factor 1 significantly increases the prediction accuracy of the Framingham Recurring-Coronary-Heart-Disease-Score, with a significant improvement in discrimination (7%, p = 0.01) and correctly reclassifying 41% of events and 37% of non-events (p = 0.005).
- **CONCLUSIONS:** Aging mitochondrial dysfunction evaluated by metabolomic profiling is associated with MACEs, independently of standard predictors. (MCAC, LCAC and alanine)

Novel proteins associated with risk for coronary heart disease or stroke among postmenopausal women identified by in-depth plasma proteome profiling

Genome Med. 2010;2(7):48.

- Proteomics in the WHI from 800 women who developed CHD or stoke
- Case versus control concentration differences in 37 proteins (l $P < 0.05$) for CHD
- For CHD, beta-2 microglobulin (B2M), alpha-1-acid glycoprotein 1 (ORM1), and insulin-like growth factor binding protein acid labile subunit (IGFALS) were best $P< 0.05$.
- Corresponding numbers for stroke were 47 proteins with $P < 0.05$
- For stroke Apo-lipoprotein A-II precursor (APOA2), peptidyl-prolyl isomerase A (PPIA), and insulin-like growth factor binding protein 4 (IGFBP4), were best $P < 0.05$. Other proteins involved in insulin-like growth factor signaling were also highly ranked. The associations of B2M with CHD ($P < 0.001$) and IGFBP4 with stroke ($P = 0.005$) were highest.
- **CONCLUSIONS:** In-depth proteomic discovery analysis of plasma samples identified B2M, ORM -1 and IGFALS as best risk markers for CHD and provided a number of other candidate markers of disease risk and candidate mediators of hormone therapy effects on CHD and stroke.

Metabolomic profile is associated with the risk of incident coronary heart disease.

Am Heart J. 2014;168(1):45-52.

- Investigated whether a single-point blood measurement of the metabolome is associated with and predictive for the risk of CHD.

- Proton nuclear magnetic resonance spectra in 79 cases who developed CHD during follow-up (median 8.1 years) was compared to 565 randomly selected individuals. In these spectra, 100 signals representing 36 metabolites were identified. A weighted metabolite score of 13 proton nuclear magnetic resonance signals that optimally predicted CHD was found.
- This metabolite score, including signals representing a lipid fraction, glucose, valine, ornithine, glutamate, creatinine, glycoproteins, citrate, and 1.5-anhydrosorbitol, was associated with the incidence of CHD independent of traditional risk factors (hazard ratio 1.50).

Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease.

Am Heart J.2012;163(5):844-850.

- 2,023 consecutive patients undergoing cardiac catheterization. Mass spectrometry profiling of 69 metabolites and lipid assessments.
- Median follow-up of 3.1 years.
- Five of 13 metabolite factors were independently associated with mortality: factor 1 (medium-chain acylcarnitines: hazard ratio [HR] 1.12 , P = .005), factor 2 (short-chain dicarboxylacylcarnitines: HR 1.17 , P = .005), factor 3 (long-chain dicarboxylacylcarnitines: HR 1.14 , P = .002); factor 6 (branched-chain amino acids: HR 0.86 , P = .03), and factor 12 (fatty acids: HR 1.19 , P = .004).
- Three factors independently predicted death/MI: factor 2 (HR 1.11, P = .04), factor 3 (HR 1.13, P = .005), and factor 12 (HR 1.18, P = .004). For mortality, 27% of intermediate-risk patients were correctly reclassified (net reclassification improvement 8.8%; for death/MI model, 11% were correctly reclassified (net reclassification improvement 3.9%).
- Conclusions: Metabolic profiles predict CVD mortality independently of standard predictors (MCAC,SCDCAC,LCDCAC, BCAA and FA).

Identification of biomarkers of stent restenosis with serum metabolomic profiling using gas chromatography / mass spectrometry

Circ J.2012;76(8):1864-73.

- Gas chromatography/mass spectrometry for serum metabolomic profiles of male patients hospitalized for follow up coronary angiography 6 months after stent implantation to correlate with stent re-stenosis.
- Of 83 serum metabolites analyzed, molecules - isobutylamine, sarcosine, homoserine, ribulose, taurine, glutamine, glucose, and tryptophan - in the major restenosis group were significantly different from those in the minor restenosis group.
- Differences in correlation among these metabolites imply possible alternations in the activated metabolic pathways.
- **CONCLUSIONS:** Serum metabolic profiling is useful in the identification of specific biomarkers of stent restenosis.

Identification of biomarkers for unstable angina by plasma metabolomic profiling.

Mol Biosyst. 2013 ;9(12):3059-67.

- Plasma samples from 45 unstable angina and 43 atherosclerosis subjects.
- Sixteen potential endogenous biomarkers for unstable angina were identified
- The plasma concentrations of 12 metabolites were higher while the concentrations of four metabolites were lower.
- Plasma metabolomics analyzed had great potential in biomarker discovery for unstable angina.

Metabolic profiles predict adverse events after coronary artery bypass grafting.

J Thorac Cardiovasc Surg.2012;143(4):873-8

- 478 subjects from the CATHGEN study who underwent coronary artery bypass grafting.
- Targeted mass spectrometry-based profiling of 69 metabolites to assess the metabolite factor levels and a composite outcome of post-coronary artery bypass grafting, myocardial infarction, the need for percutaneous coronary intervention, repeat coronary artery bypass grafting, and death.
- Mean follow-up period of 4.3 ± 2.4 years.
- Three principal components were significantly associated with an adverse outcome : short-chain dicarboxylacylcarnitines (factor 2, P = .001); ketone-related metabolites (factor 5, P = .02); and short-chain acylcarnitines (factor 6, P = .004). These 3 factors remained independently predictive of an adverse outcome after multivariate adjustment: factor 2 (adjusted hazard ratio, 1.23; P < .001), factor 5 (odds ratio, 1.17; P = .04), and factor 6 (odds ratio, 1.14; P = .03).
- **CONCLUSIONS:** Metabolic profiles are independently associated with adverse outcomes after coronary artery bypass grafting. These novel biomarkers of risk augment existing tools for risk stratification of coronary artery bypass grafting patients and elucidate CHD risk/ biochemical pathways. (SCDCAC,KRM, SCAC)

Large-scale Metabolomic Profiling Identifies Novel Biomarkers for Incident Coronary Heart Disease.

PLoS Genet. 2014 Dec 11;10(12):e1004801. doi: 10.1371/journal.pgen.1004801. eCollection 2014.

- Mass spectrometry-based non-targeted metabolomics study for association with incident CHD events in 1,028 individuals (10 y. median follow-up) with validation in 1,670 individuals (3.9 y. median follow-up).
- Four metabolites were replicated and independent of main cardiovascular risk factors: [lysophosphatidylcholine 18:1 (hazard ratio [HR] per standard deviation [SD] increment=0.77, P-value<0.001), lysophosphatidylcholine 18:2 (HR=0.81, P-value<0.001), monoglyceride 18:2 (MG 18:2; HR=1.18, P-value=0.011) and sphingomyelin 28:1 (HR=0.85, P-value=0.015)]. Together they contributed to moderate improvements in discrimination and re-classification in addition to traditional risk factors (C-statistic: 0.76 vs. 0.75; NRI: 9.2%).
- Four lipid-related metabolites with evidence for clinical utility, as well as a causal role in CHD development (LPC(2), MG, SM).

Metabolomic profile is associated with the risk of incident coronary heart disease.

Am Heart J. 2014 ;168(1):45-52.; JAMA 2013 ; 28;310(8):821-8. Genome Med. 2010;l 28;2(7):48. Circ J. 2012;76(8):1864-73; Evid Based Complement Alternat Med. 2014;2014:281829.

- **The known quantifiable serum metabolome consists of 4,229 metabolites. Only 36 (0.9%) were included in this study**
- **Dicarboxylacylcarnitines, medium and long chain acylcarnitines**
- **Fatty acids .**
- **Beta-2 microglobulin (B2M) Alpha-1-acid glycoprotein 1 (ORM1)**
- **Insulin-like growth factor binding protein acid labile subunit (IGFALS)**
- Lipid fraction: an unsaturated lipid structure
- Glucose; alpha and beta glucose
- Valine: metabolic risk factors, insulin resistance, T2DM, CVD,Alanine
- Ornithine: from the splitting of urea from arginine, reduces arginine/NO
- Glutamate and glutamine: genetic variant 1q25 and glutamate-ammonia ligase (GLUL) related to glutamic acid metabolism and CHD in T2DM
- Creatinine: risk for CKD,Glycoproteins: N-acetyl glycoprotien, fibrinogen, prothrombin,Citrate,1.5-anhydrosorbitol: short term glycemic control
- TMAO.Lactic acid, beta- hydroxy butyrate and acetone ,Methylamine, HDL,Leucine,Phenylalanine,Lysine,TyrosineProline
- Threonine,Aspartic acid, Isoleucine,Acetyl-glutamic acid
- Histidine
- Isobutylamine, Sarcosine Homoserine,Ribulose, Taurine, Tryptophan, Branched-chain amino acids: low levels Ketone-related metabolites

Metabolomic profile is associated with the risk of incident coronary heart disease. (continued)

Am Heart J. 2014 ;168(1):45-52.; JAMA 2013 ;310(8):821-8. Genome Med. 2010 ;2(7):48. Circ J. 2012;76(8):1864-73; Evid Based Complement Alternat Med. 2014;2014:281829.

- **Fatty acids**
- Beta-2 microglobulin (B2M)
- Alpha-1-acid glycoprotein 1 (ORM1)
- Insulin-like growth factor binding protein acid labile subunit (IGFALS)
- Isobutylamine
- Sarcosine
- Homoserine
- Ribulose
- Taurine
- **Tryptophan**
- Branched-chain amino acids: low levels
- Ketone-related metabolites

Metabolomic profile is associated with the risk of incident coronary heart disease.

Am Heart J. 2014 Jul;168(1):45-52.; JAMA 2013 Aug 28;310(8):821-8. Genome Med. 2010 Jul 28;2(7):48. Circ J. 2012;76(8):1864-73; Evid Based Complement Alternat Med. 2014;2014:281829.

- **Dicarboxylacylcarnitines, medium and long chain acylcarnitines**
- Lipid fraction: an unsaturated lipid structure
- Glucose; alpha and beta glucose
- Valine: metabolic risk factors, insulin resistance, T2DM, CVD
- Alanine
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- Creatinine: risk for CKD
- Glycoproteins: N-acetly glycoprotien, fibrinogen, prothrombin
- Citrate
- 1.5-anhydrosorbitol: short term glycemic control
- TMAO

Metabolomics and Hypertension

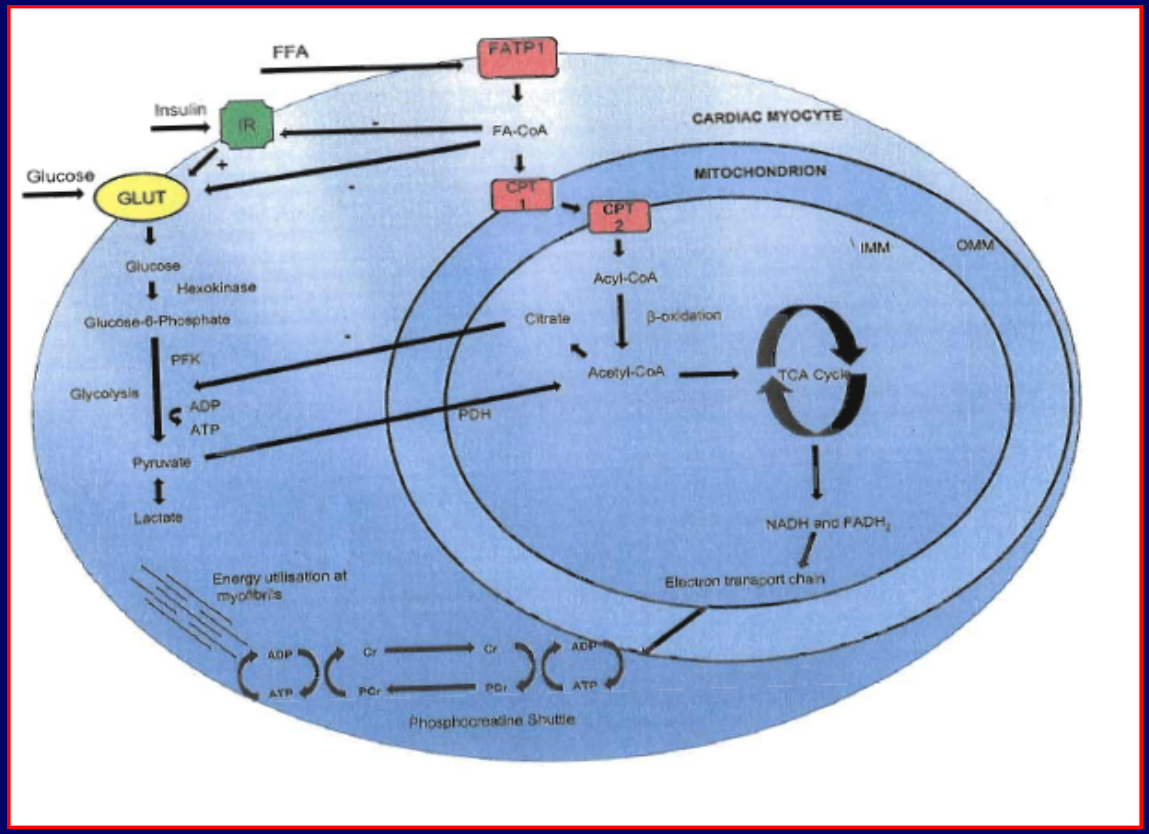
J of Hypertension 2014;32:1159

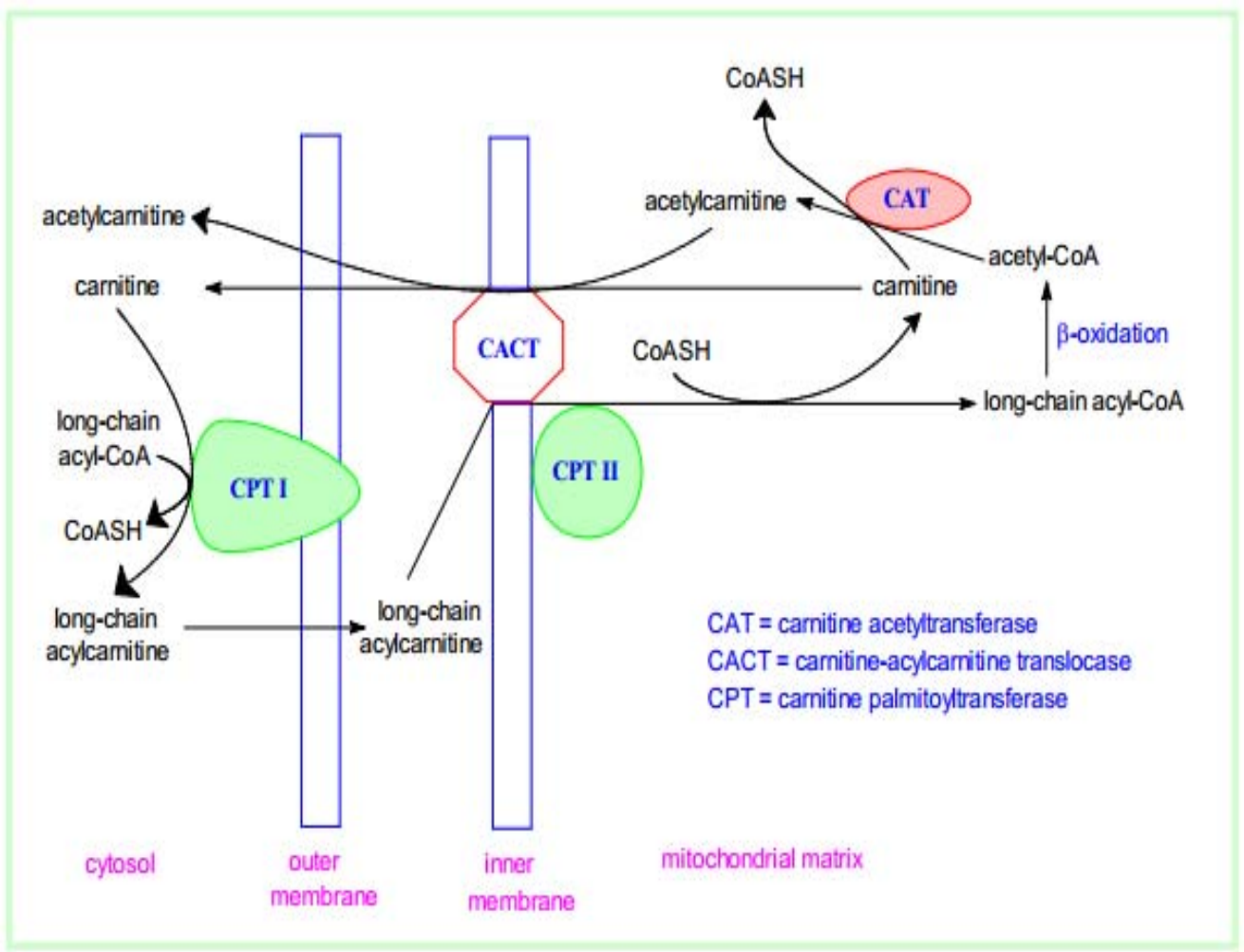
- NMR spectroscopy, LC and GC (liquid or gas chromatography)
- Blood, urine, tissue, primary cell culture
- Evaluated the metabolic perturbations of hypertension to determine the mechanisms.
- Pathophysiologic and biochemical endothelial and vascular changes precede hypertension by decades.
- The metabolites represent the final products of cellular processes including genes, mRNA, protein activity and bidirectional complex interactions of the systems biology components.
- Abnormal lipid profile: increased TC, HDL, Lp(a), Apo B, and HDL 3.
- Increased FFA
- Increased inflammation: alpha 1 acid glycoprotein
- Gut microbiome: increased urine formate, fiber, alanine (meat), low hippurate (low polyphenol intake)
- Pre-eclampsia markers

Cardiac Metabolomic Testing
CHF and CHD
Diabetes 2013;62:1-8

- Dicarboxylacylcarnitines: short and long chain forms relate to mitochondrial metabolism and beta oxidation of fatty acids and acetyl CoA. Mitochondrial dysfunction, decrease ATP, insulin resistance.
- Acylcarnitines: short, medium and long chain relate to mitochondrial metabolism and beta oxidation of fatty acids for acetyl-CoA. Mitochondrial dysfunction, decrease ATP, insulin resistance.
- Fatty acids
- BCAA (branched chain amino acids (valine, leucine and isoleucine)

Chart 1


FFA
FATP1
Insulin
IR
FA-CoA
CARDIAC MYOCYTE
Glucose
GLUT
MITOCHONDRION
CPT 1
CPT 2
IMM
OMM
Glucose
Hexokinase
Acyl-CoA
Glucose-6-Phosphate
Citrate
β-oxidation
PFK
Acetyl-CoA
TCA Cycle
Glycolysis
ADP
ATP
PDH
Pyruvate
Lactate
NADH and FADH2
Electron transport chain
Energy utilisation at myofibrils
ADP
ATP
Cr
PCr
Phosphocreatine Shuttle



Carnitine and acylcarnitines: structure, occurrence, biology and analysis. W.W. Christie © lipidlibrary.aocs.org

Mitochondria, Beta Oxidation and Carnitine. Cell Energy and Toxicity

Diabetes 2013;62:1-8

- The carnitine shuttle is key for transport of long chain FA> C12
- Acyl CoA and its intermediates are potentially toxic to the cell and must be removed by carnitine and the CPT, CAC and CACT enzymes.
- 3 forms of CPT (carnitine palmitotransferase). CPT 1B is in heart and skeletal muscle.
- Incomplete mitochondrial metabolism due to enzyme defects with accumulation of toxic acylcarnitines and acyl-CoA of various chain lengths depending on the enzyme deficiency and reduction in acetyl CoA for Krebs cycle production of ATP and energy is seen in insulin resistance (IR) Type 2 DM, CVD and other diseases.
- Acyl carnitines induce lipotoxicty and IR by numerous mechanisms.

Recommended Metabolomic Testing

Diabetes 2013;62:1-8, Atherosclerosis 2014, 232;260, Am Heart J 2012,163:844, J Thoracic Cardiovasc Surg 2012, 143:873, Genome Med 2010; 2: 48.

