Endothelial Function and Cardiovascular Prognosis

September 26, 2013

3rd Dubrovnik Cardiology Highlights

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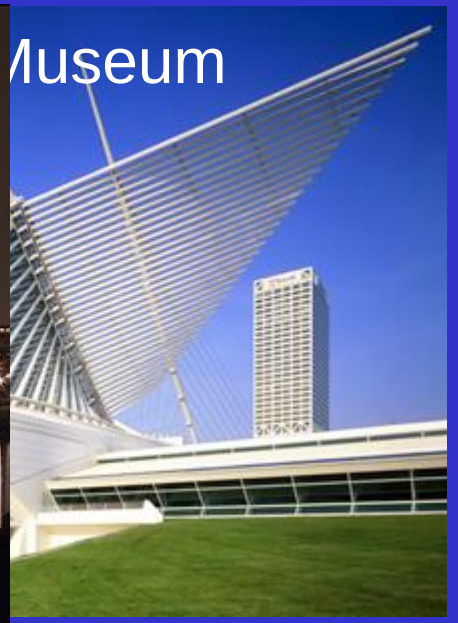
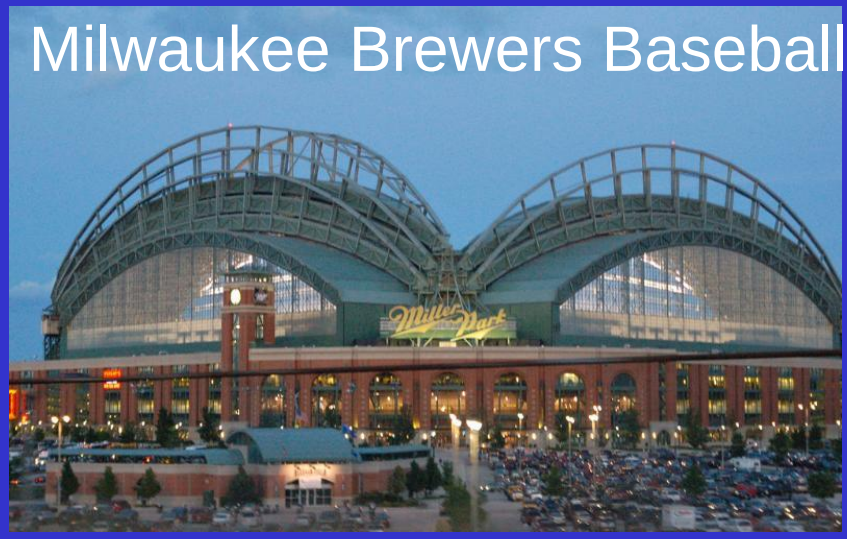


Green Bay Packers
World Football Champions, 2011



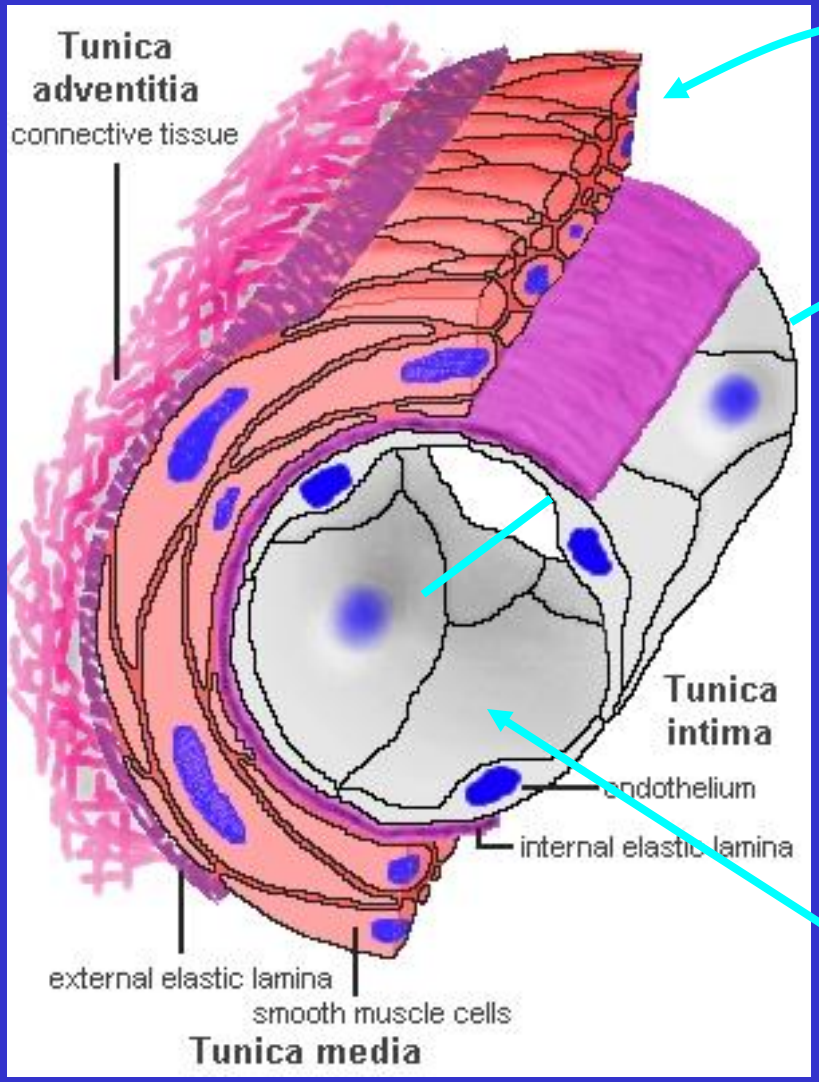
Role of the Endothelium in Managing Cardiovascular Risk