- **Dicarboxylacylcarnitines (DCAC):** short and long chain forms relate to mitochondrial metabolism and beta oxidation of fatty acids and acetyl CoA. Mitochondrial dysfunction, decreased ATP, insulin resistance, DM and lipotoxicity.
- **Acylcarnitines (AC):** short, medium and long chain relate to mitochondrial metabolism and beta oxidation of fatty acids for acetyl-CoA. Mitochondrial dysfunction, decreased ATP, insulin resistance, DM and lipotoxicity.
- **Fatty acids**
- **BCAA** (branched chain amino acids (valine, leucine and isoleucine).
- **Alanine**
- **Beta 2 Microglobulin**
- **KRM: Ketone related metabolite**
- **LPC 18:1 and 18:2: lysophatytidylcholine**
- **MG: monoglycerides**
- **SM: syhingomyelin**
- **ORM-1: alpha -1 acid glycoprotein**
- **IGFBP4-ALS: Insulin growth factor binding protein- acid labile subunit.**

Gene Expression Testing (GET)
Gene Expression Score (GES): Corus CHD

Gene Expression Testing (GET)
Gene Expression Score (GES): Corus CHD

Cath Lab Digest 2013;21:8 Circulation 2008;1:31
Critical Pathways in Cardiology 2013;12:37
Circulation: Cardiovascular Genetics 2013;6:154
J of Cardiovascular Translation Research 2012;3:366
Am Heart J 2012;164:320 ; Circ Cardiovas Genet 2008;1:31

- Gene expression test that measures changes in WBC mRNA levels that are sensitive to the presence of coronary plaque.
- Measures and integrates the mRNA expression levels of 23 genes expressed in cells of the innate and adaptive arms of the immune system which are grouped into 6 categories.
- Highly correlated with quantitative coronary artery angiogram and degree of coronary artery stenosis. (IMPACT, PREDICT, COMPASS clinical trials).

Gene Expression Testing (GET)
Gene Expression Score (GES) Corus CHD

Cath Lab Digest 2013;21:8 Circulation 2008;1:31
Critical Pathways in Cardiology 2013;12:37
Circulation: Cardiovascular Genetics 2013;6:154
J of Cardiovascular Translation Research 2012;3:366
Am Heart J 2012;164:320; Circ Cardiovas Genet 2008;1:31

Measures expression levels of 23 genes grouped into 6 categories

- Cellular and neutrophil apoptosis and necrosis, inflammation , immune function and signaling pathways (term 1).
- Neutrophil to lymphocyte ratio and cell necrosis (term 2).
- NKTc (Natural killer T cell)activation. (term 3).
- Inflammatory cell biology and cell migration into atherosclerotic plaque with T and B lymphocytes and their ratio (term 4).
- Innate and adaptive immune response to LDL oxidation and other inflammatory processes (term 5).
- Associated biological pathways to CHD yet unknown (term 6).

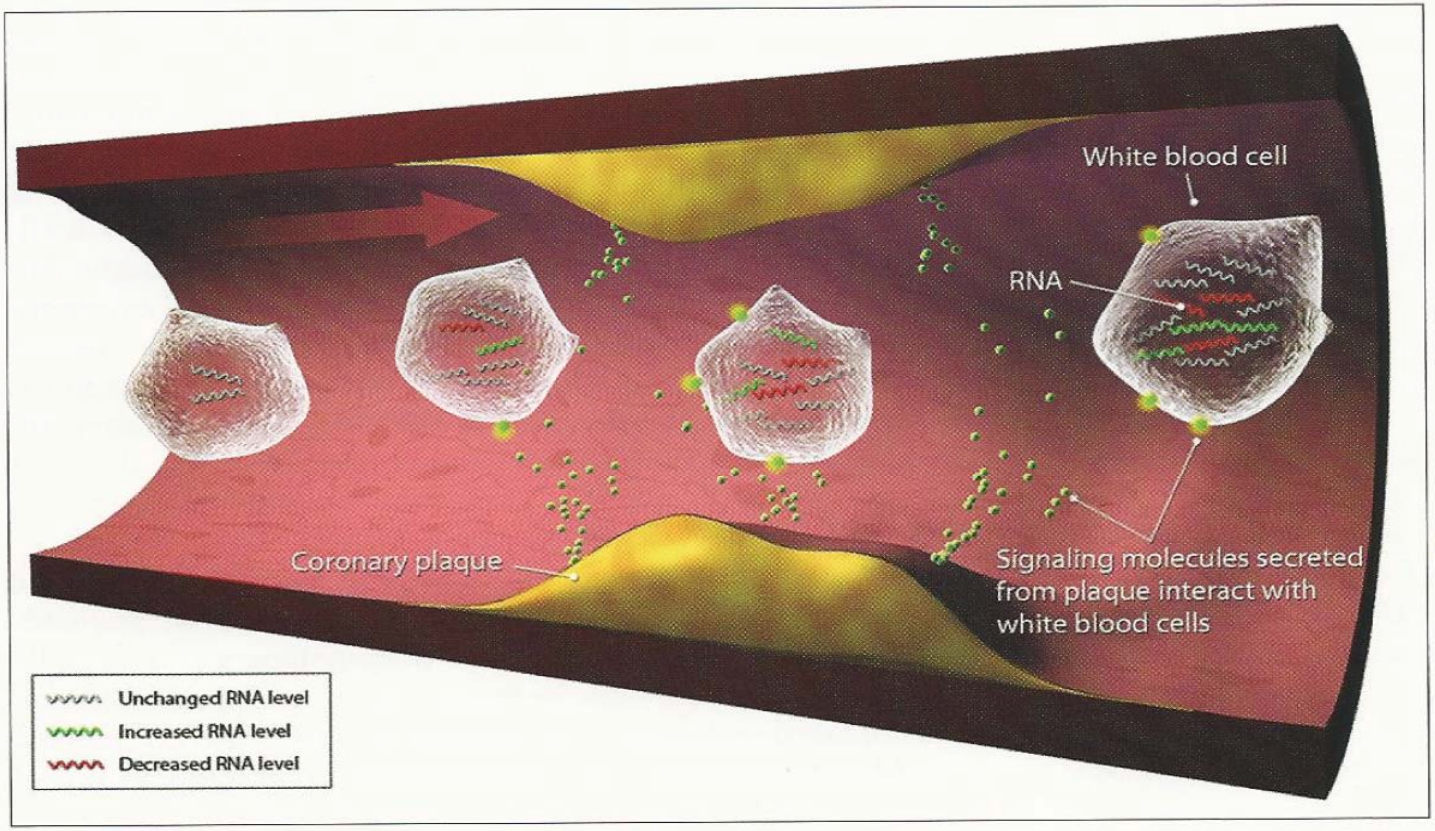
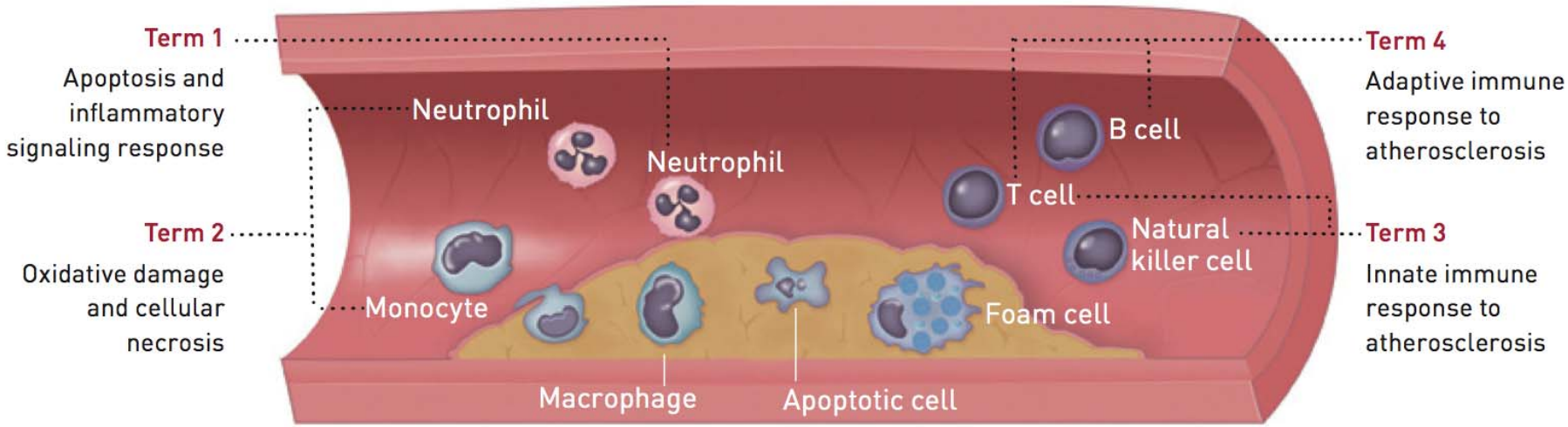


Figure 1. The gene expression test measures changes in blood cell RNA levels that are sensitive to the presence of coronary plaque.

Circ Cardiovas Genet 2013;6:154

FIGURE 1: CORUS CAD GENE TERM SCHEMATIC



PREDICT TRIAL
Gene Expression Testing: GES (Gene Expression Score) Corus CHD

Ann Intern Med 2010;153:425
J of Cardiovascular Translation Research 2012;3(5):366
Am Heart Journal 2012;164:320

- Correlated coronary angiogram with Corus gene expression.
- Higher the Corus score (0-40) the greater the chance of a 50% or greater stenosis in one major coronary artery and greater risk of future MACE (major adverse CV event).
- Score < 15 = Low risk for CHD
- Score 28 = 50% chance of major CHD
- Score 40 = 68% chance of major CHD

IMPACT Trial

J Am Board Fam Med 2014;27:258
Crit Pathways in Cardiol 2013;12:37

- Prospective study 251 non diabetic subjects with stable non-acute chest pain and related symptoms.
- GES done in all patients. Average score 16 (1-38).
- GES directed additional diagnostic CV testing and improved accuracy of diagnosis.

COMPASS TRIAL
Gene Expression Testing: (GES) Gene Expression Score: Corus CHD
Circ Cardiovasc Genet 2013;6:154

- Compared Corus gene expression to nuclear stress testing or myocardial perfusion imaging (MPI) in 537 patients.
- All patients had coronary angiogram or CT angiogram.
- Corus outperformed MPI in accuracy for predictive CHD. Corus 96% negative predictive value, 89% sensitive and 53% specificity and MPI was 88% negative predictive value.
- Strong discrimination for CHD, proportional to angiogram stenosis, better than MPI, good agreement with both CT angiogram and coronary angiogram.

PULS(Protein Unstable Lesion Signature) Cardiac Test (CHL)

Curr Med Res Opin 2012;28:1819-30

Elevated score related to:

- CHD development
- Presence of unstable or vulnerable arterial plaque
- Increased near-term risk of myocardial infarction

Biomarkers:

- MCP-3: immune cell direction and activity
- sFas: prevents apoptosis
- Fas Ligand: initiates cell recycling and death
- Eotaxin: activates immune cells at areas of injury
- CTACK: Helps to clean up damaged cells
- IL-16: recruits and activates immune cells, inflammation
- HGF: stimulates tissue repair.

Normal less than 3.5. Borderline 3.5 -7.49. Elevated > 7.5

Computerized Arterial Pulse Wave Analysis (CAPWA) Arterial Compliance (AC) and Pulse Wave Velocity (PWV)

Mayo Clinic Proc 2010;85;460-472

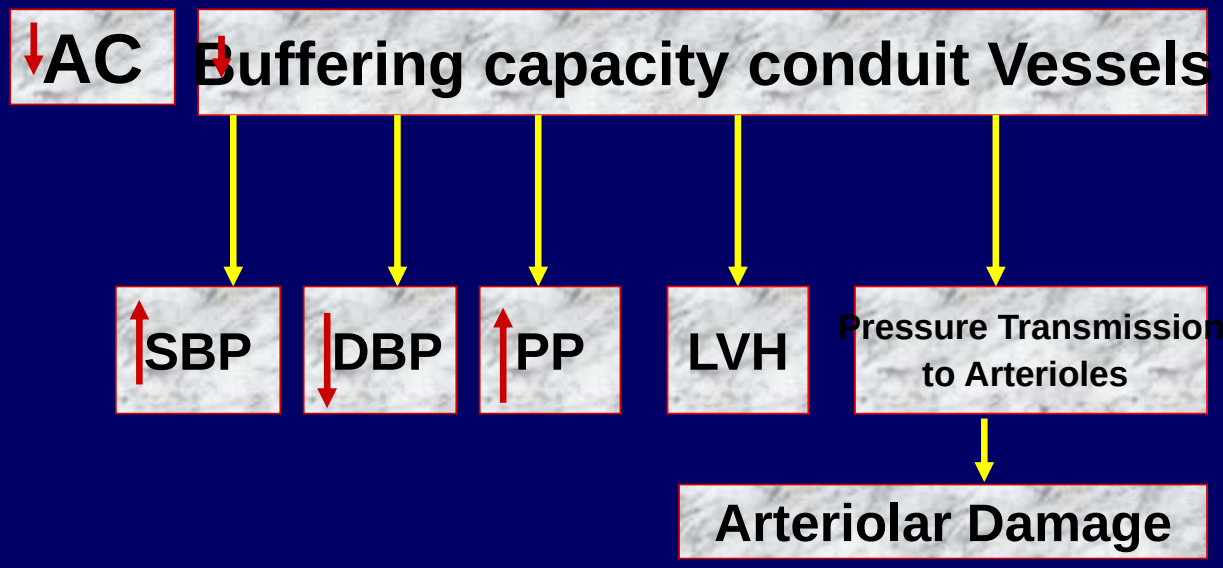
Therapeutic Advances in Cardiovascular Disease 2009;3:367-378

Arterial Compliance is the change in arterial distensibility defined by the change in volume / change in pressure

Vascular Compliance: Introduction and Overview

- Function and structural alterations of the arterial wall precede atherosclerosis and cardiovascular events.
- Endothelial dysfunction is the earliest marker of these changes.
- Arterial muscle changes and compliance of small and then large arteries are next changes.
- Pharmacological treatment of hypertension has reduced CVA to predicted levels but CHD reduction has been sub-optimal. This "CHD GAP" may be due to lack of therapeutic response in improving:
  1. Endothelial dysfunction (ED)
  2. Arterial compliance (AC)
  3. Concomitant risk factors
  4. Micronutrient depletion treatment
  5. Hemodynamic dysfunction
  6. Cardiovascular genetics and genomics with gene expression

Vascular Compliance: Introduction and Overview



Vascular Compliance: Arterial Structure

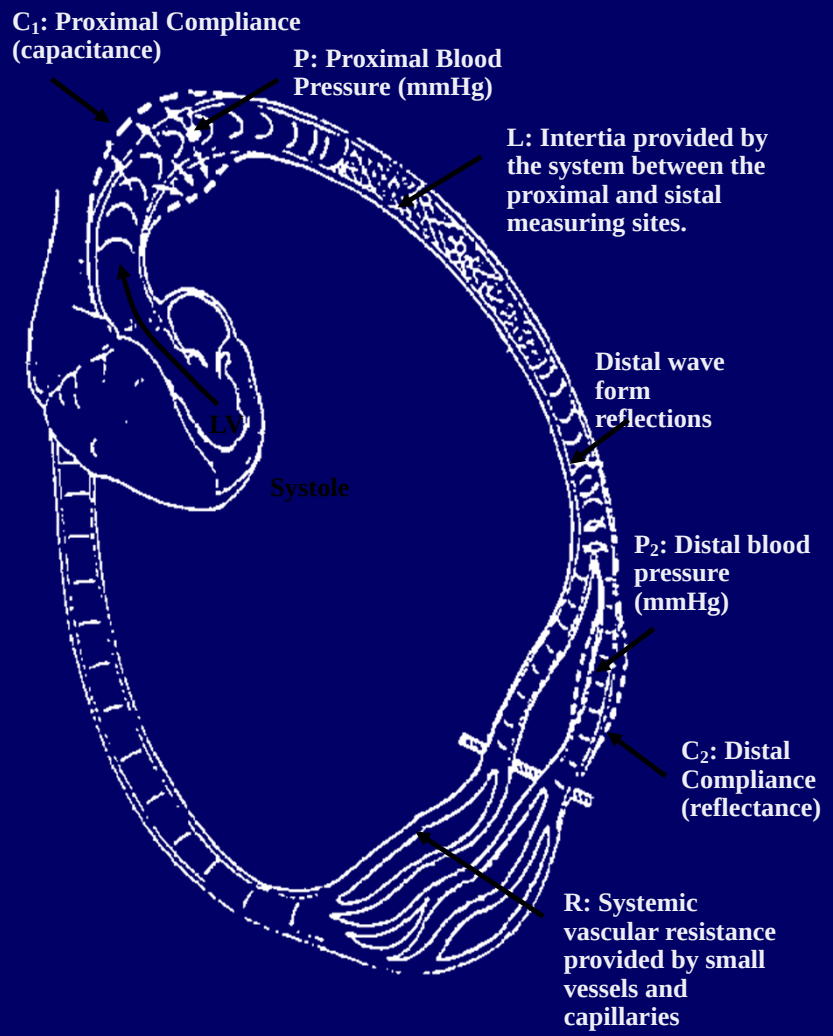
Artery Structure Determines Vascular Stiffness/Compliance

- **Intima:** Endothelium
  Subendothelium
- **Media:** Elastic SM fibers
  Protein matrix: elastin + collagen
  Internal elastic membrane
- **Adventitia:** Fibrous tissue (strong vessel shape large arteries)

Vascular Compliance: Artery Types

- **Conduit (Capacitive):** C1 (store blood in systole) (buffer) (thin endothelium with thick elastin and collagen) ↓VSM
- **Branch (Oscillatory):** C2 (pressure oscillations/reflected waves) (intermediate structure)
- **Arterioles (Resistance):** C2 (control blood flow) VSM + endothelium primarily with minimal elastin or collagen) NO dependent. Early marker vascular disease (HBP, HLP, DM)
- Endothelium role is greatest in thin wall vessels – (oscillatory and resistance). ED earlier and greatest in C2 vessels.

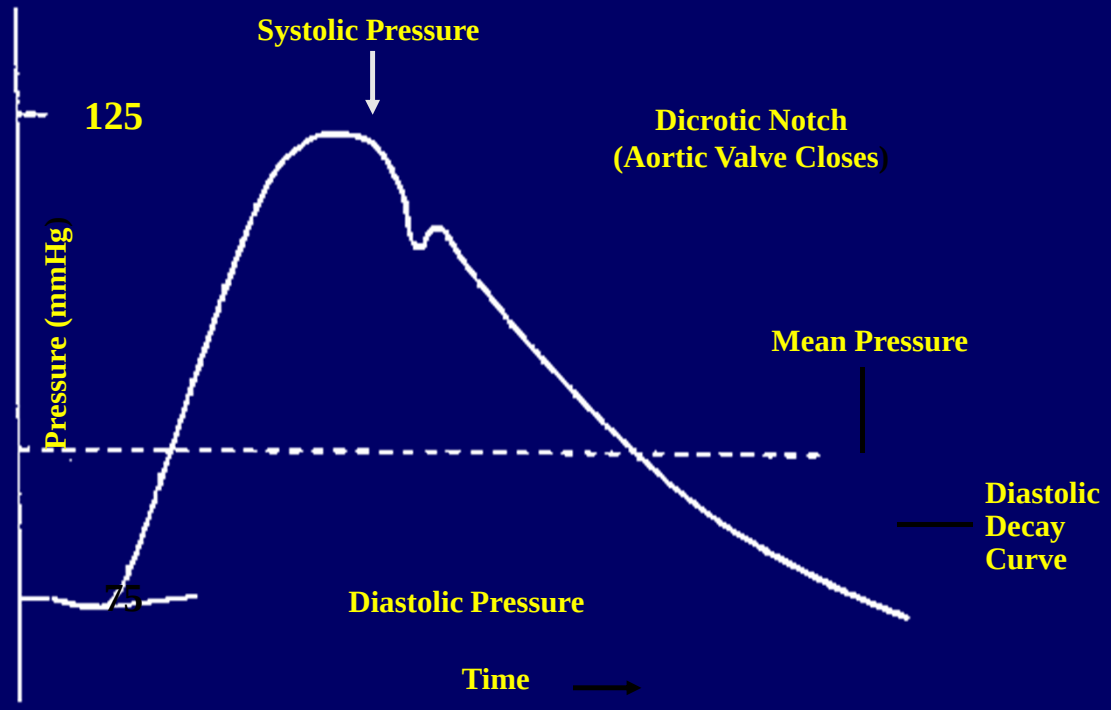
Illustration of Circulatory System



Buffering System of Vasculature

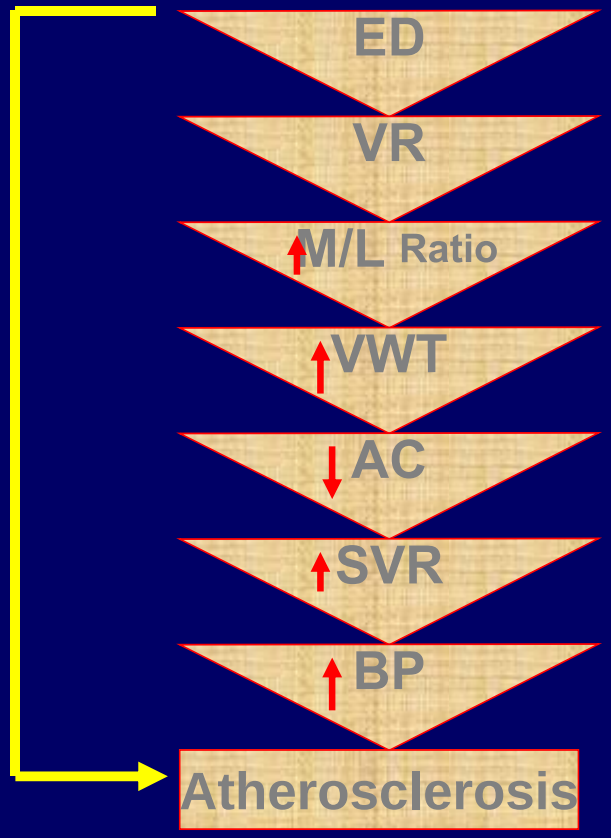
- In systole there is rapid infusion of SV
  - 20 – 30% is forward flow
  - 70 – 80% is stored in large conduit (capacitive) arteries, then released to periphery during diastole
- Converts pulsatile flow in aorta to continuous flow in capillaries (Windkessel effect)
- Loss of buffering with ↓ AC causes reduced continuous flow but increased pulsatile flow to precapillary and capillary vasculature inducing small vessel damage, end organ dysfunction and damage (↑↑ PWV)
- Out of phase propagation of flow and pressure waves.
  - Pressure wave faster and distorted
  - Reflected wave
  - Augmentation index (systole)

Arterial Blood Pressure Waveform



An arterial pressure pulse waveform with the limits of the pressure excursion over the cardiac cycle marked. Systolic pressure represents the peak pressure attained while diastolic pressure represents the trough occurring during the cardiac cycle.