1. Detail translational research findings that showed the potential clinical importance of endothelial function

2. Show clinical examples of how understanding endothelial function can alter patient care.

3. Propose new therapies aimed at preventive detection of, and therapy for, atherosclerosis.

Vascular Structure-Function Relationship

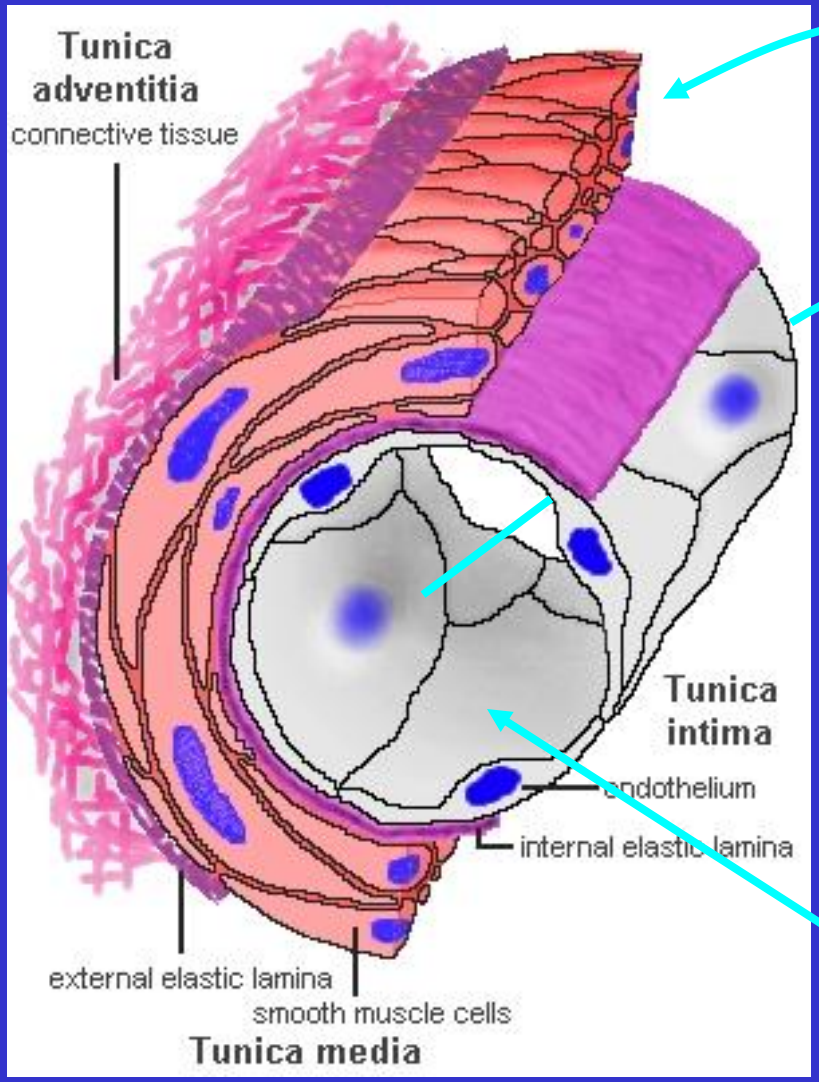


EDRF

1980: Furchgott identified the critical role of endothelium in vasodilation

- Shear Stress from normal blood flow
- Acetylcholine
- Bradykinin

Vascular Structure-Function Relationship



NO

- Shear Stress from normal blood flow
- Acetylcholine
- Bradykinin

1980: Furchgott identified the critical role of endothelium in vasodilation

1990: Furchgott and others showed that the dilator compound released from endothelium is Nitric Oxide (NO)

1998: Nobel Prize awarded to Furchgott, Ignarro, and Murad

Physiological Properties of NO

- vasodilation
- inhibit platelet aggregation
- inhibit leukocyte adhesion to endothelial cells
- anti-inflammatory: prevent white cell margination
- prevent smooth muscle proliferation and intimal migration
- inhibit the oxidation of LDL cholesterol
- induce apoptosis of smooth muscle cells
- inhibits cell adhesion molecule expression

Each of these properties is antiatherogenic in coronary and systemic vessels

NO Hypothesis for Atherosclerosis

- Nitric oxide released from the endothelium maintains vascular integrity and prevents the development of atherosclerosis

- Corollary: Arteries would become atherosclerotic if not for nitric oxide being released from the endothelium

Testing the NO Hypothesis

Must show that:

1. **Risk factors for cardiovascular disease are associated with endothelial dysfunction and loss of NO**