Vascular Remodeling



(Endothelial Dysfunction)

Vascular Remodeling)

(Media / Lumen Ratio)

Vascular Wall thickness

(Arterial Compliance

Systemic Vascular Resistance)

(Blood Pressure

Pulse Wave Velocity and Arterial Compliance and Elasticity CV Profiler

J Am Coll Cardiol 2002;39 abstract 3523
Blood Pressure Monitoring. 2002.7: 231
Am Heart J 2003;146:679;J Hypertens 2010;28:1935
J of Clinical Hypertension 2015;00:1-11

- C2 compliance identifies the presence of endothelial dysfunction in the microvascular circulation, the very small arterioles and arteries. (range 4-9)
- C1 compliance is a measure of the elastic behavior of the aorta and larger arteries (range 8-17)
- Lower numbers indicate diseased arteries and are age and gender adjusted
- Improves risk stratification beyond usual risk factors including MAU ,ECHO and Carotid IMT.
- Low C2 and increased PWV predict **CVD/CHD**

CV Profile Report (patient)

CVProfile™ Report

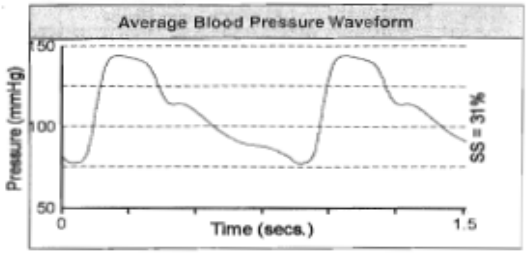
CVProfiler®
Profile for life.

Profile by: ST THOMAS MEDICAL GROUP

~~ID#: 41[redacted]~~

Name: [redacted]

SSN:
Date: Jan 01, 1997
Time: 00:17
Age: 47 years
Gender: Female
Height: 5 ft 7 in
Weight: 180 lbs
BSArea: 1.93 meters²
Body Mass Index: 28.2



PARAMETER		VALUE
Systolic Blood Pressure	(mmHg)	144
Diastolic Blood Pressure	(mmHg)	78
Mean Arterial Blood Pressure	(mmHg)	104
Pulse Pressure	(mmHg)	66
Pulse Rate	(beats/min)	71
C1 – Large Artery Elasticity Index (Capacitive Arterial Compliance)	(ml/mmHg x 10)	10.2
C2 – Small Artery Elasticity Index (Oscillatory or Reflective Arterial Compliance)	(ml/mmHg x 100)	3.5

MEDICAL HISTORY

CV Disease: N
CV Medications: ?
Diabetes: ?
Relatives CV Disease: ?
Tobacco: ?
Race: ?

CLINICAL COMMENTS:

Abnl C2
ED
Normal > 7.6

Form: 00017-002K 02/04 Toll-Free: 888-785-7392 C-VP1 Serial # DO 005268

Arterial Elasticity

Arterial Elasticity Guidelines

Instructions: (1) Circle the gender and age range of the individual tested.
(2) Write the C1 and C2 arterial elasticity index values printed on the CVProfile™ Report in the brackets at the top of the guideline table which matches individual's gender.
(3) In the same row as the individual's range, circle the C1 and C2 table values which match those written in the brackets in order to interpret the individual's vascular health.

MALE	C1 – Large Artery [ ] Elasticity Index Range			C2 – Small Artery [ ] Elasticity Index Range		
Age Range	Abnormal	Borderline	Normal	Abnormal	Borderline	Normal
15 - 19	< 10	10 – 17	> 17	< 6	6 – 9	> 9
20 - 29	< 9	9 – 16	> 16	< 6	6 – 8	> 8
30 - 39	< 8	8 – 14	> 14	< 6	6 – 8	> 8
40 - 49	< 7	7 – 12	> 12	< 5	5 – 7	> 7
50 - 59	< 6	6 – 11	> 11	< 5	5 – 7	> 7
60 - 69	< 5	5 – 10	> 10	< 4	4 – 6	> 6
> 70	< 5	5 – 9	> 9	< 4	4 – 5	> 5

FEMALE	C1 – Large Artery [ ] Elasticity Index Range			C2 – Small Artery [ ] Elasticity Index Range		
Age Range	Abnormal	Borderline	Normal	Abnormal	Borderline	Normal
15 - 19	< 9	9 – 15	> 15	< 6	6 – 8	> 8
20 - 29	< 8	8 – 14	> 14	< 5	5 – 7	> 7
30 - 39	< 7	7 – 12	> 12	< 4	4 – 6	> 6
40 - 49	< 6	6 – 10	> 10	< 4	4 – 6	> 6
50 - 59	< 5	5 – 10	> 10	< 3	3 – 5	> 5
60 - 69	< 4	4 – 9	> 9	< 3	3 – 5	> 5
> 70	< 4	4 – 8	> 8	< 2	2 – 4	> 4

Endothelial Function: ENDO-PAT

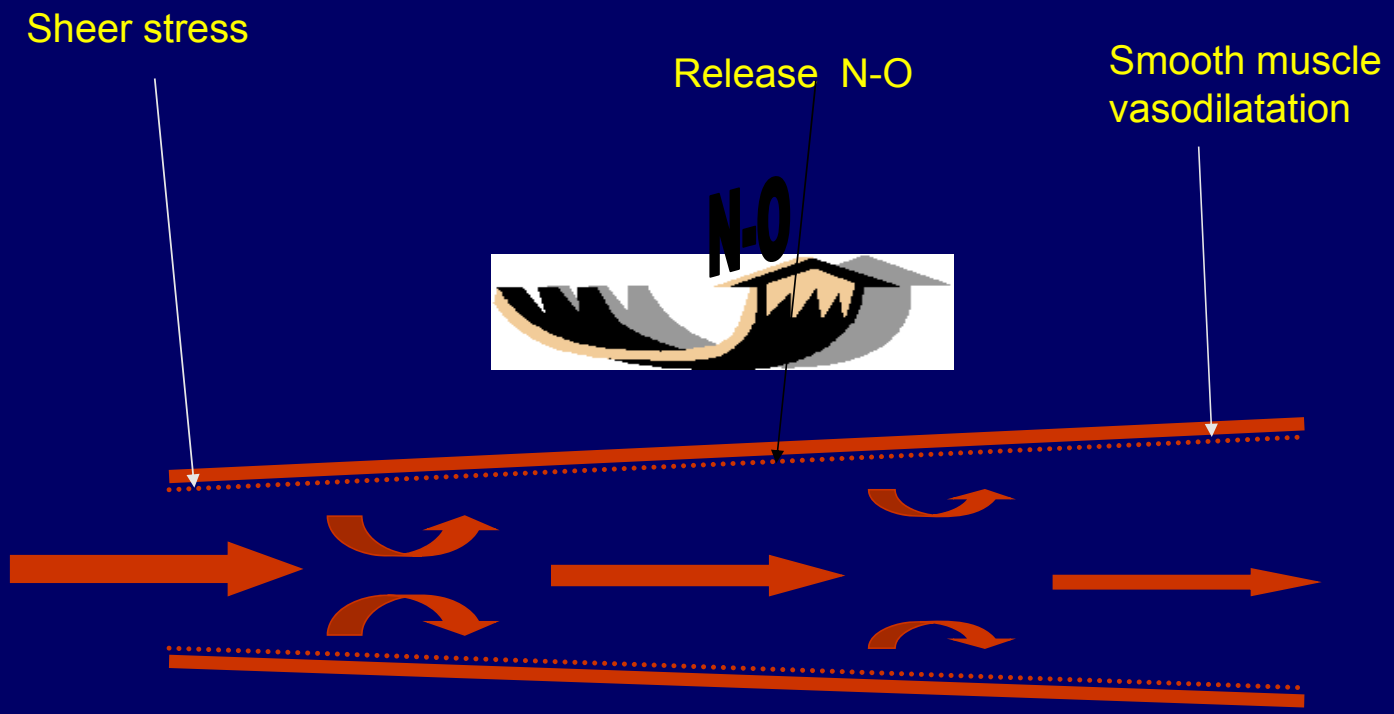
Endothelial Function: ENDO-PAT
Endothelial Dysfunction, Hypertension and CVD

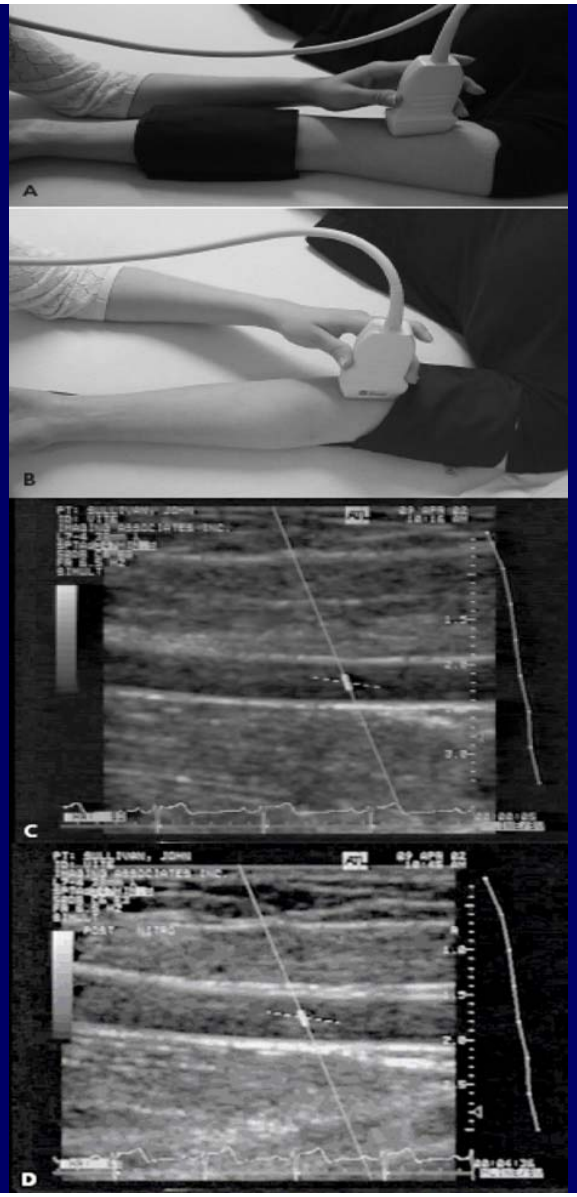
JACC 2010;55:1688

JACC 2004;44:2137 Circulation 2008;117:2467

- Measures reactive hyperemia and endothelial function and dysfunction. FDA approved.
- 5 minute occlusion of brachial artery with BP cuff.
- Digital measurement for endothelial and flow mediated dilation (FMD) as increase in signal amplitude.
- Measure pre and post occlusion ratio index.
- Index of 1.67 has sensitivity of 82% and specificity of 77% to diagnose coronary endothelial dysfunction and highly correlates to brachial artery FMD(r=.0.33 to 0.55). Predicts hypertension and CHD.

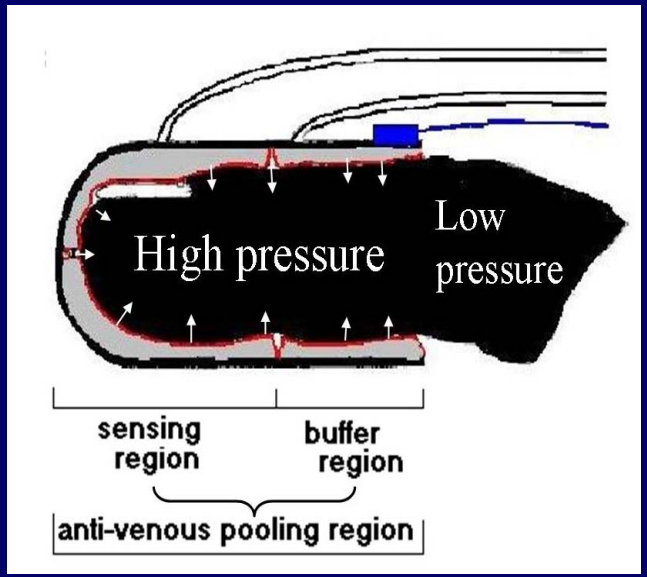
Physiology of Endothelium-Mediated Vasodilation

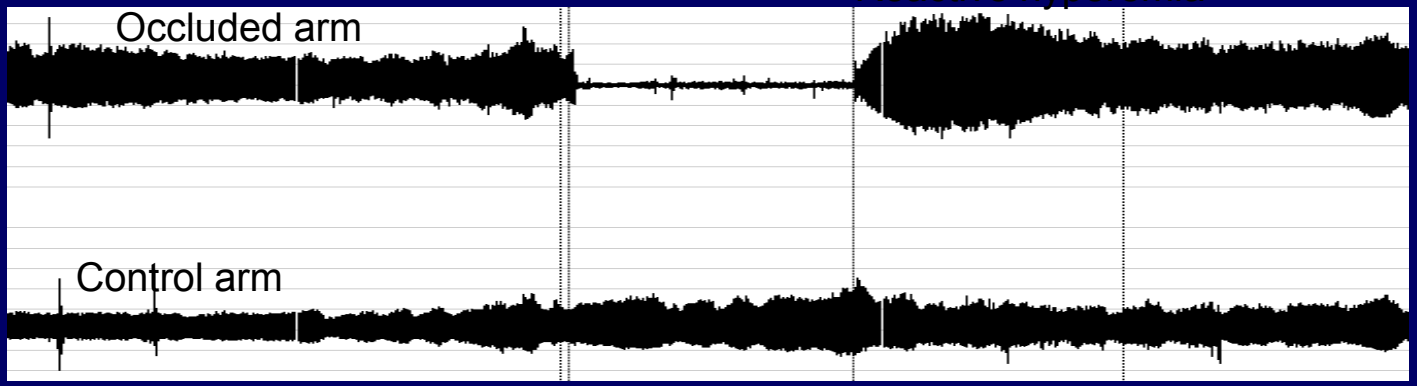



A
B
C
D

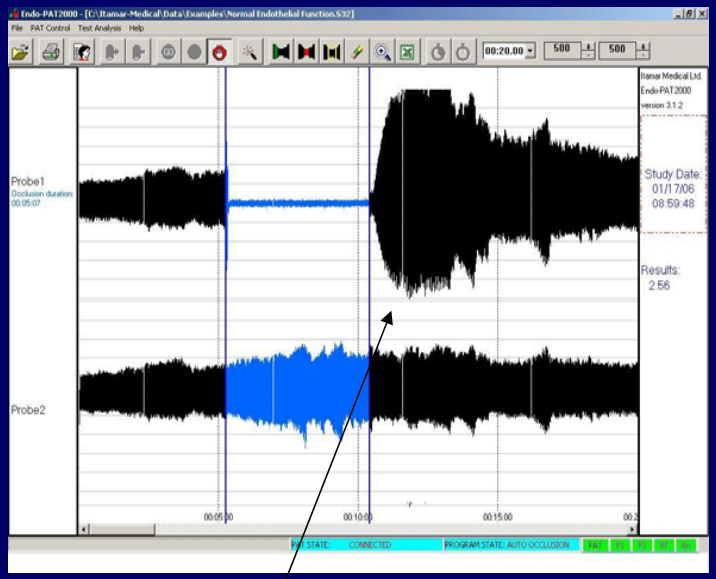
PAT Bio-sensor - unique design
Applies uniform pressure to entire finger surface

- Prevents venous blood pooling
  - Eliminates veno-arteriolar constrictor reflex
- Clamps probe to finger
  - Reduces noise
- Buffers measuring site
  - Buffers noisy venous waves
- Unloads arterial wall tension
  - Increases dynamic volume range

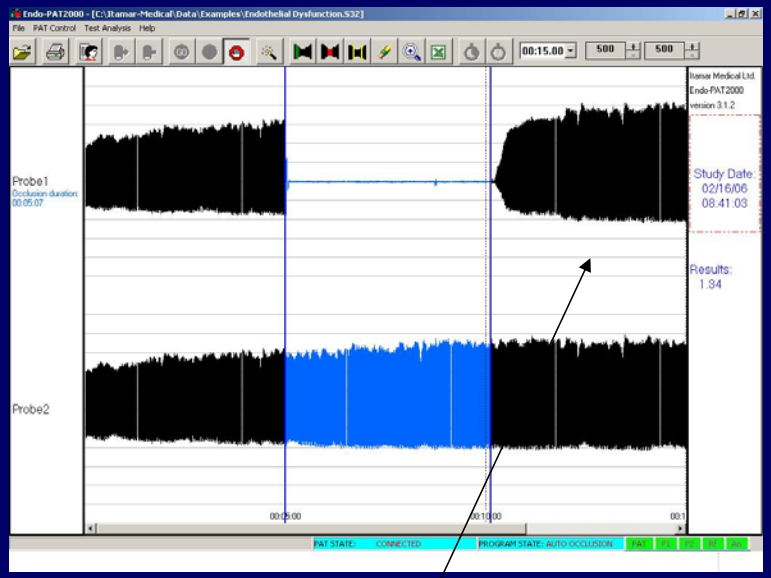



Reactive hyperemia
Occluded arm
Control arm

ENDOPAT Good and poor results



Normal EF



Poor EDF

Endo-PAT2000

Test Date: 06/03/13 07:41:07

Patient Information

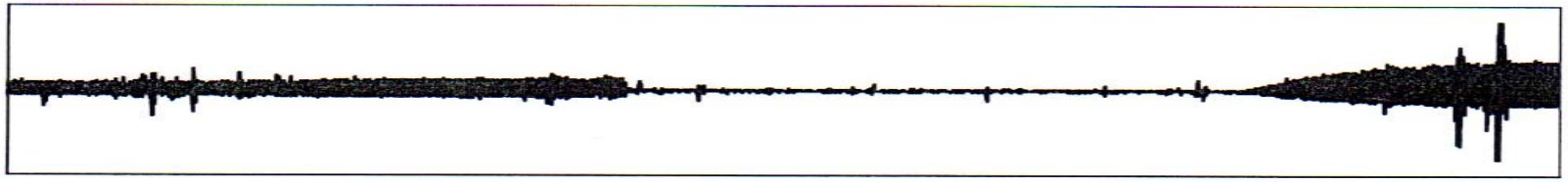
ID:	[redacted] mch 2	Name:	[redacted]	Systolic BP	130 mm Hg
Age:	75	Gender:	Female	Diastolic BP:	76 mm Hg
Height:	5' 3"	Weight:	140 lb	BMI:	24.8
User Field 1:		User Field 2:			
Comments:					