2. **Endothelial dysfunction precedes atherosclerosis**

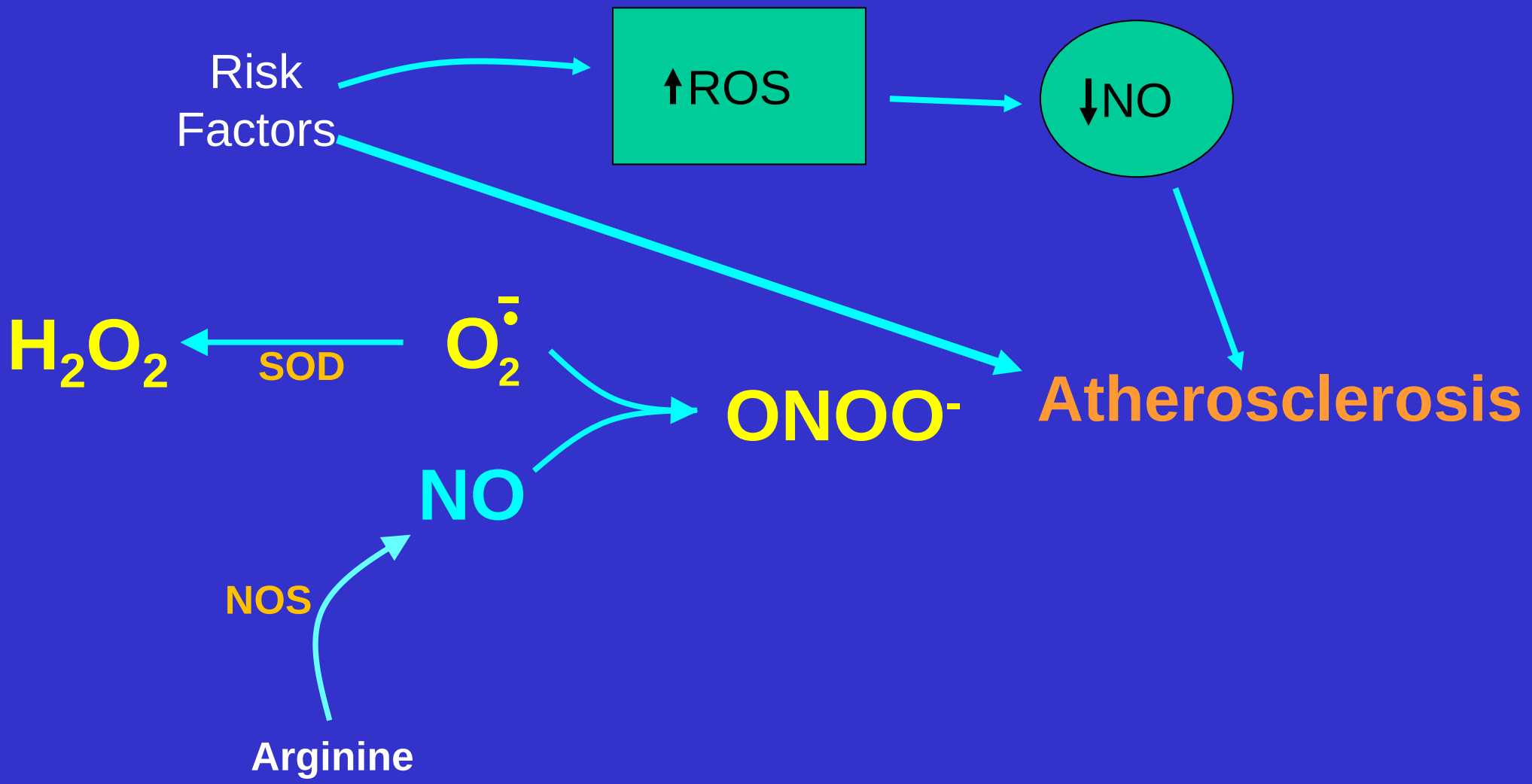
3. **Improving endothelial function and restoring NO production prevents or reduces atherosclerosis**

4. **Endothelial dysfunction portends bad prognosis in humans**

Risk Factors and Endothelial Function

	Risk for CAD	Endothelial Dysfunction	Loss of NO
Diabetes	✓	✓	✓
HTN	✓	✓	✓
Hypercholesterolemia	✓	✓	✓
Family History of CAD	✓	✓	✓
Tobacco Use	✓	✓	✓
Male Gender	✓	✓	✓
Menopause	✓	✓	✓
Mental Stress	✓	✓	✓
Hyperhomocysteinemia	✓	✓	✓

Common Link between Risk Factors and NO



Testing the NO Hypothesis

Must show that:

1. ✓ Endothelial dysfunction with loss of NO is associated with risk factors for cardiovascular disease
2. Endothelial dysfunction leads to atherosclerosis
3. Improving endothelial function and restoring NO production leads to improved outcomes
4. Abnormal endothelial function portends bad prognosis

Endothelial Dysfunction Leads to Atherosclerosis

	normal	↑Chol	Non-Obst.	Obst. CAD
Epicard. Anat: Dilator				
Ach	↑↑	↓↓	↓↓	↓↓
Cold Pressor	↑↑	↑↑	↓↓	↓↓
FMD	↑↑	↑↑	↓↓	↓↓
	N=11	N=9	N=9	N=9