Study Information

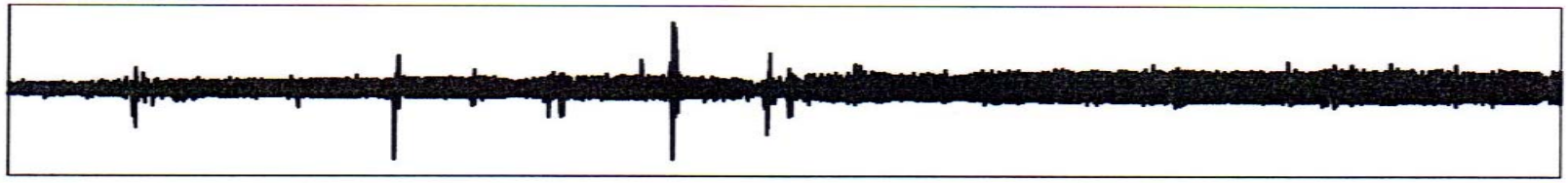
Test Duration:	00:14:20	PATographer:	NK		
Recording Ver:	3.4.4	Analysis Ver:	3.4.4	Occ. Borders:	Automated

PAT Signals

Occluded Arm



Control Arm



Baseline(05:43) Occlusion(05:35) Dilatation(03:02)

Study Results

RHI: 1.58 Endothelial Dysfunction

Heart Rate: 50 bpm

Recommendations

Endo-PAT2000

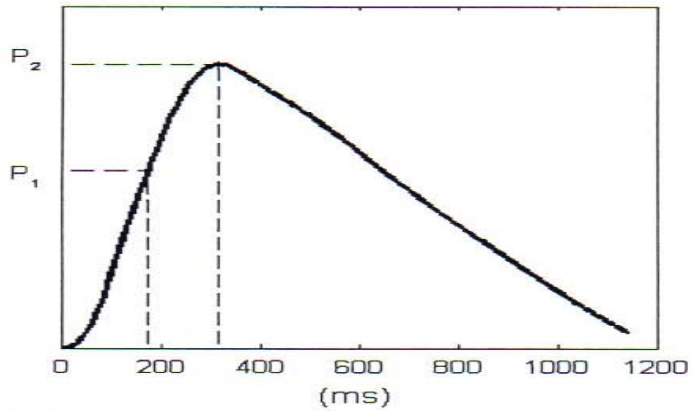
Test Date: 06/03/13 07:41:07

Augmentation Index (AI) - a measure of Arterial Stiffeness

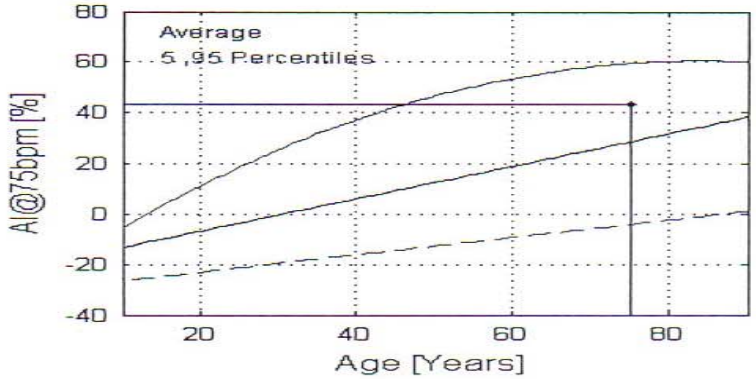
AI: 59%
AI@75bpm: 44%
AI = (P2-P1)/P1 x 100 [%]

Averaged - 123 pulses

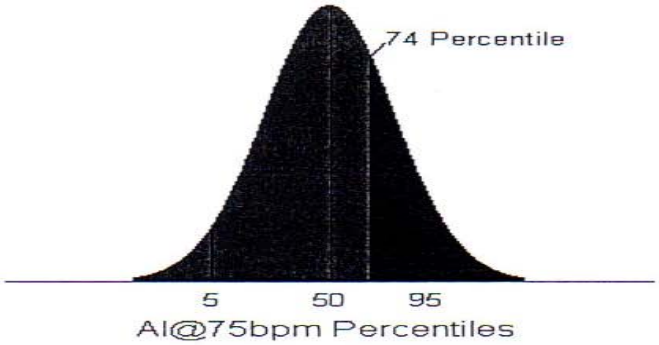
Average PAT Waveform (from baseline segment)



AI@75bpm in female population as function of age



Patient Relative to Age and Gender matched distribution



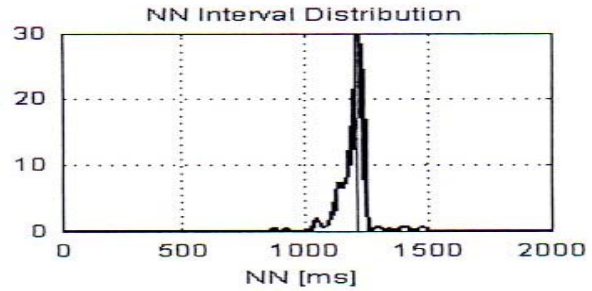
Endo-PAT2000

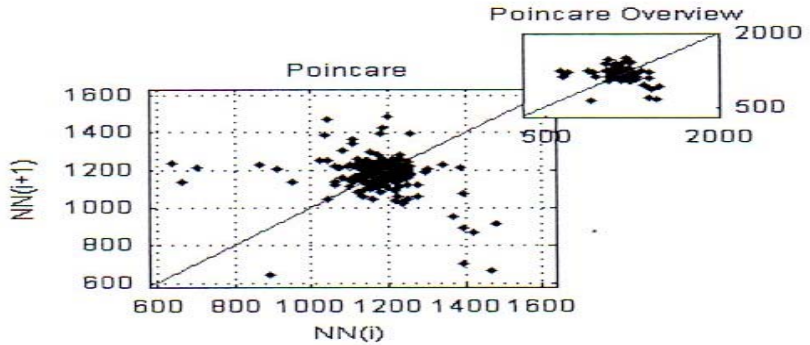
Test Date: 06/03/13 07:41:07

Heart Rate Variability (HRV)

Time Domain

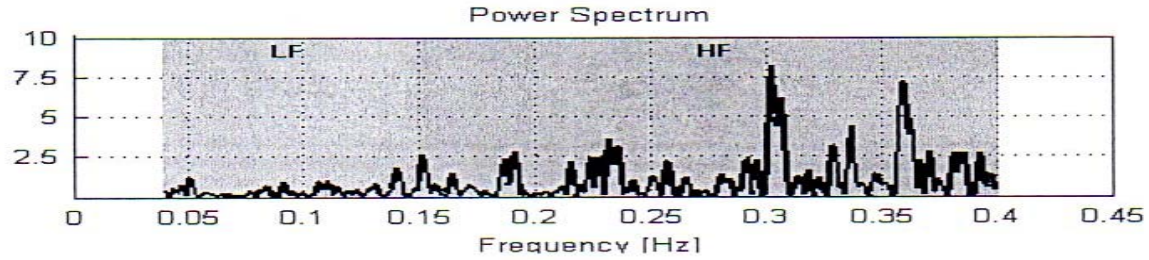
Mean NN:	1196	ms
SDNN:	63.59	ms
RMSSD:	95.00	ms
NN50:	35	
pNN50:	14.58	%
Triangular Index:	8.13	





Frequency Domain

LF (0.04-0.15 Hz):	36.83	ms²
HF (0.15-0.4 Hz):	279.11	ms²
LF/HF:	0.13	



ENDOPAT
J Am Coll Cardiol 2004;44:2137– 41

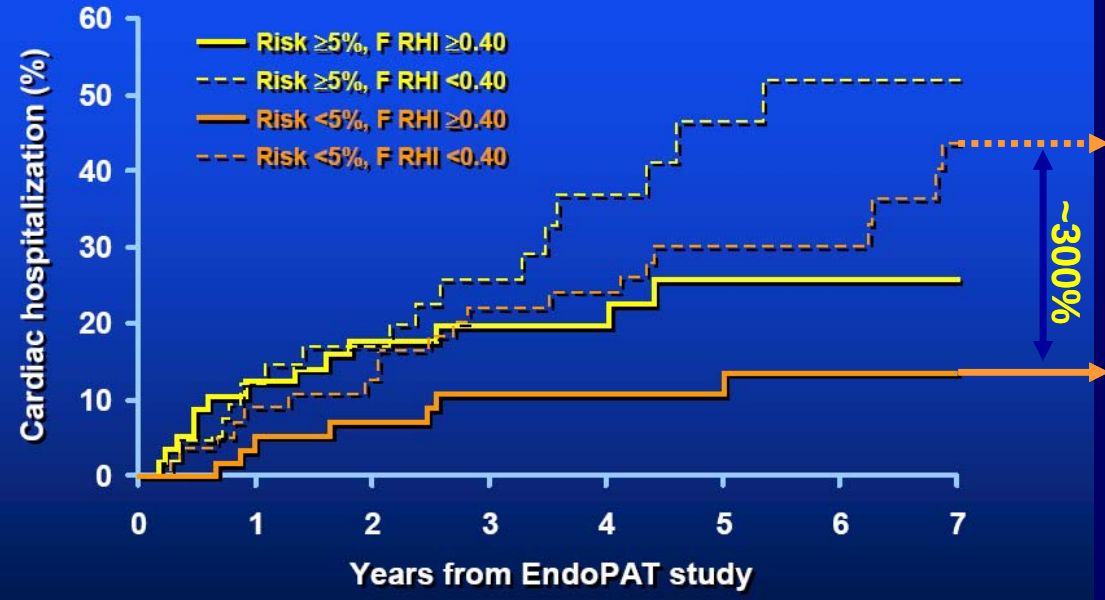
- Study designed to assess EndoPAT RHI v. Coronary Endothelial Function.
- 94 Patients without angiographic CAD
- All underwent Coronary Endothelial Function Testing with Acetylcholine.
- 39 were Normal (CBF increased by 50% or more after Ach infusion).
- 55 were Abnormal.
- EndoPAT RHI Measured in all Patients. Average RHI was 1.27 in Patients with Coronary Endothelial Dysfunction and 1.78 in those without Coronary ED.
- Conclusion: EndoPAT RHI score correlated well with coronary endothelial function testing and is therefore a valid predictive model for subsequent CVD events.

ENDOPAT AND FRAMINGHAM RISK SCORE AND CHD RISK

European J Cardiol 2010;31:1142

EndoPAT vs. Framingham Risk Score

Mayo Clinic, 2010



~300%

Low risk but <u>with</u> endothelial dysfunction

Low risk & <u>normal</u> endothelial function

n=270, Intermediate risk patients
Mayo Clinic & Tufts Medical Center

All CV Deaths were in Patients with Endothelial Dysfunction

MAYO CLINIC

ENDO-PAT AND CVD OUTCOMES

Eur Heart J 2010 ;31:1142
Am J Cardiol 2012;109:1711

- 270 patients over 7 years : ED and Framingham risk score
- Abnormal Index predicted cardiac events such as cardiac death, MI cardiac hospitalization and CABG: 48% vs 28% (p=0.03). This was independent of Framingham risk score.
- Also correlates with risk factors
- The more severe the CAD the worse the index
- Lower EP scores with ED correlate with IVUS of Coronary Arteries with more necrotic core, more calcium and higher risk of plaque rupture. (AJC)

Associations of Endopat and IVUS assessed coronary plaque morphology in CHD

Am J Cardiology 2012;109:1711

- An abnormal reactive hyperemia (ED) by Endopat is associated with plaque structure that is more prone to rupture as measured by IVUS.
- More necrotic core
- More dense calcium
- Less fibrous tissue

Endopat

1. Vasc Med 2012;17(2):79-84
2. Am Heart J 2003;146:168
3. Vasc Med 2007;12:13-16
4.J of Hypertension 2013;31:1570-74

- In patients with CHD, FMD and PAT have high correlation (p< 0.001) and reproducibility on same day (18%) and between day (11%). Need 2 hours between tests to allow for endothelial recovery. (1)
- PWA, FMD and PAT have high correlations to assess vascular endothelial function. (2)
- PAT correlates with FMD and extent of CHD and presence or absence of CHD. (3)
- PAT, FMD and EDV (acetylcholine)) all correlate with each other and with endothelial function. (4)

Persistent Impairment of Endothelial Vasomotor Function has a Negative Impact on Outcome in Patients with CHD

JACC 2009;53:323

- Persistent impaired FMD less than 5.5 % vs those with improved FMD over 5.5% was an independent risk factor for CV events with HR of 2.9 over 36 months

Peripheral Endothelial Function and Cardiovascular Events in High Risk Patients

J of American Heart Assoc 2013;2. Nov 2 2013 Epub

- 528 patients with high risk for CV events over 5 years.
- EndoPat RHI was measure before and after coronary angiogram
- RHI, BNP and CV score by SYNTAX were all independent risk predictors for all future CV events such as MI, CV death, unstable angina, ischemic CVA, CABG, CHF and PAD.
- When RHI was added to FRS, BNP and SYNTAX the net reclassification index was significantly improved by 27.5 % with a significant increase in in C-statistic from .728 to .766.

Impaired periperal endothelial function as assessed by digital reactive hyperemia peripheral arterial tonometry and risk of in-stent restenosis (ISR)

AHA abstract 1106-86 March 29, 2014. Komura N et al

- 258 consecutive patients with PCA and stents for CHD.
- Repeat coronary angiogram at 6 and 9 months
- ISR defined as over 50% restenosis
- ISR patients had lower follow-up Endopat scores (1.75 +/- 0.47 vs 1.99+/- 0.56, p = 0.02) but there was no significant difference at baseline Endopat.
- Endopat was strongest independent predictor of ISR

ED and risk prediction

If you improve endothelial function regardless of the risk factors you can decrease carotid IMT and CHD risk.

Cardiopulmonary Exercise Testing (CPET)

Clinician's Guide to Cardiopulmonary Exercise Testing in Adults
A Scientific Statement From the American Heart Association
Circulation July 13, 2010 ;122, Issue 2

Exercise testing remains a remarkably durable and versatile tool that provides valuable diagnostic and prognostic information regarding patients with cardiovascular and pulmonary disease.

When combined with exercise testing, adjunctive imaging modalities offer greater diagnostic accuracy, additional information regarding cardiac structure and function, and additional prognostic information. Similarly, the addition of ventilatory gas exchange measurements during exercise testing provides a wide array of unique and clinically useful incremental information that heretofore has been poorly understood and underutilized by the practicing clinician.

The reasons for this are many and include the requirement for additional equipment(cardiopulmonary exercise testing [CPX] systems),personnel who are proficient in the administration and interpretation of these tests, limited or absence of training of cardiovascular specialists and limited training by pulmonary specialists in this technique, and the lack of understanding of the value of CPX by practicing clinicians.

Modern CPX systems allow for the analysis of gas exchange at rest, during exercise, and during recovery and yield breath-by-breath measures of oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and ventilation ($\dot{V}E$). These advanced computerized systems provide both simple and complex analyses of these data that are easy to retrieve and store, which makes CPX available for widespread use.

These data can be readily integrated with standard variables measured during exercise testing, including heart rate, blood pressure, work rate, electrocardiography findings, and symptoms, to provide a comprehensive assessment of exercise tolerance and exercise responses. CPX can even be performed with adjunctive imaging modalities for additional diagnostic assessment.

Hence, CPX offers the clinician the ability to obtain a wealth of information beyond standard exercise electrocardiography testing that when appropriately applied and interpreted can assist in the management of complex cardiovascular and pulmonary disease.

WatchPat for Obstructive Sleep Apnea (OSA)

AHI Apnea / Hypopnea Index	Wake / Sleep Detection True Sleep Time
	REM / Deep / Light Sleep Stages True Sleep Time
ODI Oxygen Desaturation Index	Body Position
RDI Respiratory Disturbance Index	Snoring

- WatchPAT is an FDA-approved portable diagnostic device that uniquely uses finger based physiology and innovative technology to enable simple and accurate Obstructive Sleep Apnea (OSA) testing while avoiding the complexity and discomfort associated with traditional air-flow based systems
- OSA causes hypertension, MI, CHF, CVA, A. Fib, DM, sudden death, obesity, fatique

Sleep Study Report

Sleep Summary

Start Study Time:	11:09:35PM
End Study Time:	5:48:33AM
Total Study Time:	6 hrs, 38 min
Sleep Time	**4 hrs, 50 min**
% REM of Sleep Time:	14.4

Respiratory Indices

	REM	NREM	All Night
pRDI:	47.1	22.0	**25.6**
pAHI:	47.1	14.0	**18.8**
ODI:	35.7	7.5	**11.6**

Indices are calculated using valid sleep time of 4 hrs, 50 min.
pRDI/pAHI are calculated using oxi desaturations ≥ 3%

Oxygen Saturation Statistics

Mean:	96	Minimum:	86	Maximum:	99
Mean of Desaturations Nadirs (%):					93

Oxygen Desatur. %:	**4-9**	**10-20**	**>20**	**Total**
Events Number	55	1	0	56
Total	98.2	1.8	0.0	100.0

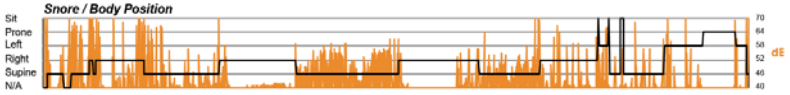
Oxygen Saturation	**<90**	**<88**	**<85**	**<80**	**<70**
Duration (minutes):	0.8	0.3	0.0	0.0	0.0
Sleep %	0.3	0.1	0.0	0.0	0.0

Pulse Rate Statistics during Sleep (BPM)

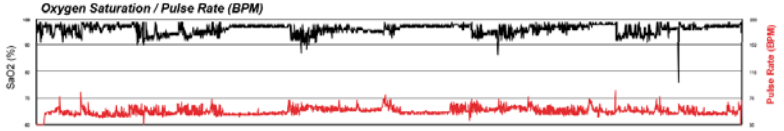
Mean:	55	Minimum:	N/A	Maximum:	75

PAT Respiratory Events

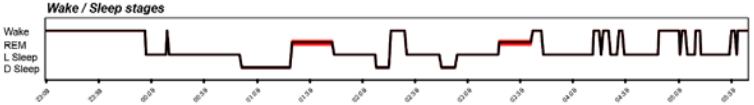
Snore / Body Position



Oxygen Saturation / Pulse Rate (BPM)



Wake / Sleep stages



Sleep Study Report

Body Position Statistics

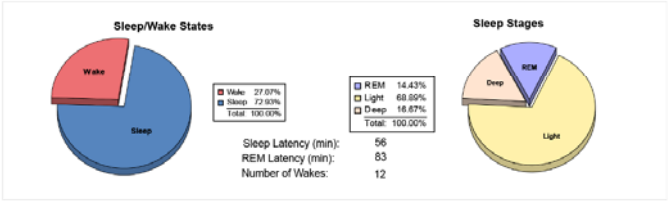
Position	Supine	Prone	Right	Left
Sleep (min)	150.3	17.0	114.7	9.0
Sleep %	51.7	5.8	39.4	3.1
pRDI	33.6	0.0	19.4	N/A
pAHI	28.8	0.0	10.0	N/A
ODI	18.4	0.0	5.2	N/A

Snoring Statistics

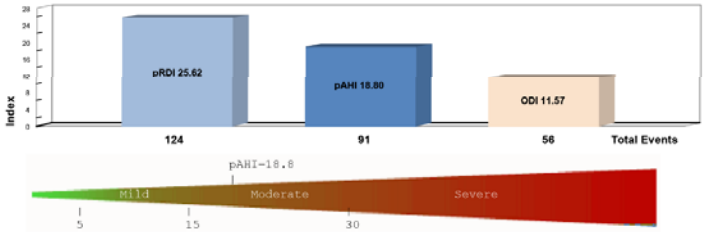
Snoring Level (dB)	>40	>50	>60	>70	>80	>Threshold (45)
Sleep (min)	95.5	21.3	1.5	0.0	0.0	39.8
Sleep %	32.8	7.3	0.5	0.0	0.0	13.7

Mean: 42 dB

Sleep Stages Chart



Respiratory Indices Chart



* Reference values are according to AASM guidelines

Coronary Artery Calcification (CAC)
Coronary CT Angiogram

Vascular Calcification: Pathogenesis

Circulation1995;92: 2029-32;JCI 1994;93:2106-13
Arch Int Med 2007;167:879-85;Free Radic Biol Med 2001;31:509-19
Curr Opin Nephrol Hypertens 2013;22:405-412.
European Heart J 2014;35:1515

- Active process by calcifying vascular cells in vascular smooth muscle
- Mediated by signaling molecules
  1. Osteocalcin
  2. Osteopontin
  3. Bone morphogenic protein BMP-2
  4. Matrix Gla protein
  5. Fibroblast growth factor (FGF 23)
  6. Fetuin A
  7. Osteoprogerin(OPG)
  8. Inorganic pyrophosphate

Associated with all cause mortality and CVD events (CHF and CVA). Alters Vitamin D metabolism and RAAS.