- 38 patients

- from Zeiher, Drexler et al., Circulation, 1991

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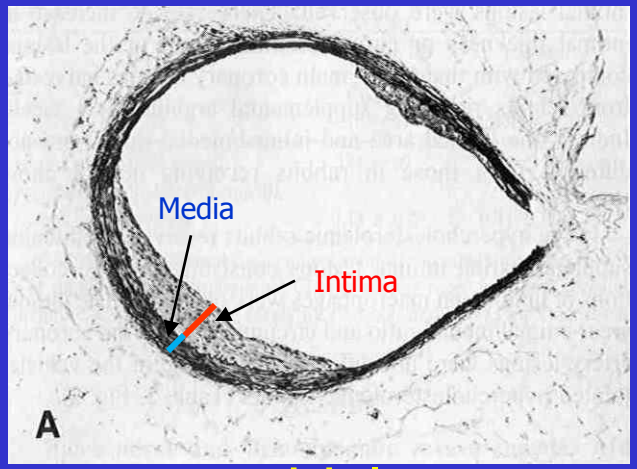
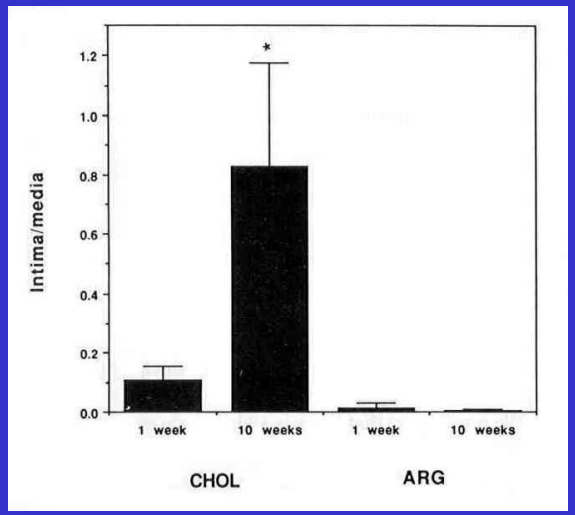
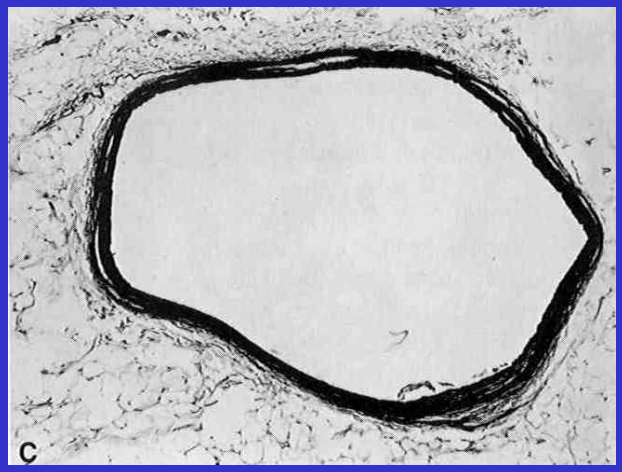
Reversal of Atherosclerosis in Hypercholesterolemia



vehicle





L-arginine

Treat the pathology of hypercholesterolemia without changing the cholesterol!

- Male rabbits
- 10 weeks HC diet
- Aortic histomorphometry

- Cooke, et al. JCI, 1992

Testing the NO Hypothesis

Must show that:

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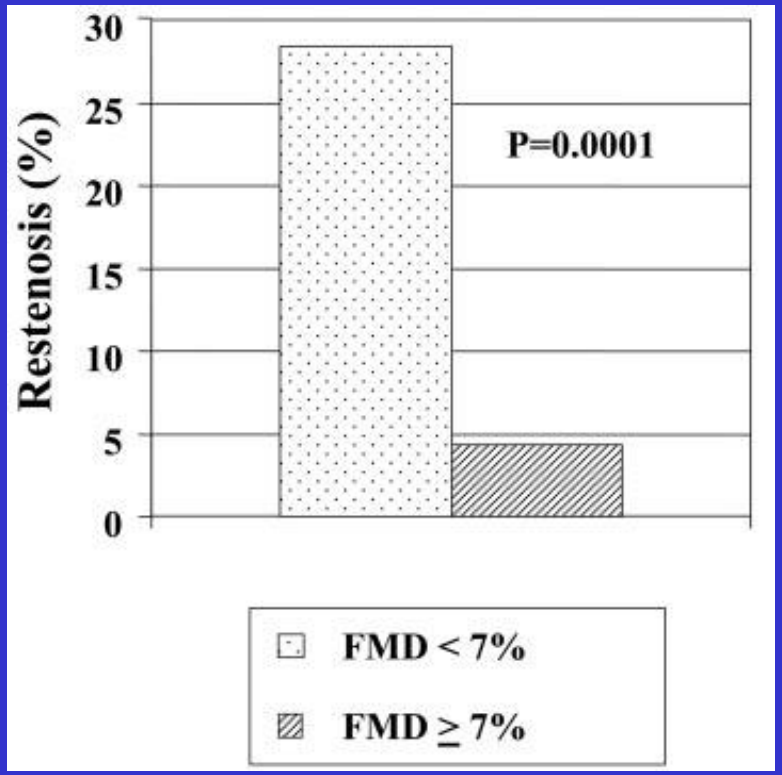
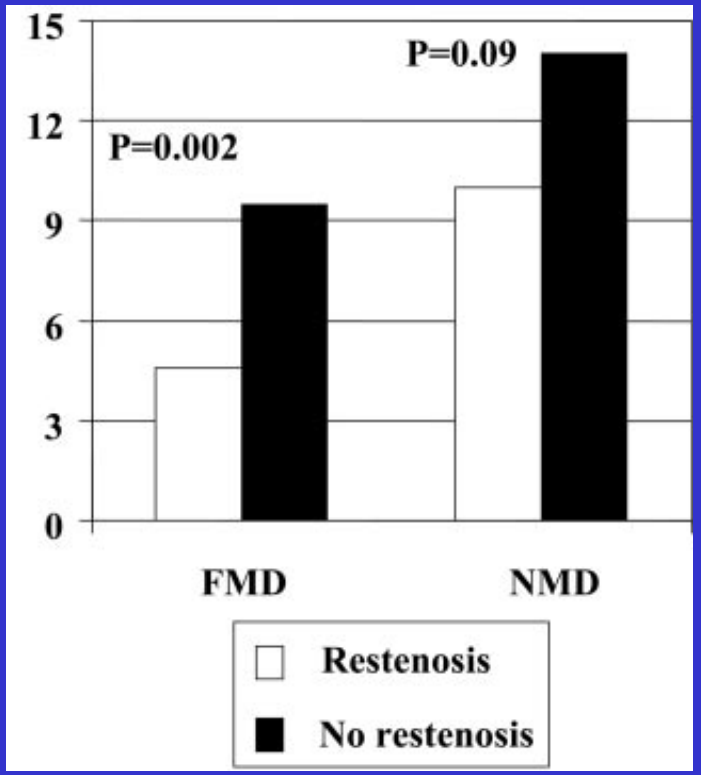
In Vivo Measurement of Endothelial Function