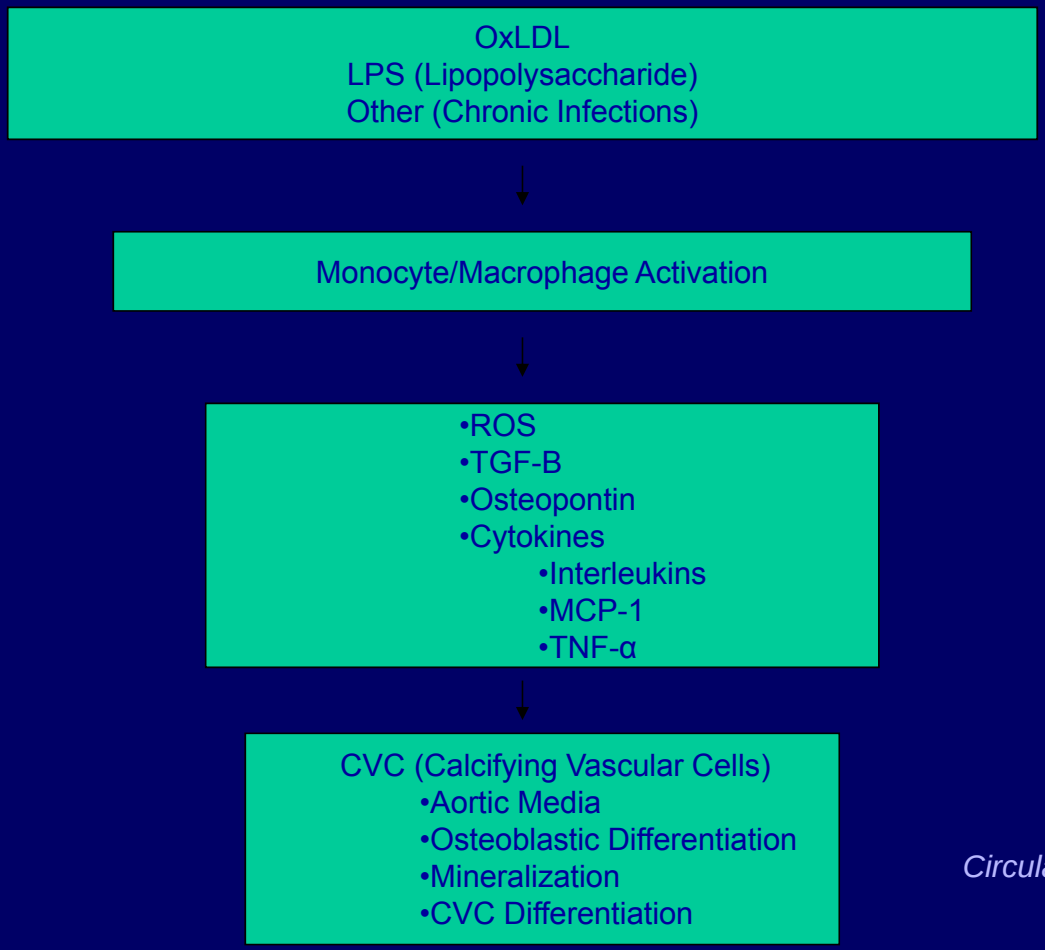
Signals are induced by inflammatory and lipid mediators such as TGF beta and oxLDL which lead to transformation of vascular smooth muscle cells into osteoblast-type cells, PTH, vitamin D, calcium and phosphate are all key elements.

Vascular Calcification: Pathogenesis

Circulation1995;92: 2029-32;JCI 1994;93:2106-13
Arch Int Med 2007;167:879-85;Free Radic Biol Med 2001;31:509-19
Curr Opin Nephrol Hypertens 2013;22:405-412.

- Imbalance between inducers and inhibitors
- Various stimuli lead to dedifferentiation of VSMC into osteoblast/chondroblast-like cells
- Multipotent vascular stem cells
- Micro-RNA involved
- FGF 23/Klotho axis
- Vitamin D and K
- BMP 2
- Serum phosphorous and calcium
- CKD

Vascular Calcification and Atherosclerosis



Circulation 2002; 105:650-655

Coronary Artery Calcification.(CAC)

JAMA 2010;303:1610;Am J Cardiology 2015;116:520
Am J Cardiol 2010;105:459
Arterioscler Thromb Vasc Biol 2004;24:1272
Clin Cardiol;2010;33:658;JAMA 2014;311:271

- CAC progression over 15 % annually provides increase CHD risk analysis with 17 fold increase in CVD.
- CAC is composite of volume and density of calcium
- Higher calcium density lowers CHD risk and is seen in statin treated patients due to reduction in lipid core and plaque stabilization.
- Baseline CAC score predicts CHD risk beyond traditional risk factors. CAC score of over 300 has hazard ratio of CHD of 10
- Positive CAC increases risk of major cardiac event by 6-35 fold
- CAC is tip of iceberg :90% of noncalcified plaque below
- CAC correlates with traditional risk factors but also with increased oxidative stress , autoantibodies to oxLDL and apoB-immune complexes.
- Correlates with glycemic load and index.
- Progression from zero calcium score to calcification does not occur until 5 years and this occurs in 25%
- Low radiation .5mSv

Inflammation Biomarkers and CAC

Am J Cardiol 2016:118:311

- Only PAI-1 is associated with the presence and progression of CAC in women compared to other inflammatory biomarkers such as hsCRP, fibrinogen, and factor VII.
- Other studies show hsCRP in women with normal BMI is associated with CAC
- Higher BMI in women with obesity is associated with CAC.
- ACEI and ARB and Spironolactone reduce PAI-1

Coronary Artery Calcification and Carotid IMT (MESA)

Arch Intern Med 2008;168:1333.

- Coronary artery calcification was associated more strongly than carotid IMT with risk of incident CVD. (n=6698) over 5.3 years
- CAC: CVD risk increased 2.1 fold per one SD
- Carotid IMT: CVD risk increased 1.3 fold per one SD

CT Angiogram (CTA) and CAC

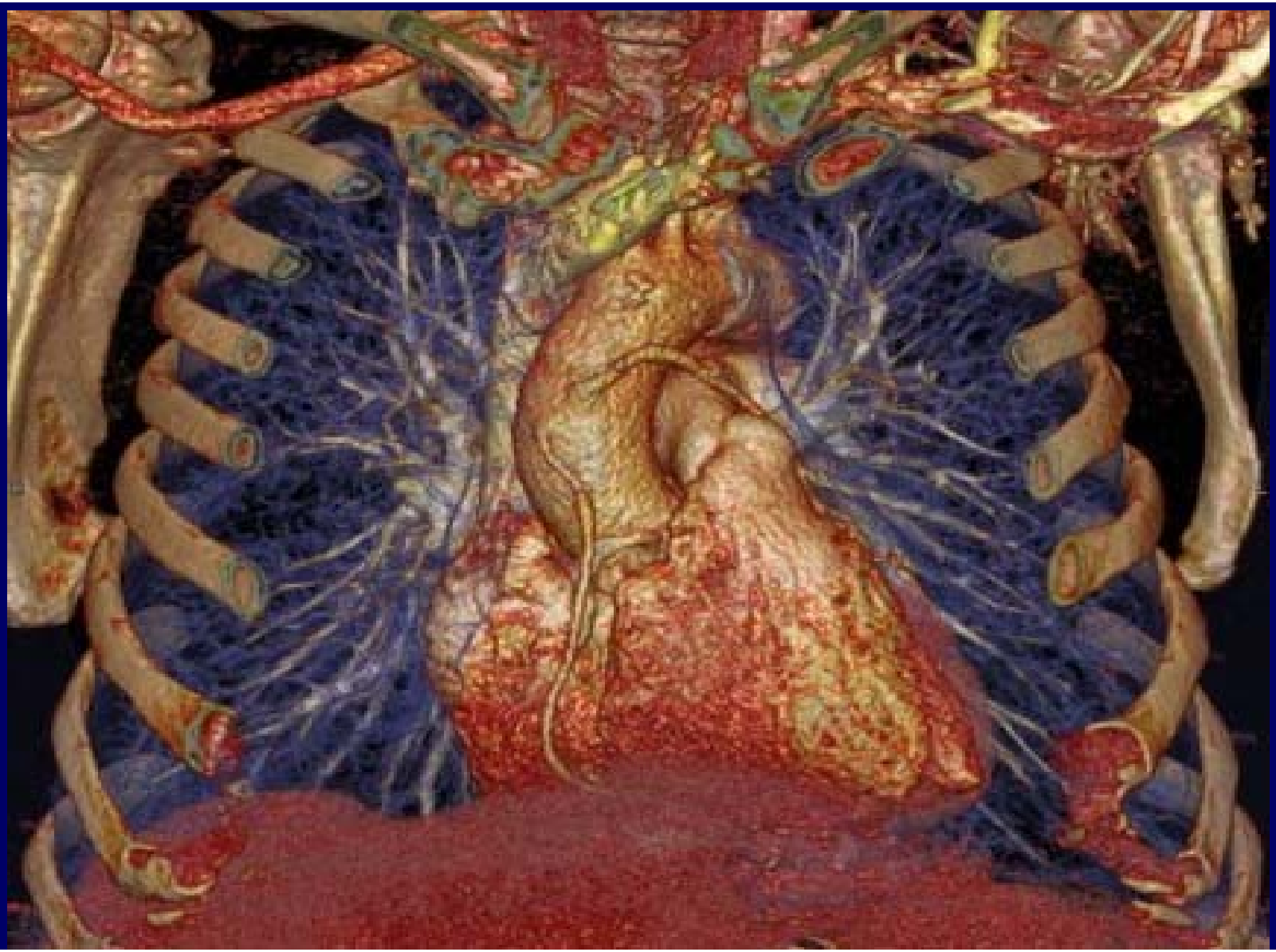
Am J Cardiol 2010;106:1574; Am J Cardiology 2011;107:799;Am J Cardiol 2012;109:1449; Mayo Clinic Proc 2014;89(10): 1350-59
Am J Card 2104;114:1707; Am Heart J 2016;177:17
Am J of Cardiology 2017;120:2154

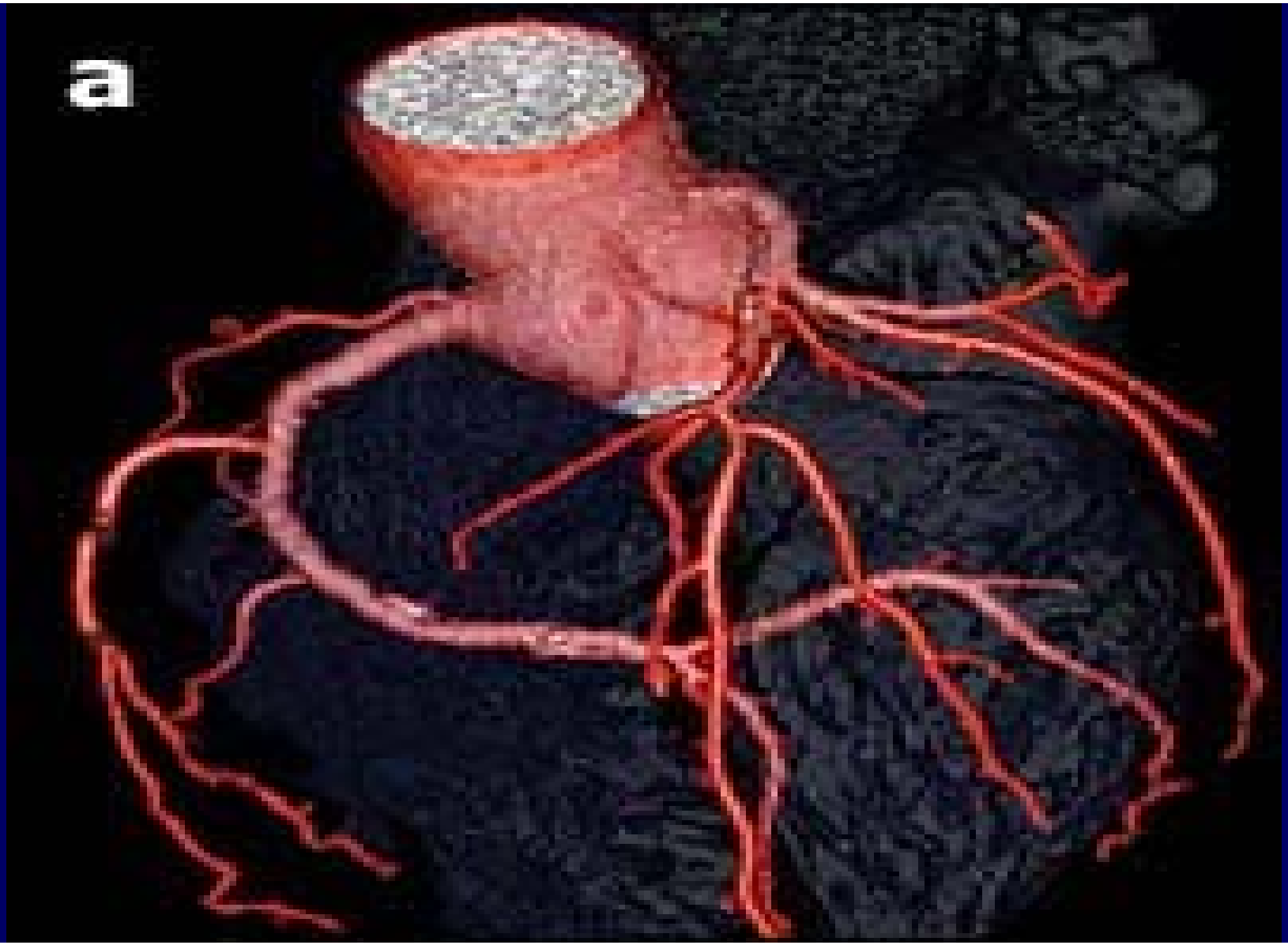
- The risk of major CV events or death increased in a graded manner with the degree of coronary atherosclerosis as defined by CTA even in the absence of high grade coronary artery stenosis
- Both the CAC score and the number of calcified plaques improve risk strategication
- CAC is superior to predict future CHD events compared to the Framingham risk score and other biomarkers for CHD. Predicts increase risk A. Fib.
- CAC imparts increased CHD risk in younger and elderly individuals, across all age groups.
- Sugar –sweetened beverages have the highest correlation with CAC of food groups.

CTA vs Angiogram

Am J Cardiology 2016:117:1863

- Coronary CTA detects approximately twice as many coronary segments with plaque compared to coronary angiograms.
- This results in 52% of patients being assigned to a greater risk category.

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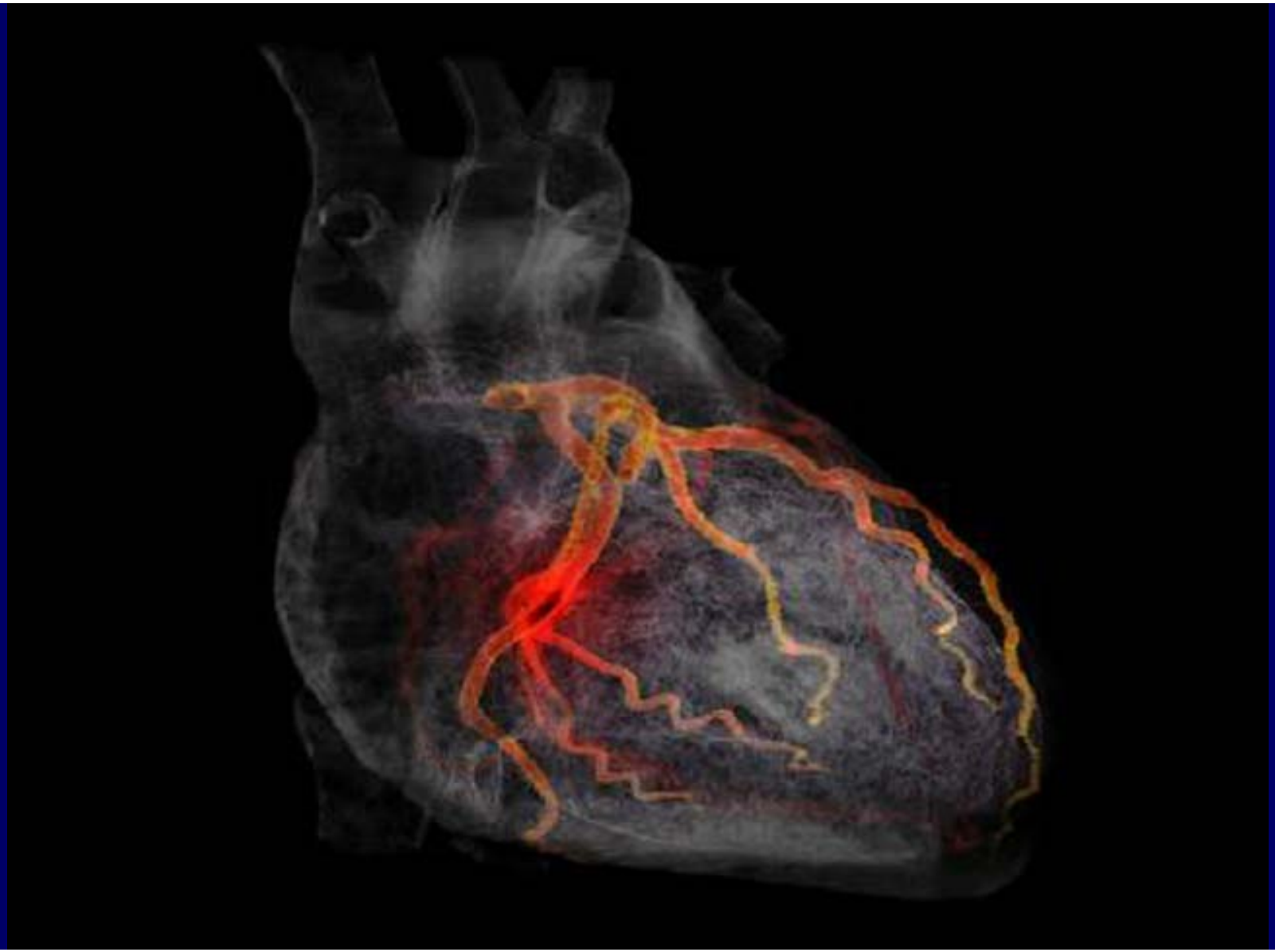


Premier Radiology Belle Meade
A member of the Saint Thomas Imaging Network

Premier Radiology Belle Meade * 28 White Bridge Pike, Suite 111 * Nashville, TN 37205
Phone: (615)356-3999 * Fax: (615)353-0462

Name:	[redacted]	**Exam Date:**	[redacted]
Patient ID:	[redacted]	**Referrer:**	
Secondary ID:		**2nd Referrer:**	
DOB:	2/9/1953	**3rd Referrer:**	
Acc#:	3169322		

PROCEDURE: CARDIAC CT FOR CORONARY ARTERY CALCIUM SCORING

TECHNIQUE: Multi-detector computed tomography of the heart was performed during suspended respiration, and without the administration of contrast material. Post-processing was performed on a workstation to measure the amount of coronary vascular calcium. CPT 75571

HISTORY: Screening

COMPARISON: None

RESULTS:
Thorax: No significant abnormalities identified in the lungs or mediastinum. Note that the CT examination is limited to the heart and the adjacent lung and mediastinum.

CALCIUM SCORING:

Left main coronary artery (LMCA): 12
Left anterior descending artery (LAD): 103
Circumflex artery: 0
Right coronary artery (RCA): 0

TOTAL AGATSTON SCORE: 115

The probability of a nonzero score in a person of the same age, sex, and race/ethnicity is 68 percent.

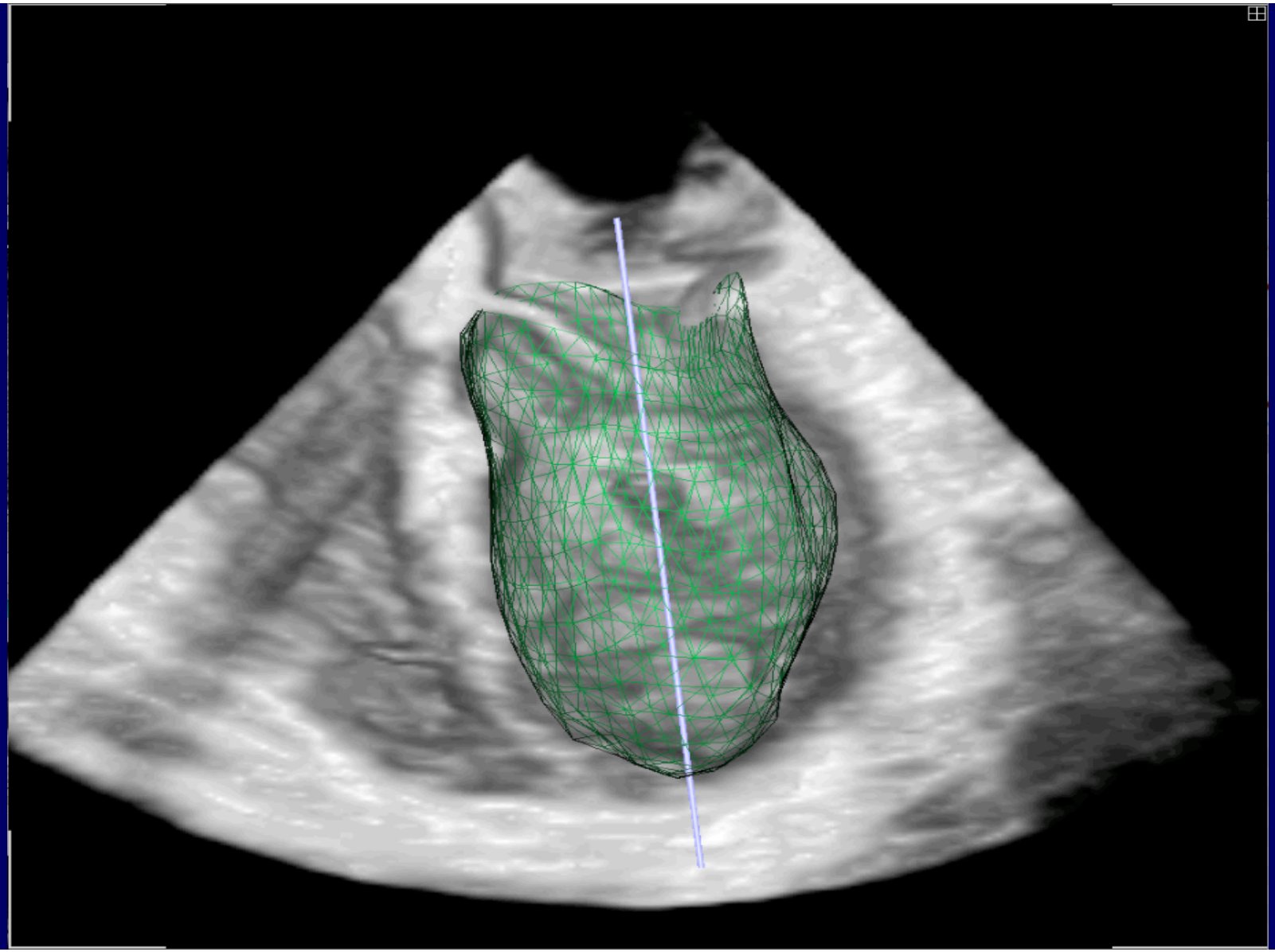
The above-stated Agatston score is at percentile 70 for subjects of the same age, gender, and race/ethnicity who are free of clinical cardiovascular disease and treated diabetes. This indicates that 30 percent of individuals of the same age, sex, and race/ethnicity will have the same or higher score.

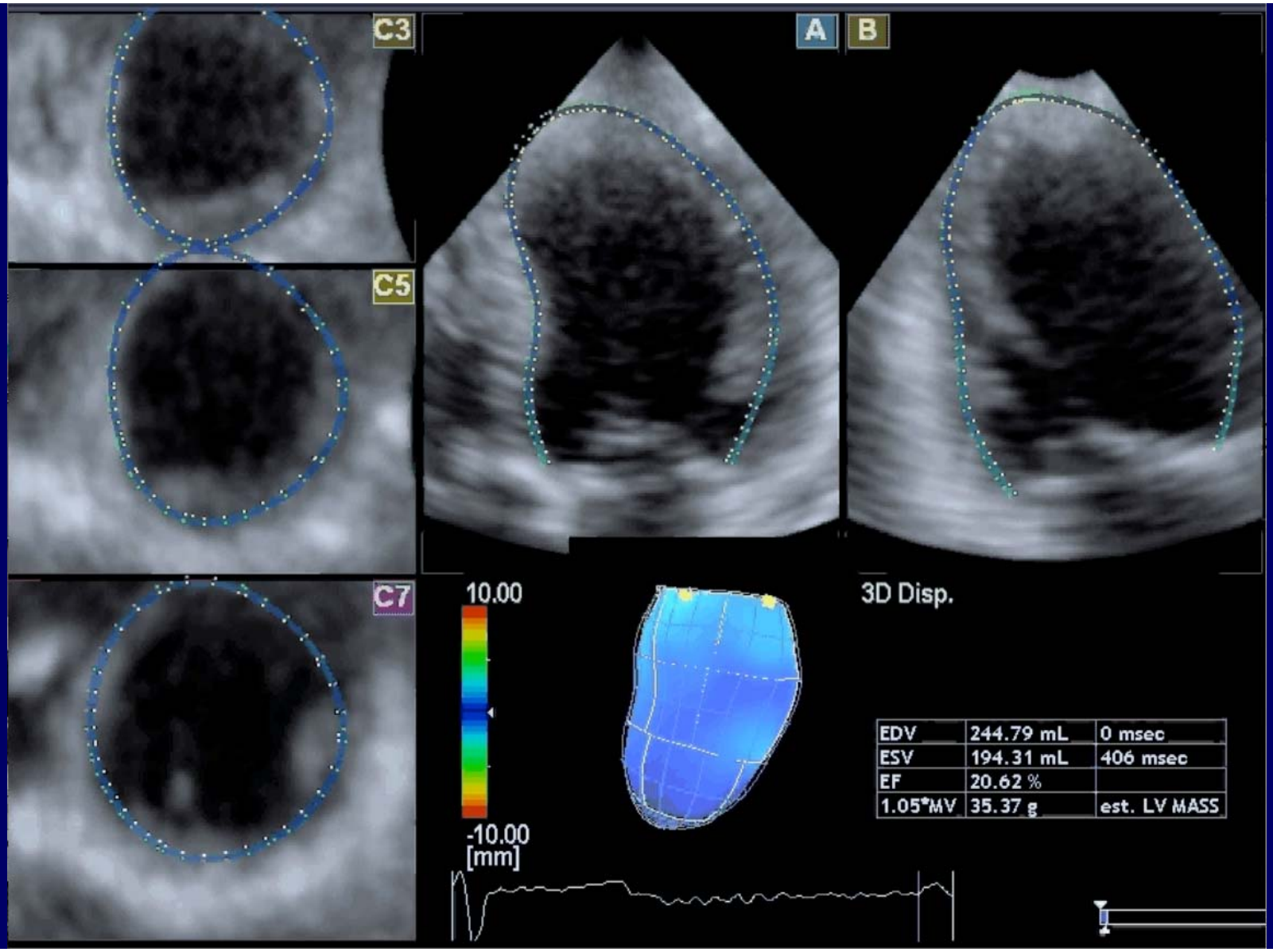
Calcium Score (CACS) Interpretations (Agatston Score):
0 (zero): No identifiable atherosclerotic plaque. Very low cardiovascular disease risk. Less than 5 percent chance of presence of coronary artery disease (CAD). A negative examination.
1-10: Minimal plaque burden. Significant CAD very unlikely.
11-100: Mild plaque burden. Likely mild or minimal coronary stenosis (obstruction).
101-400: Moderate plaque burden. Moderate non-obstructive CAD highly likely.
Over 400: Extensive plaque burden. High likelihood (>90 percent) of at least one major coronary vessel with a significant stenosis (>50 percent diameter).

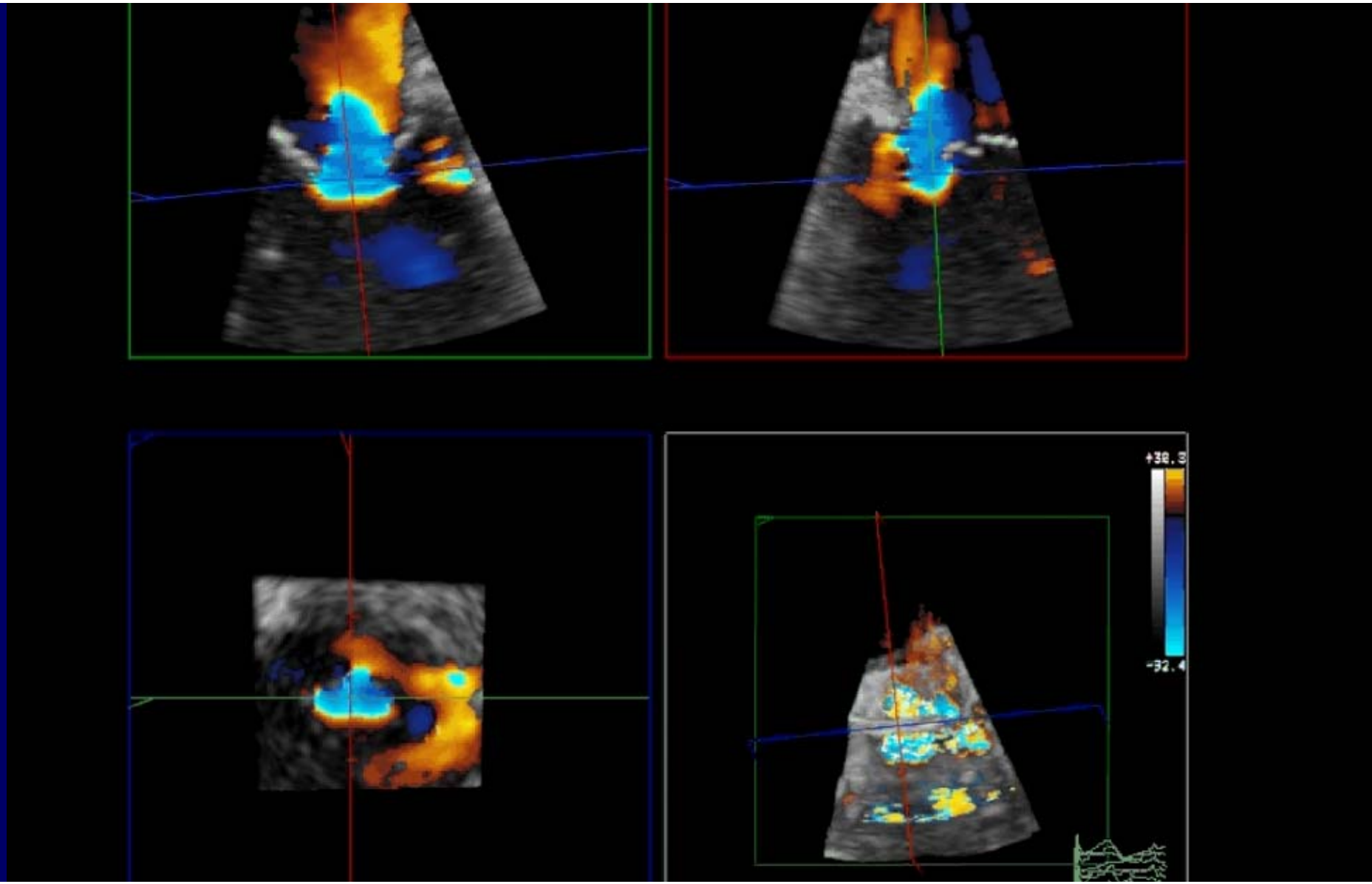
Note: This examination is not to be considered a substitute for a clinical examination by a physician. Coronary artery calcium scoring is intended to be a risk assessment test for coronary artery disease only, and the results of this examination should be taken into careful consideration by the patient's own physician in the context of other factors such as relevant history, physical examination, and other indicated or related investigations.

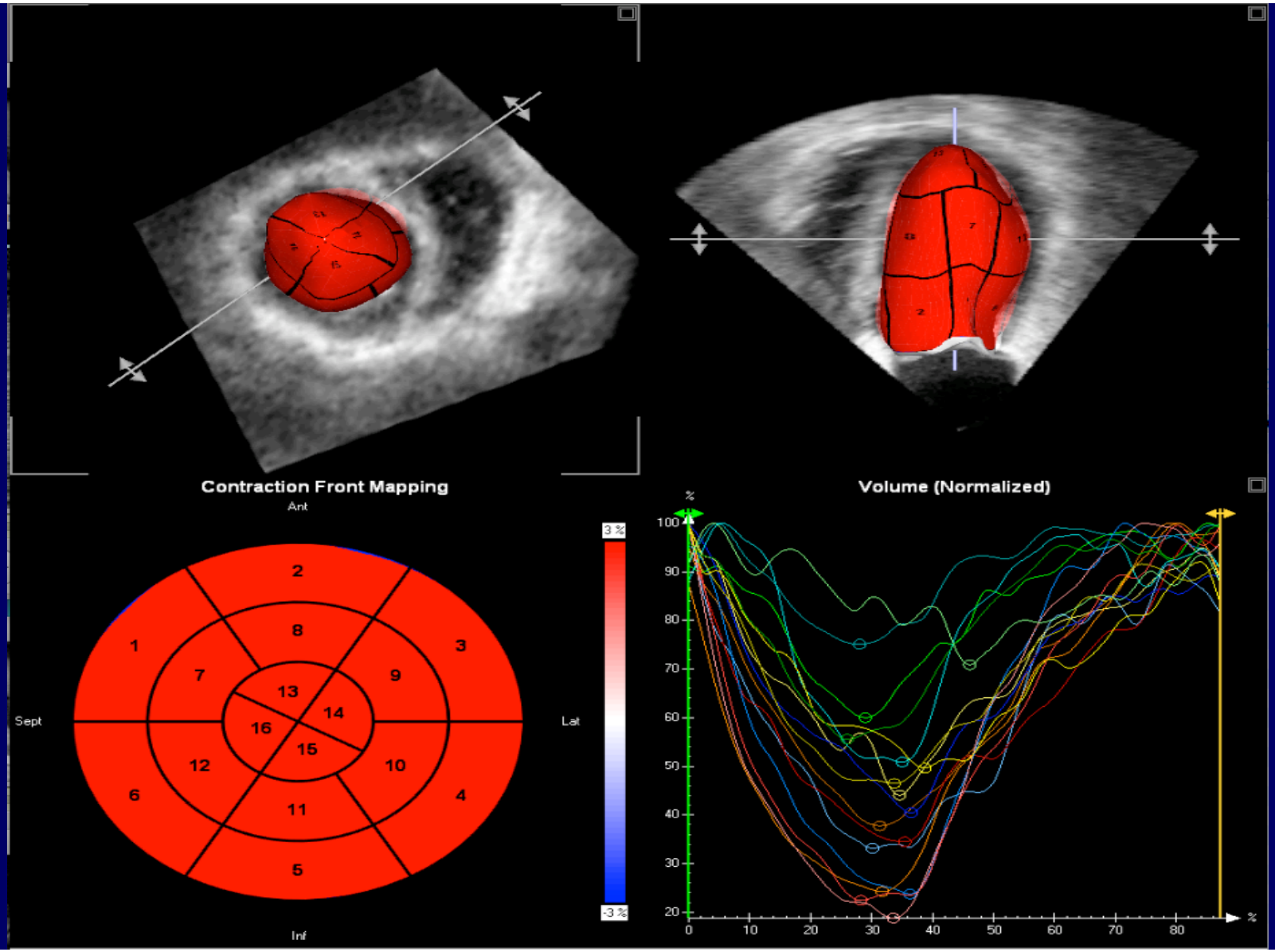
ECHO and Stress ECHO

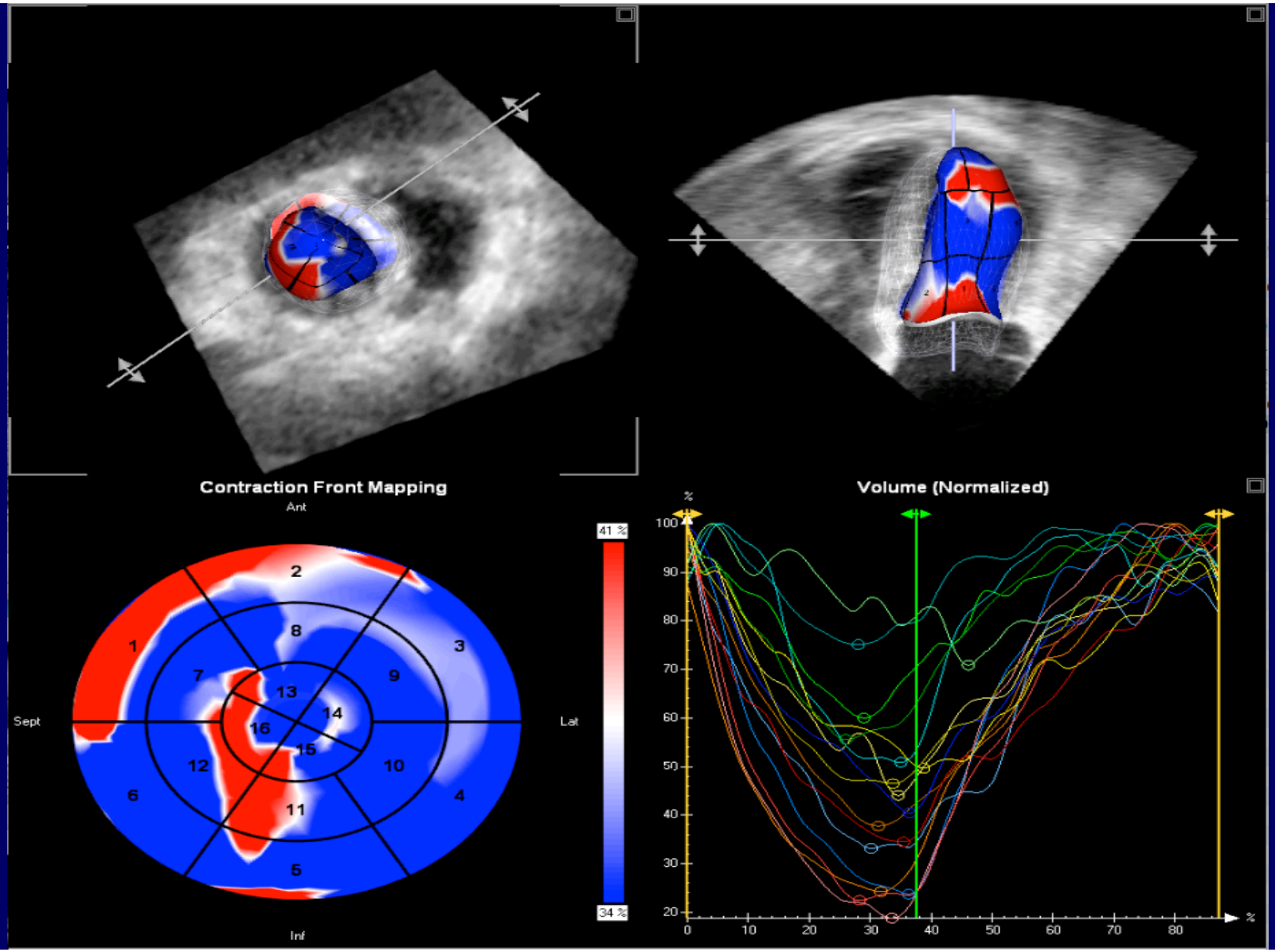
- Left Ventricular Hypertrophy
- Left Atrial Hypertrophy
- Diastolic Dysfunction
- Valve function
- Ejection Fraction
- Wall Motion abnormalities
- 85-90 % sensitive with stress ECHO for CHD