Prognostic Importance of FMD in PCI Patients



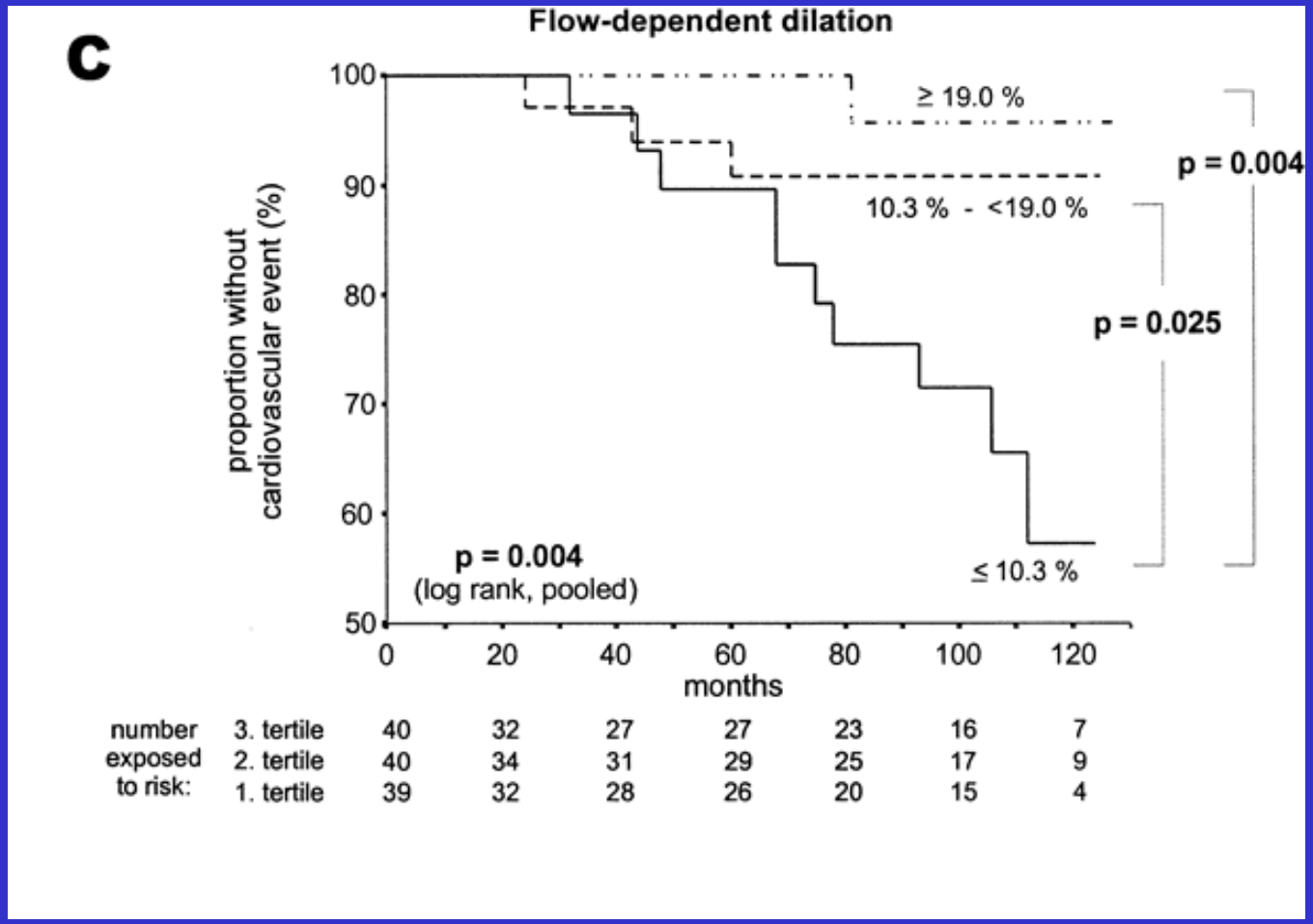


Patients: - 136 following PCI for single vessel disease
- FMD 30 days after PCI
- 6 mo. f/u

FMD was the strongest predictor of restenosis (OR 4.5) by multivariate analysis

Patti et al. Circ. 2005

Prognosis and Endothelial Function

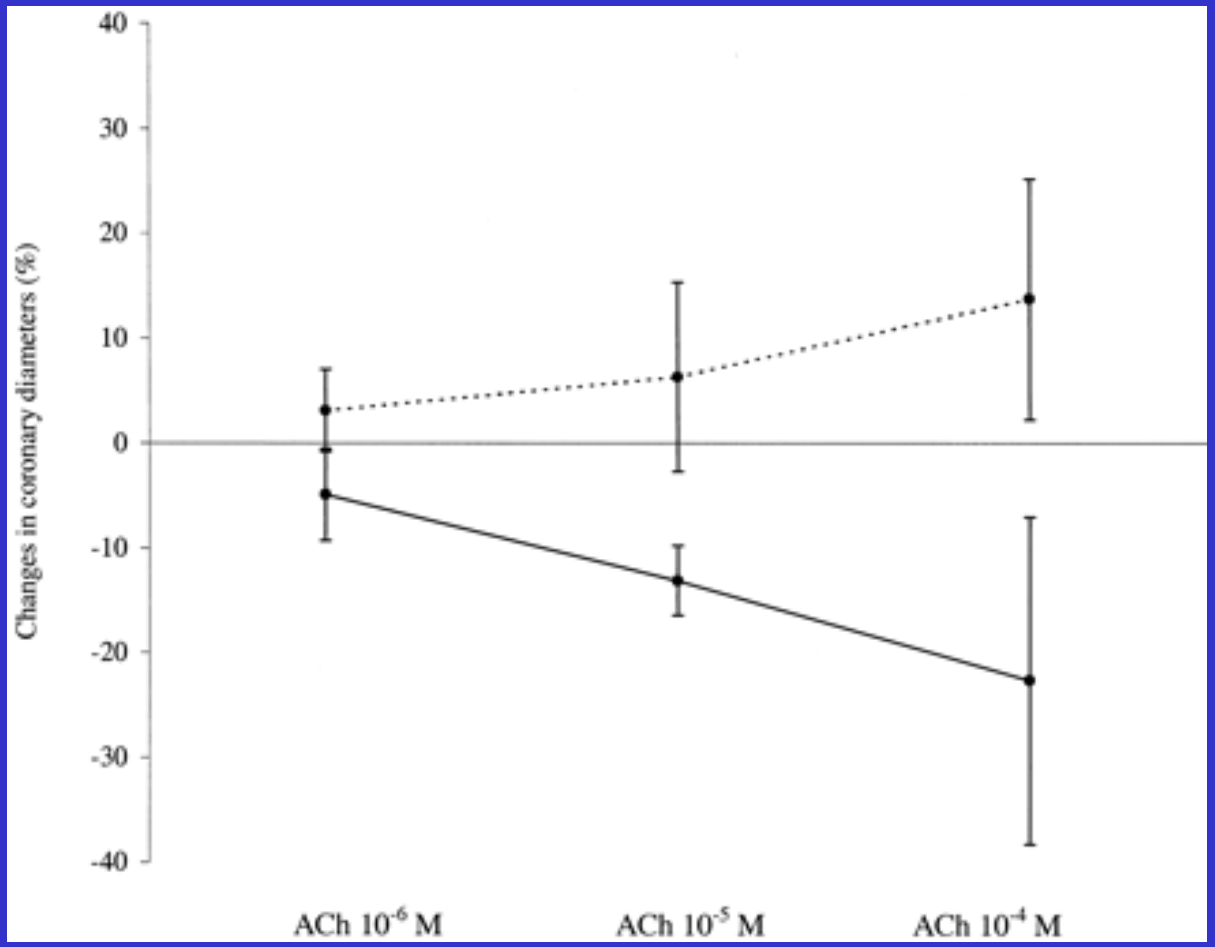


- 147 consecutive patients with cath
- -measured coronary endothelial function
- 7.7 year follow-up for cardiovascular events (MI, UA, death, PCI, CABG, CVA)

- Endothelial function was an INDEPENDENT predictor of prognosis

- Schachinger, et al. Circulation, 2000

Prognosis and Endothelial Function



-42 women with chest pain + SPECT scans and normal coronary arteries at cath.