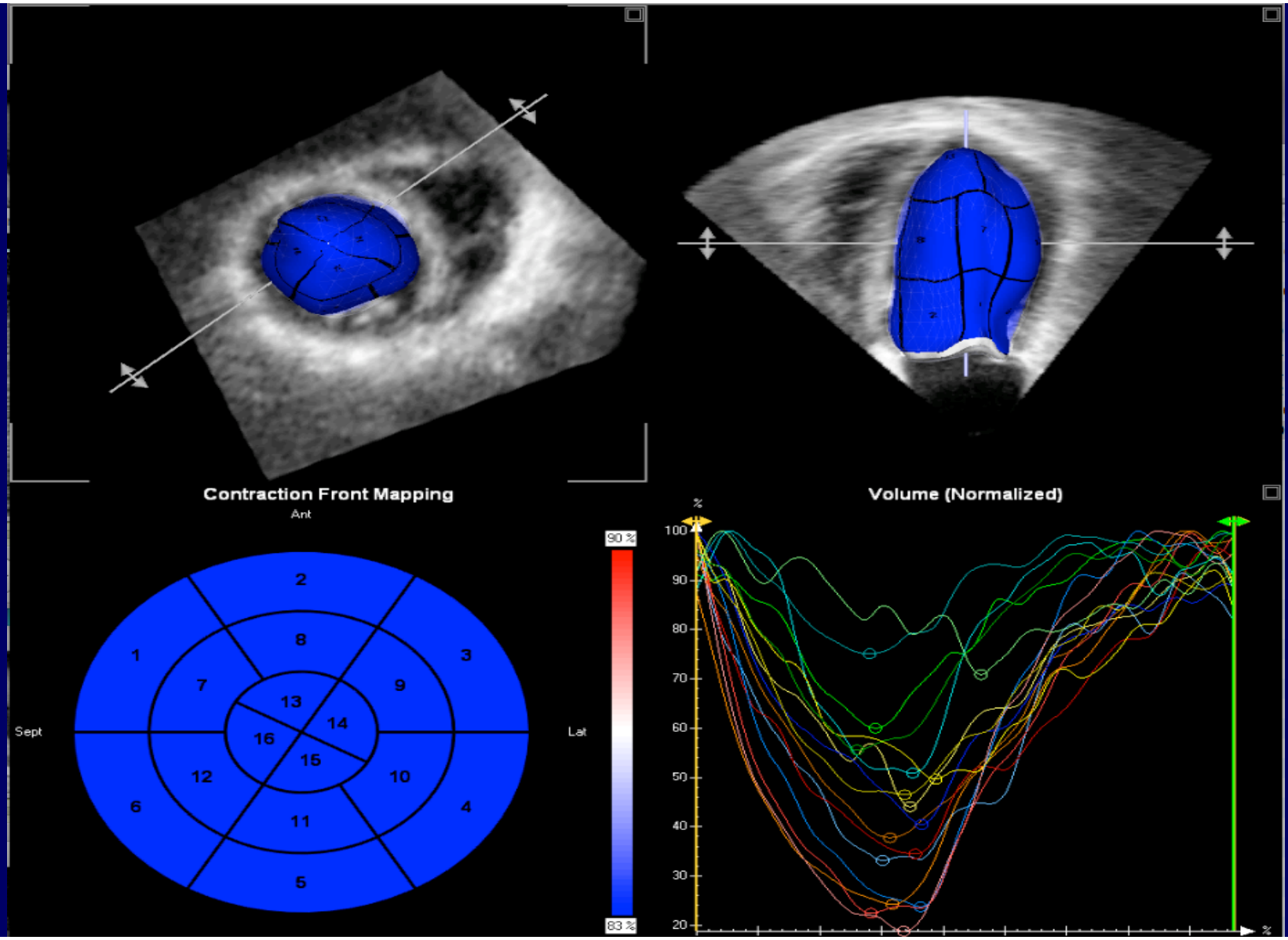



C3
C5
C7
A
B
10.00
-10.00
[mm]
3D Disp.
EDV 244.79 mL 0 msec
ESV 194.31 mL 406 msec
EF 20.62 %
1.05*MV 35.37 g est. LV MASS


+38.3
-32.4


Contraction Front Mapping
Ant
Sept
Lat
Inf
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
3 %
-3 %
Volume (Normalized)
%
100
90
80
70
60
50
40
30
20
0
10
20
30
40
50
60
70
80
%


Contraction Front Mapping
Ant
Sept
Lat
Inf
41 %
34 %
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Volume (Normalized)
%
100
90
80
70
60
50
40
30
20
0
10
20
30
40
50
60
70
80
%


Contraction Front Mapping
Ant
Sept
Lat
90 %
83 %
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Volume (Normalized)
%
100
90
80
70
60
50
40
30
20
%

Carotid Intimal Medial Thickness (IMT) and Carotid Atherosclerosis

Carotid IMT

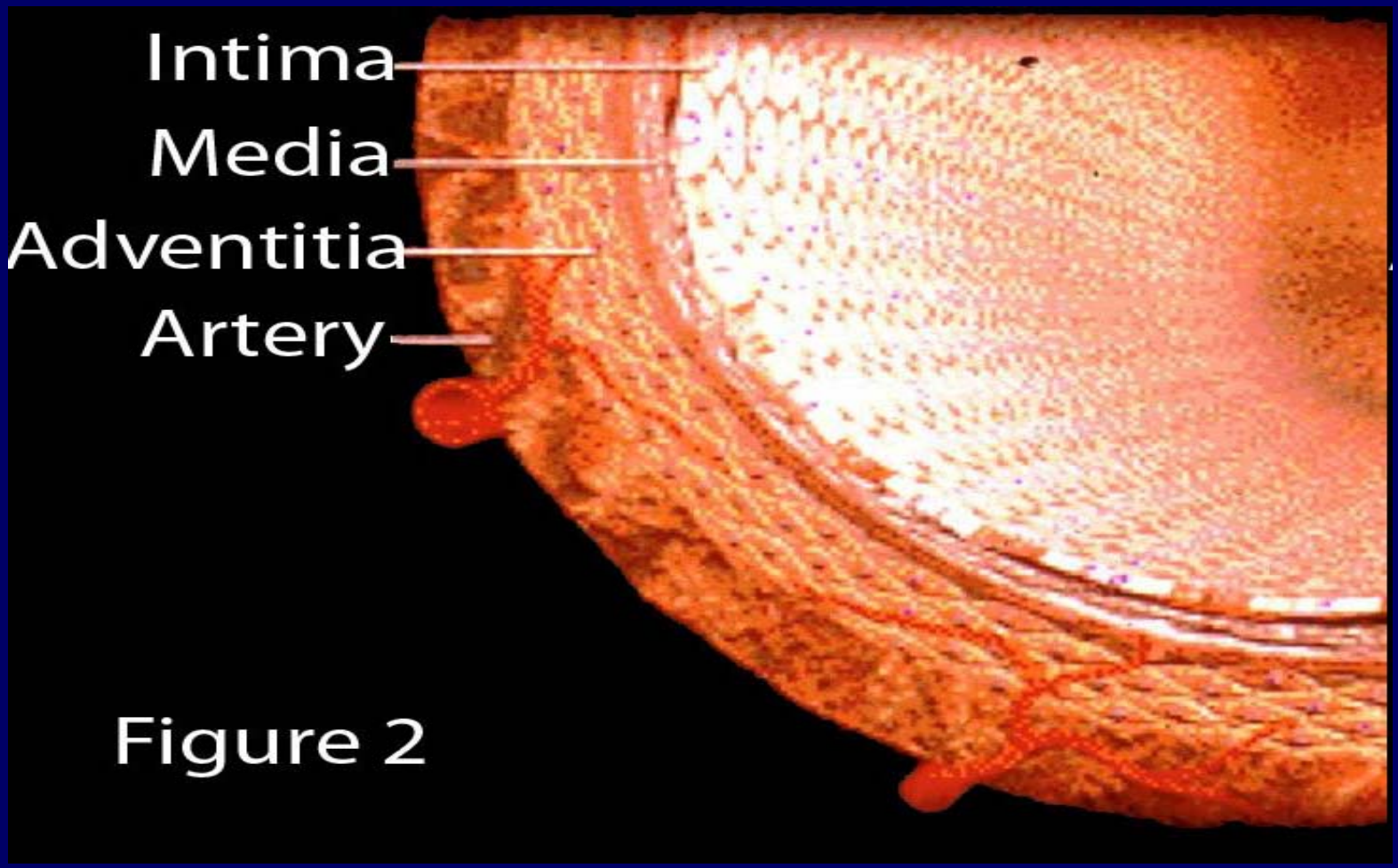
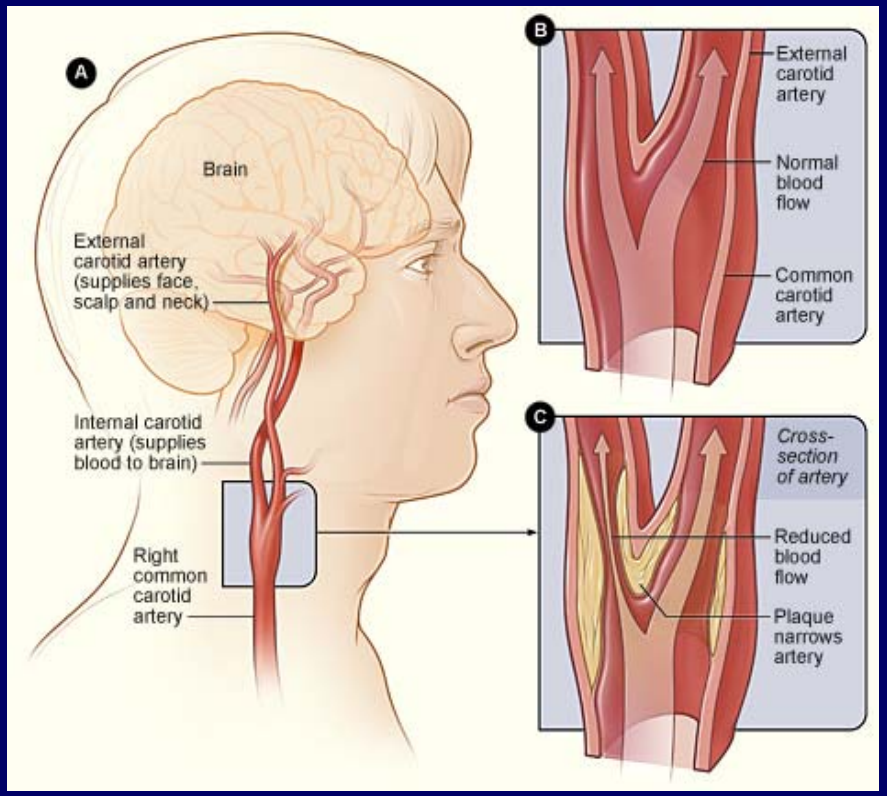


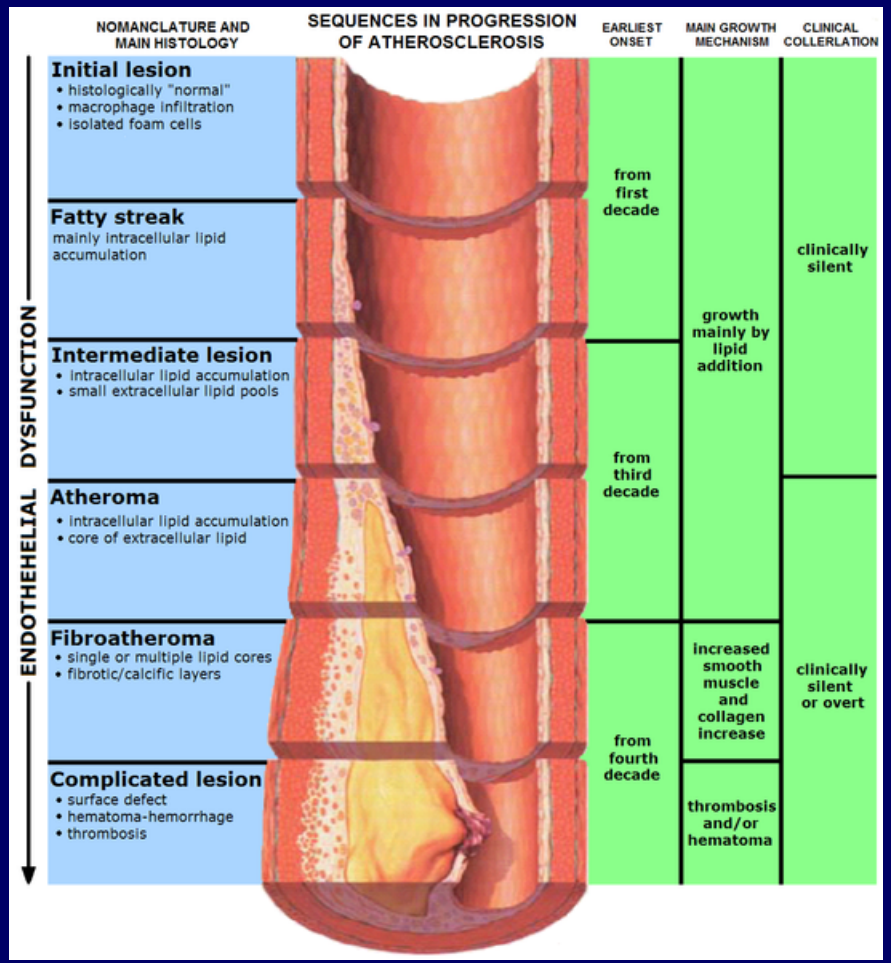
Figure 2

Carotid Artery Structure



This illustrates the structure of the carotid artery tree that is scanned in the IMT test. The measurement is taken in the common carotid artery which provides the best look at the degree of atherosclerosis.

Progression of Atherosclerosis



This illustrates the progression of disease that is seen when measuring IMT.

Plaque Assessment

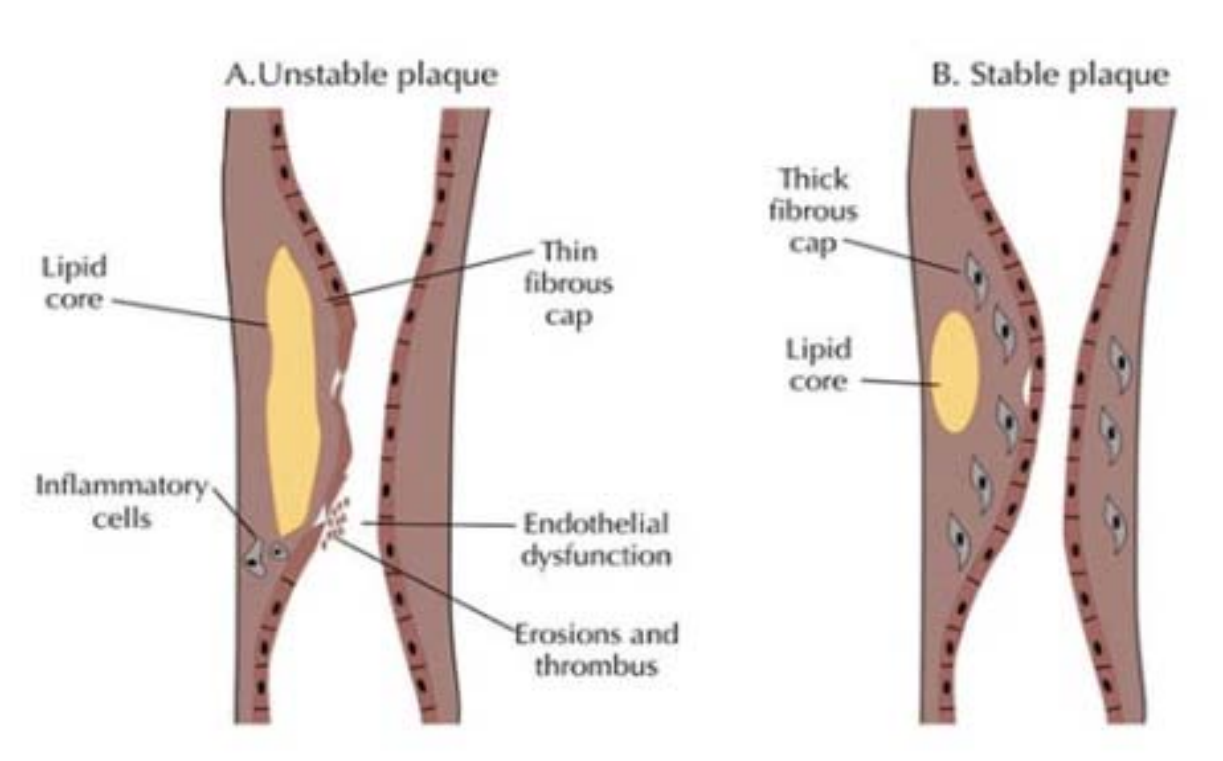
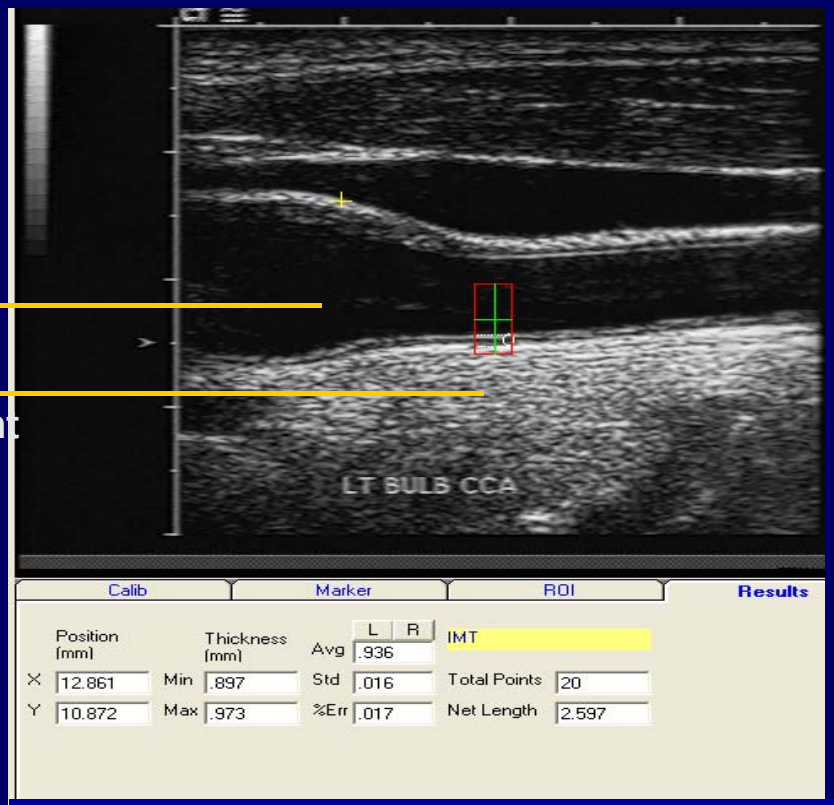


Image Measurement and Analysis



Left Common Carotid Artery

The beginning of the bulb is always on the left side

This shows the ideal view of the area used for analysis.

Note: The double-line seen at both the top and bottom show scan is “on-axis.”

Carotid IMT

Cerbrovasc Dis 2007;23:75
Curr Cardiol Rep 2009;11:21.
J of Hypertension 2012;30:1690

- **Normal values without any plaque present but must be adjusted for age and gender:**

- **Less than 0.6 mm : Normal low risk**
- **0.6 to 0.7mm : Moderate risk**
- **0.7 to 0.95 mm : High risk**

- **The normal IMT accretion rate (CIMTAR) is less than 0.016 mm / year.**

Carotid IMT

Cerbrovasc Dis 2007;23:75 Curr Cardiol Rep 2009;11:21.

- Carotid IMT reflects not only early atherosclerosis but also non-atherosclerotic compensatory enlargement with largely medial hypertrophy as a result of smooth muscle cell hyperplasia and fibrocellular hypertrophy
- Atherosclerosis, CVD, future CV events such as CHD MI ,TIA and stroke and correlates well with risk factors
- Carotid IMT and plaque have different natural history, patterns of risk factors and prediction of cardiac and cerebral events.
- Carotid IMT is most closely correlated with hypertension and ischemic CVA
- Carotid plaque is most closely correlated with hyperlipidemia and smoking
- Total plaque area is the most strongly predictive of CV risk of the ultrasound phenotypes

Carotid IMT and Future MI: Meta-analysis

Circulation 2007;115:459

- Age adjusted and sex adjusted overall estimates of relative risk for future events: 37,197 subjects
- Nonlinear risk, but linear models fitted relatively well for moderate to high IMT values

Myocardial Infarction

- 1.26 (95% CI 1.21-1.30) per one SD common carotid artery IMT difference
- 1.15 (95% CI 1.12-1.17) per 0.10 mm common carotid artery IMT difference over 5 years

Retinal Scans and ED

J of Hypertension 2012;30:1169 Am J Cardiol 2012;110:246
J of Hypertension 2014;32:2120; Am J Cardiol 2015;115:609

- Fundus examination of retinal arterioles with SLDF (scanning laser doppler flowmetry) correlates highly with micromyographic biopsies of the MLR in subcutaneous small arteries.
- Retinal pathology indicates microvascular disease even after adjustment of renal dysfunction and traditional CVD risk factors.
- Retinal microvascular endothelial dysfunction assessed with flicker light of retinal veins and arteries is a NO dependent phenomenon and predicts CHD.
- Correlates with hypertension and CVD.

Ocular Pulse Amplitude (OPA)
J of Am Acad Opthalmology 2012

- Reliably detects carotid artery stenosis by measuring inside the eye during systole and diastole and calculating the difference as the OPA.
- Low OPA means little difference between SBP and DBP indicating carotid artery stenosis.

Ankle Brachial Index

Diabetes Care. 2006;29:637-42
J Am Coll Cardiol 2008;52;1736
Ren Fail 2004;26: 433
Korean Circ J 2010;40:224
JAMA 2008;300:197

- Low ABI < 0.9 and PAD are associated with increased risk of CVD and CHD independent of the metabolic syndrome and other major CVD risk factors and predicts CKD
- 10 year CV mortality with ABI < 0.9 is 4 x greater than normal ABI.
- Improves CV risk prediction beyond Framingham Risk Score (FRS)

Ankle Brachial Index

Atherosclerosis, Thrombosis and Vascular Biology. 2005;25:1463
Blood Pressure 2010;19:308

- Meta-analysis of 22 studies 28,000 patients with low ABI outcomes
- CHD: 16.5 % sensitive, 92. 7% specific
- Stroke: 16% sensitive, 92.2 % specific
- Cardiovascular mortality: 41% sensitive and 87.9% specific
- Incidence of PAD in patients with previous CHD or CVA is 35%.