- ~Half showed coronary constriction to Ach and ~½ showed dilation at baseline.

-Followed for 10 years with repeat angiogram.

-<u>At 10 year follow-up:</u>
-None that originally dilated to Ach developed CAD.
-13/17 who originally constricted to Ach developed angiographic CAD.

- Bugiardini, et al. Circulation, 2004

Testing the NO Hypothesis

Must show that:

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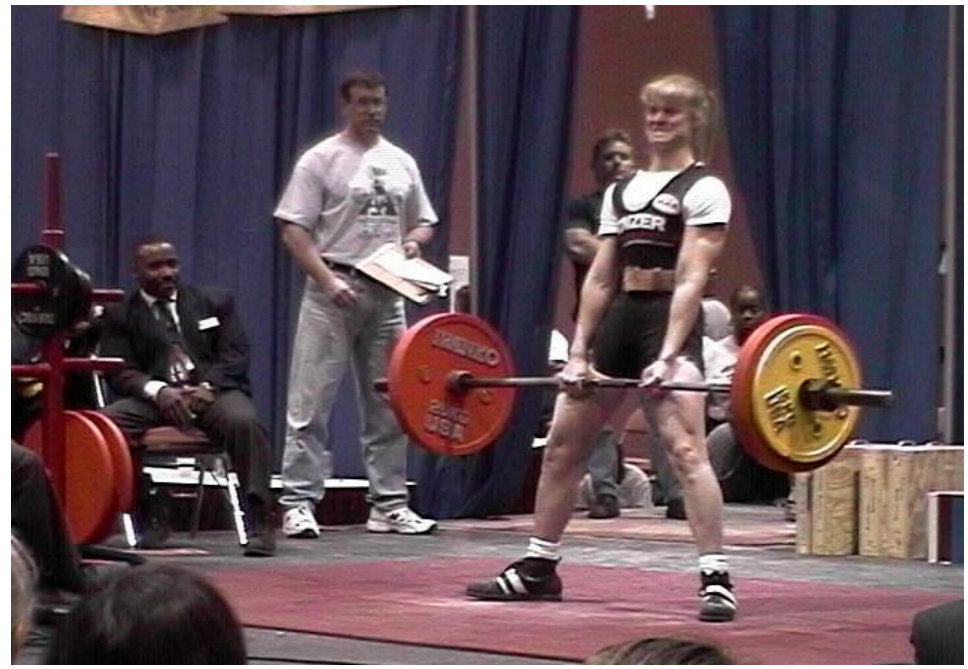
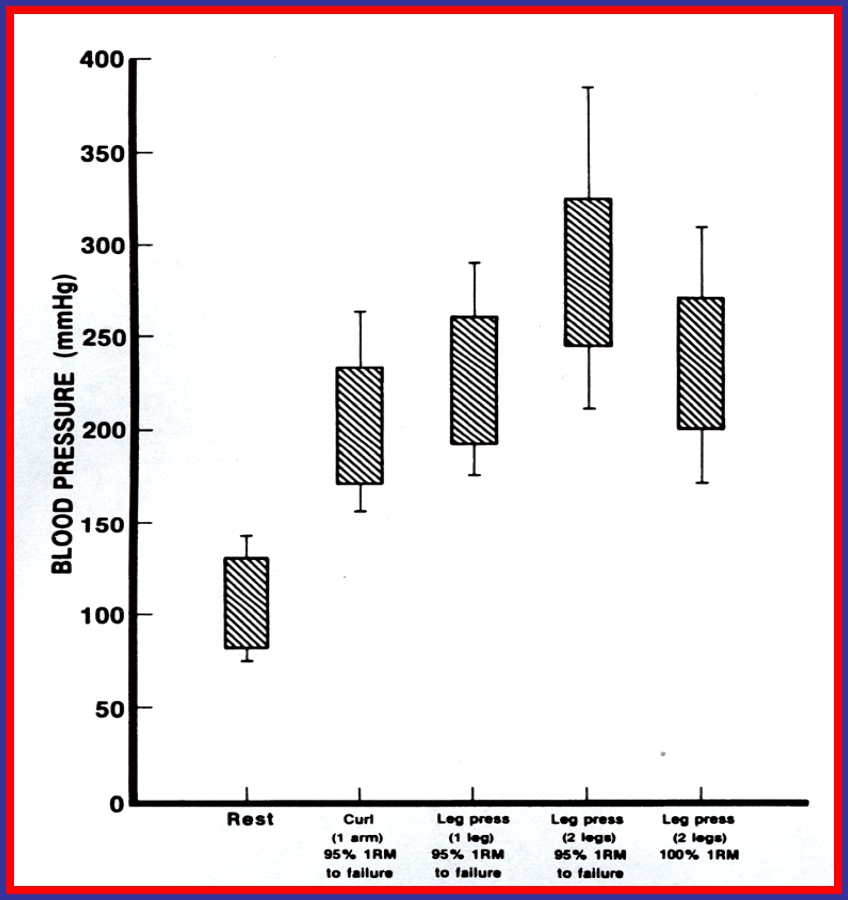
Are All Forms of Exercise Healthy?