Post-exercise ABI predicts all-cause mortality

Am J Cardiol 2011;107:778

- Post exercise ABI is a powerful independent predictor for all-cause mortality and provides additional risk stratification beyond the ABI at rest.
- HR 1.67 with p <0.0001.
- Defined as ABI < 0.85.

24 Hour Ambulatory BP

- Dipping pattern
  Dippers (10-20 % difference) vs non dippers (0-10% difference in night and day BP)
  Excessive dipping increases ischemic CVA and reverse dipping increases ICH.
  Non dippers have increased platelet volume
  Reverse dipping (0 %) and extreme dipping (20%)
- Nocturnal BP drives the risk for CHD.
- AM BP surges increase CVA risk and MI risk and LVH (>20 mm Hg SBP) J of Hypertension 2017,35:1554
- BP Load of with over 15% BP readings > 140/90 mm Hg increases CVD
- BP variability and lability increases CV risk
- White coat hypertension increases CV risk
- Masked hypertension (also with higher glucose and MAU) 10 % incidence, M>F (J Clin Hypertension 2010;12 **Rev Cardiovasc Ther. 2010;8:260** 578.

Heart Rate Measurements

- Interconnections of CNS, PSNS, SNS and CV system
- Resting HR, heart rate variability (HRV) and heart rate recovery time (HRRT) post exercise
- Neurologic, biochemical, biophysical and EMF communications.
- Activation of PSNS reduces inflammation and improves HRV. Role of trained respiration
- Accupuncture increases PSNS activity

Resting Heart Rate

- Resting HR of 62 is ideal. For each increase of 4 beats/ min the risk of CHD death increases 7-10 %
- At 76 bpm the CHD risk is increased 68%
- Also increases risk for CVA

Nocturnal Heart Rate and CVD
J Hypertension 2014;32:1016

- A meantime heart rate over 66bpm is a good predictor of cardiovascular risk.
- Seen also more in women, young patients, hypertension, greater BMI, DM and smokers.

Heart Rate Variability: HRV

J of Hypertension 2014;32:374

Global Advances in Health and Medicine;2015;4 (1):46-61.

- Underactive vagal tone and PSNS will increase IL 6, TNF alpha, HS-CRP, fibrinogen, resting HR and alter HRV.
- Heart varies with respiration. This respiratory sinus arrhythmia (RSA) is normal and if decreased will increase CV morbidity and mortality. Neurologic, biochemical, biophysical and EMF communications.
- Activation of PSNS reduces inflammation and improves HRV. Role of trained respiration
- Abnormal HRV occurs with aging, hypertension, DM and CHF
- Acupuncture increases PSNS activity
- Increased dietary sodium intake adversely effects HRV, especially during mental stress. Also increases RHR

Heart Rate Recovery Time (HRRT)

Am J Cardiol 2012;110:45
Nutrients 2013; ISSN 2072-6643 EPBUB
AHJ 2018;199:163-69

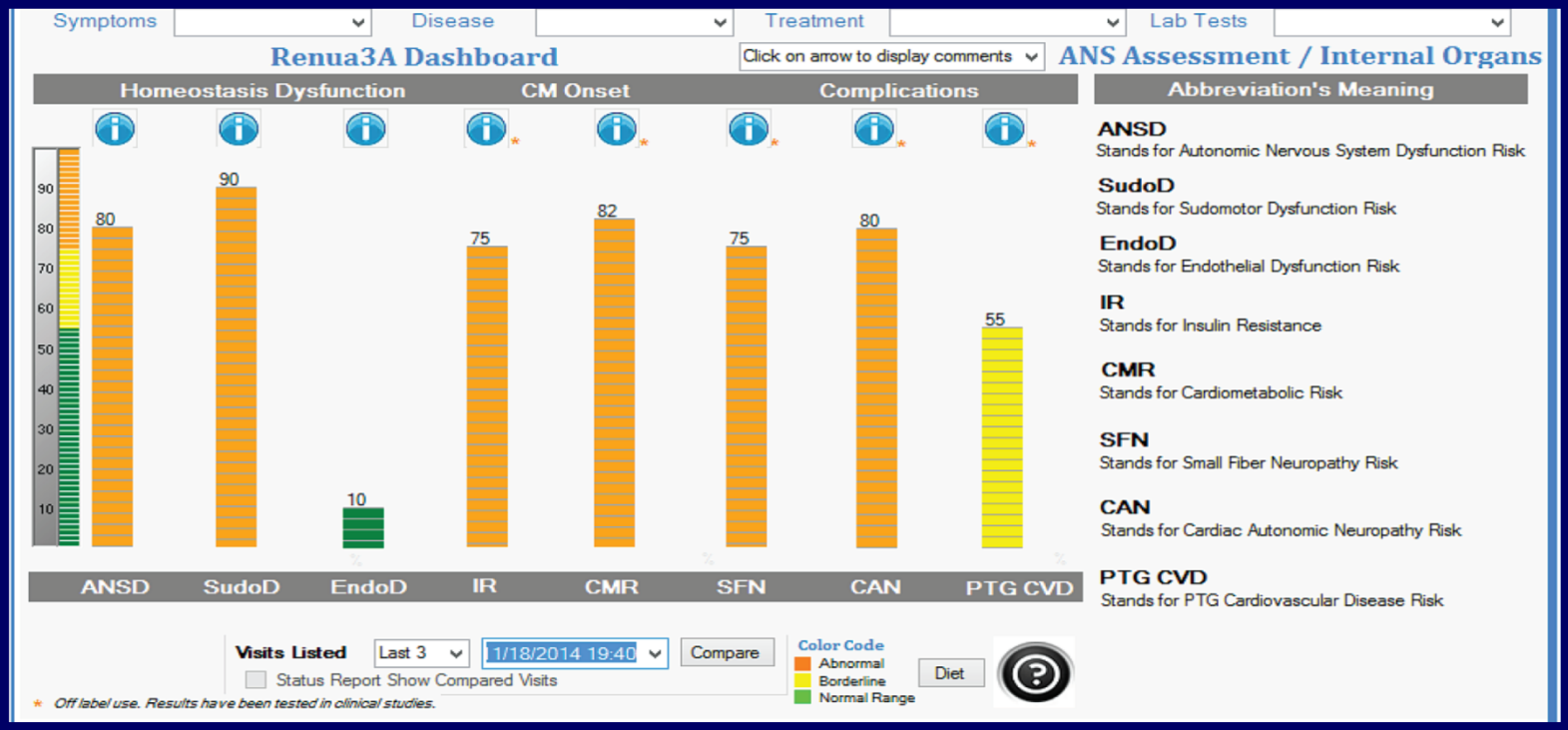
- Both early rapid 1 minute and late slower 5 minute recovery predict future CV events
- HR should decrease by at least 12-16 beats per minute after peak exercise.
- Early recovery at 1 and 2 minutes predicts sudden death and CVD. Reflects PNS reactivation post exercise. PNS activity disapates at 2 minutes then SNS is predominant.
- At 2 minutes if HRR is < 22 bpm the HR is 2.6 for SCD or CVD.
- By quartiles the 5 minute recovery showed reduction in 9 year death by about 5 % per quartile .
- Vitamin C levels over 28 umol/L improves HRRT 19% and increase physical activity 32% (both <0.001). Increases vagal tone releasing AcH in cardiac tissue.

Magnetocardiography (MCG)SQUID Superconducting Ouantum Interference Device

Ann Noninvasive Electrochardiol 2010;15(4):360
Circ J 2010;74(7):1424-30

- Multifunction Cardiogram (MCG) is a computational electrophysiologic system to detect abnormal stress and strain between the myocardium (visco-elastic solid) and intracardiac blood flow (non-Newtonian fluid at low and intermediate shearing states) from a two lead (II and V5) resting ECG.
- Detects myocardial ischemia in 82 second analysis
- Maps the heart's electrical activity to predict early CHD, ACS , MI and arrhythmias
- Sensitivity of 88% and specificity of 88% (range 80 to 100%) for early diagnosis of CHD depending on degree of stenosis.

Autonomic Function Testing



Body Composition Results

Region	Fat Mass (g)	Lean + BMC (g)	Total Mass (g)	% Fat
L. Arm	984	2066	3050	32.3
R. Arm	1094	2123	3217	34.0
Trunk	6750	20123	26874	25.1
L. Leg	2345	7055	9409	25.0
R. Leg	7258	9783		25.8

Normal for male <16% BF
Normal for female < 22 % BF

CHD Risk Scoring Analysis and Systems

CHD RISK SCORING: COSHEC
(Merged Framingham, PROCAM and INDANA Data Tables)

Houston MC et al Am J Medical Sciences 2005;329:276-291 and 292-305

Risk Factors: Men = 17, Women = 12

- Being male
- Age (years)
  Extra for cigarette smoking
- Systolic blood pressure (mm Hg)
- Total cholesterol conc. (mg / dL)
- LDL cholesterol (mg / dL)
- HDL cholesterol (mg / dL)
- Triglyceride (mg / dL)
- Height (inches)
- Creatinine conc. (mg / dL)
- Homocysteine (µmol / L)
- Prior MI
- Family history of MI pre- 60
- Prior Stroke
- LVH
- Diabetes
- Non-Diabetic, FBS (mg / dL)

COSHEC ABSOLUTE RISK ANALYSIS FOR DEATH FROM CHD IN 5 YEARS

Risk Score	% dying from cardiovascular disease in 5 years
0	0.04
5	0.07
10	0.11
15	0.19
20	0.31
25	0.51
30	0.84
35	1.4
40	2.3
45	3.7
50	6.1
55	9.8
60	15.6
65	24.5

COSHEC ABSOLUTE RISK CALCULATION

Houston MC et al Am J Medical Sciences 2005;329:276-291 and 292-305

- VERY LOW RISK: SCORE 0-10
- LOW RISK: SCORE 10-20
- MODERATE RISK SCORE 20-30
- MODERATE/HIGH SCORE 30-40
- HIGH RISK SCORE 40-50
- VERY HIGH RISK SCORE > 50
- NOTE TRIPLE RISK WITHIN EACH 10 POINT RISK SCORE

Rasmussen Center CV Scoring

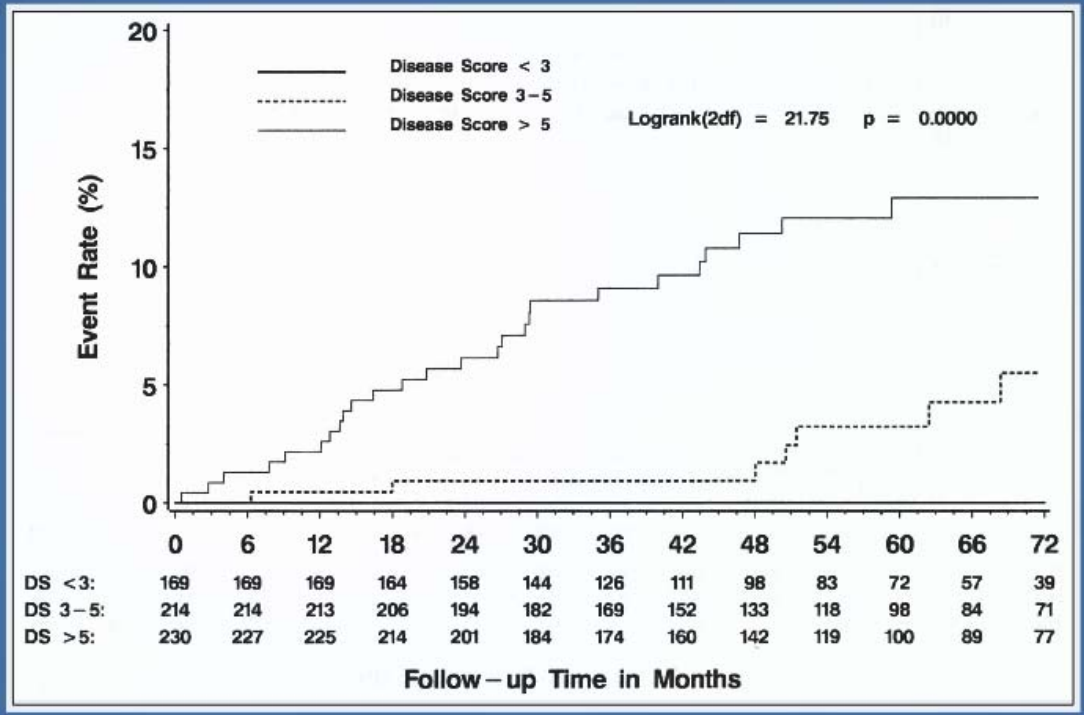
J Am Society of Hypertension 2011;5:401

- Disease score 0-2 : no CV events in 6 yrs
- Disease score 3-5: 5%CV events in 6 yrs
- Disease score over 6 : 15 % CV events in 6 yrs
- Superior to Framingham risk score
- Variables measured: CAPWA, BP at rest and exercise, LV mass by ECHO, microalbuminuria, BNP, retinal score, Carotid IMT and US, EKG .

Rasmussen Center CV Scoring

J Am Society of Hypertension 2011;5:401

Test	Normal	Borderline	Abnormal
Score for each test	0	1	2
Large artery elasticity		(age- and gender-dependent)	
Small artery elasticity		(age- and gender-dependent)	
Resting BP (mm Hg)	SBP <130 and DBP <85	SBP 130–139 or DBP 85–89	SBP ≥140 or DBP ≥90
Treadmill exercise BP (mm Hg)	SBP increase <30 and SBP ≤169	SBP increase 30–39 or SBP 170–179	SBP increase ≥40 or SBP ≥180
Optic fundus photography retinal vasculature	A/V ratio >3:5	A/V ratio ≤3:5 or mild A/V crossing changes	A/V ratio ≤1.2 or A/V nicking
Carotid IMT		(age- and gender-dependent)	
Microalbuminuria (mg/mmol)	≤0.6	0.61–0.99	≥1.00
Electrocardiogram	No abnormalities	Nonspecific abnormality	Diagnostic abnormality
LV ultrasound LVMI (g/m^2)	<120	120–129	≥130
NT-proBNP (pg/dl)	<150	150–250	>250



Kaplan-Meier curves of time morbid events during 6 years of follow-up in the three Rasmussen Disease Score (DS) Groups. The difference among the curves (P = .0000) is highly significant. Two events after 72 months are not depicted.

Credit: D. A. Duprez et al. / Journal of the American Society of Hypertension 5(5) (2011) 401 - 409

CHAN2T3 CHD Risk Score

Am Heart J 2017;193:95

Risk Factors

- HS-CRP
- Homocysteine
- Albuminuria
- N terminal prohormone of BNP
- Troponin-T

Ten year risk of CHD event per risk factor above

0 = 2.09 %

1 = 4.16 %

2 = 6.09 %

3 = 6.95 %

4= 10.22 %

5= 25 %

Comparison of Novel Risk Markers to Improve CV Risk Assessment

JAMA 2012;308:788

- **6814 participants in MESA study evaluating CAC, Carotid IMT, ABI, BFMD, HS-CRP and FH of CHD compared to the FRS.**

Results in multivariable analysis:

- **CAC: HR 2.60 (p<0.001) : BEST (also best in Rotterdam study, Kavousi study and others)**
- **FH: HR 2.18 (p<0.001)**
- **HS CRP: HR 1.28 (p< 0.05)**
- **Carotid IMT: HR 1.17 (p=.13)**
- **BFMD: HR .93 (p=.52)**
- **ABI: HR .79 (p= .01)**

The Microbiome and CVD

Proc Nutr Soc.2014 ;73(2):172-85. **Dig Dis 2013;31:278; Mayo Clinic Pro 2014;89:107;Br J Nutrition 2012;017:1505;Eur J Clin Nutr 2012; 66:1234; J AM Coll Cardiol 2005;45:185 International Journal of Cardiology 2015;179: 348,** Hypertension. 2014;64(4):897-903**,**

- The human gut microbiota is a possible novel CVD risk factor.
- The flux of metabolites derived from gut microbial metabolism of choline, phosphatidylcholine and l-carnitine contributes to CVD pathology, providing one explanation for increased disease risk of eating too much red meat.
- Diet with fermentable fibers, prebiotics, probiotics and plant polyphenols favorably regulates microbial activities within the gut.
- **Some selected probiotic strains show that ingestion of viable microorganisms with the ability to hydrolyze bile salts can improve serum lipids, BP, DM ,alter metabolites and may improve CVD risk.**
- **Increases in Pseudomaoadaceae (Gammaproteobacteria of Proteobacteria) in CVD patients. Firmicutes (Staphylococcaceae of Firmicutes) species are lower in CVD patients. CHD plaque also has higher ratio of Pseudomaoadaceae to Firmicutes bacteria and DNA.**
- **L. reuteri improves lipids**
- **Saccharomyces boulardii increases EF by 20%(1000 mg /d) in CHF.**
- **Some probiotics reduce blood pressure**

Lab Testing

- CBC with differiental
- Urinalysis
- Complete Metabolic Profile (CMP 12)
- Free T4, Free T3, TSH, RT3, thyroid antibodies
- Magnesium RBC
- Iron, TIBC and Ferritin
- Fibrinogen
- HsCRP
- Homocysteine
- Uric acid
- Microalbuminuria
- GGTP and hepatic profile
- Myeloperoxidase (MPO)
- Plasma viscosity
- IGF-1

Lab Testing

- Advanced Lipid Testing with oxLDL and reverse cholesterol transport(RCT)
- Micronutrient Testing (MNT)
- PRA and Aldosterone
- F2 isoprostanes
- Galectin 3
- LpPLA2
- B12
- Folate
- BNP and ProBNP
- Dysglycemia labs: adiponectin, leptin, FBS, 2hr GTT, insulin, proinsulin, C-peptide, A1C, HOMA IR
- Markers for inflammation, oxidative stress and immune function
- Thrombosis markers
- Renal function markers: CrCl, MAU, Cystatin C, SDMA
- Toxicology, heavy metals, POPs screen
- Omega 3 index
- Telomere test
- Gluten testing
- ADMA and SDMA
- TMAO
- Vitamin D 3
- Hormone Profile: Free testosterone, SHBG, estradiol, estriol,progesterone, DHEA and DHEAS
- PTH

Questions, Answers and Rationale

1. Genomics and Nutrigenomics of the CV system allow improved identification of patients at risk for CHD. True or False> Answer is True

Rationale: CV genetics is crucial to provide precision and personalized medicine

2. What does the CAPWA analysis provide?

   a. DM risk

   b. Lipids

   c. Arterial Compliance

   d. Risk for future CV events

   e. Both C and D.

Answer is E

Rationale: It measures both small and large arterial compliance to predict future CHD, MI and stroke.

Questions, Answers and Rationale

3. The EndoPAT provides information on:

a. Endothelial function

b. Augmentation index

c. Heart rate variability

d. All the above

Answer D

Rationale: All three of these tests can be done at the same time

4. The resting ECHO provides information on ejection fraction. T or F

Answer is T

Rationale: The ejection fraction measures cardiac contractility and risk for CHF

Questions, Answers and Rationale

5. The most common abnormality in metabolomics for CHD is
    a. Abnormal acylcarnitines
    b. Abnormal LDL composition
    c. Low taurine levels
    d. Low COQ10 levels

Answer: A

Rationale: Mitochondrial dysfunction allows for increase acylcarnitines

6. The normal body fat for a male is 22 %. T or F Answer F

Rationale. Males BF should be 16 % or less

Questions, Answers and Rationale

7. The CPET provides information on

- **a. BP response to exercise**
- **b. Pulmonary Function**
- **c. Stress TMT**
- **d. HRRT**
- **e. All the above**

Answer: E

Rationale: CPET allows measurement of all the parameters during the test

Questions, Answers and Rationale

8. The coronary artery calcium score provides predictive risk best for

a. Stroke

b. MI

c. CHF

d. PAD

Answer: B

Rationale: Calcium in the coronary arteries has on of the highest predictions for future MI.

Questions, Answers and Rationale

9. Evaluation of the microbiome can predict

a. **Dyslipidemia**
b. **Hypertension**
c. **CHD**
d. **Diabetes Mellitus**
e. **All the above**

Answer E

Rationale: Clinical trials indicate a vast array of CV connections to the gut

Questions, Answers and Rationale

10. The CHD risk scoring systems which indicate high risk for CHD are

a. COSHEC with a score over 40

b. Rasmussen with a score over 6

c. Corus GET with a score below 10 and a PULS score below 1.0

d. A and B

Answer is D

Rationale: higher scores of A and B indicate greater risk for CHD. Lower CORUS and PULS scores indicate at lower risk

11. Nocturnal BP drives the risk for CHD. T or F Answer T

Rationale: Higher BP at night increases arterial damage and predicts CHD better than the daytime BP