- It has long been known that aerobic exercise is healthy.
- What about resistance exercise like weightlifting?



Arterial Blood Pressure Responses to Heavy Resistance Exercise

- Leg Press Exercises
- Peak blood pressure 480/350 mmHg



MacDougall et al. J Appl. Phys. 1985.

Hypothesis

Acute hypertension associated with weight lifting impairs vascular endothelium-dependent dilation in humans.

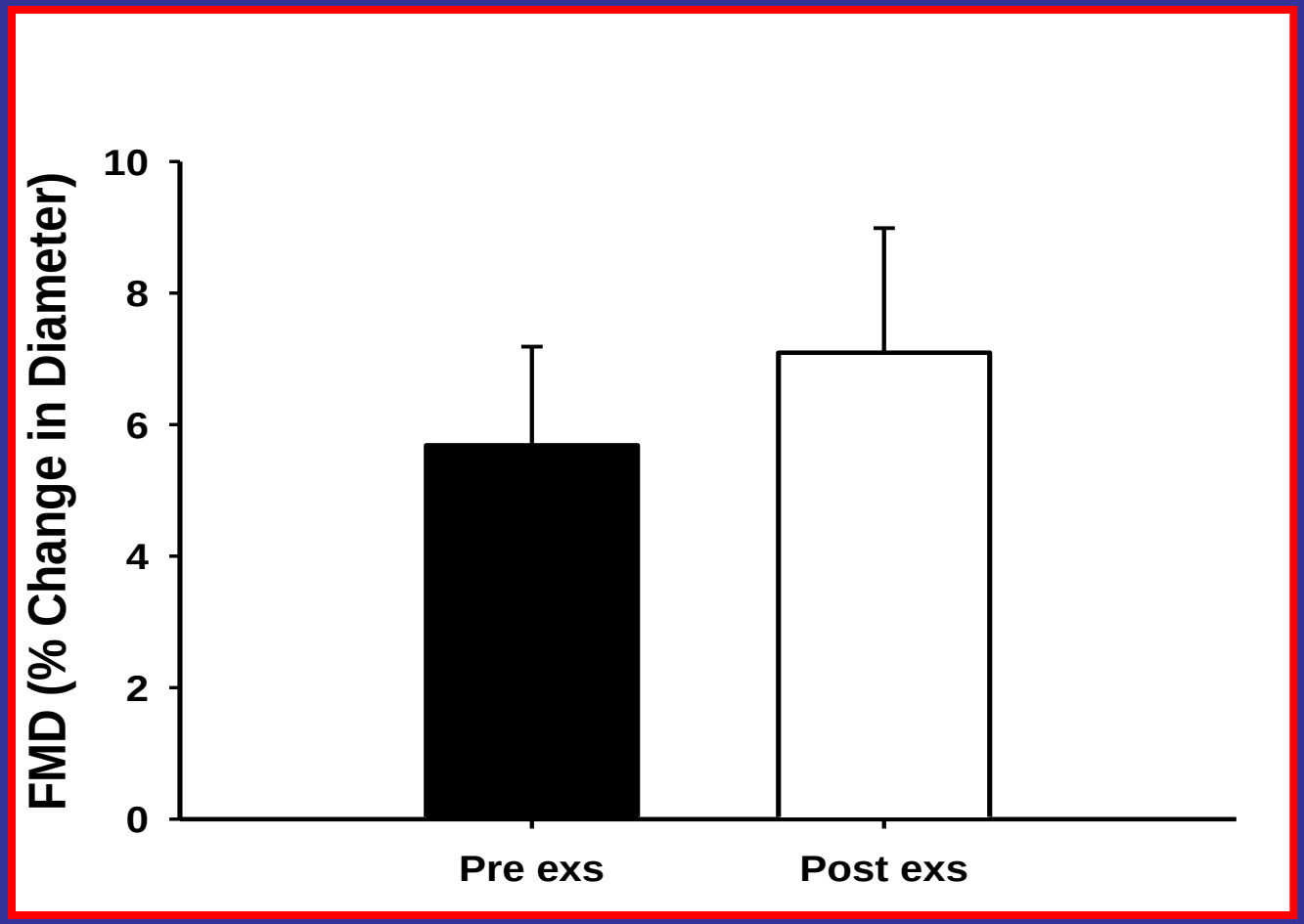
Subject Characteristics

Age (years)	28 ± 2
Sex	5 female, 9 male
BMI	27 ± 2
Resting SBP (mmHg)	124± 4
Brachial Artery Diameter (mm)	4.2 ± 0.2
Baseline FMD (%)	5.6 ± 1.5
Max Pounds Lifted	439 ± 57
Peak Exercise SBP	213 ± 8

Protocol

FMD → Exercise → FMD

Effect of Weight Lifting on Flow Mediated Dilation in Conditioned Weight Trainers



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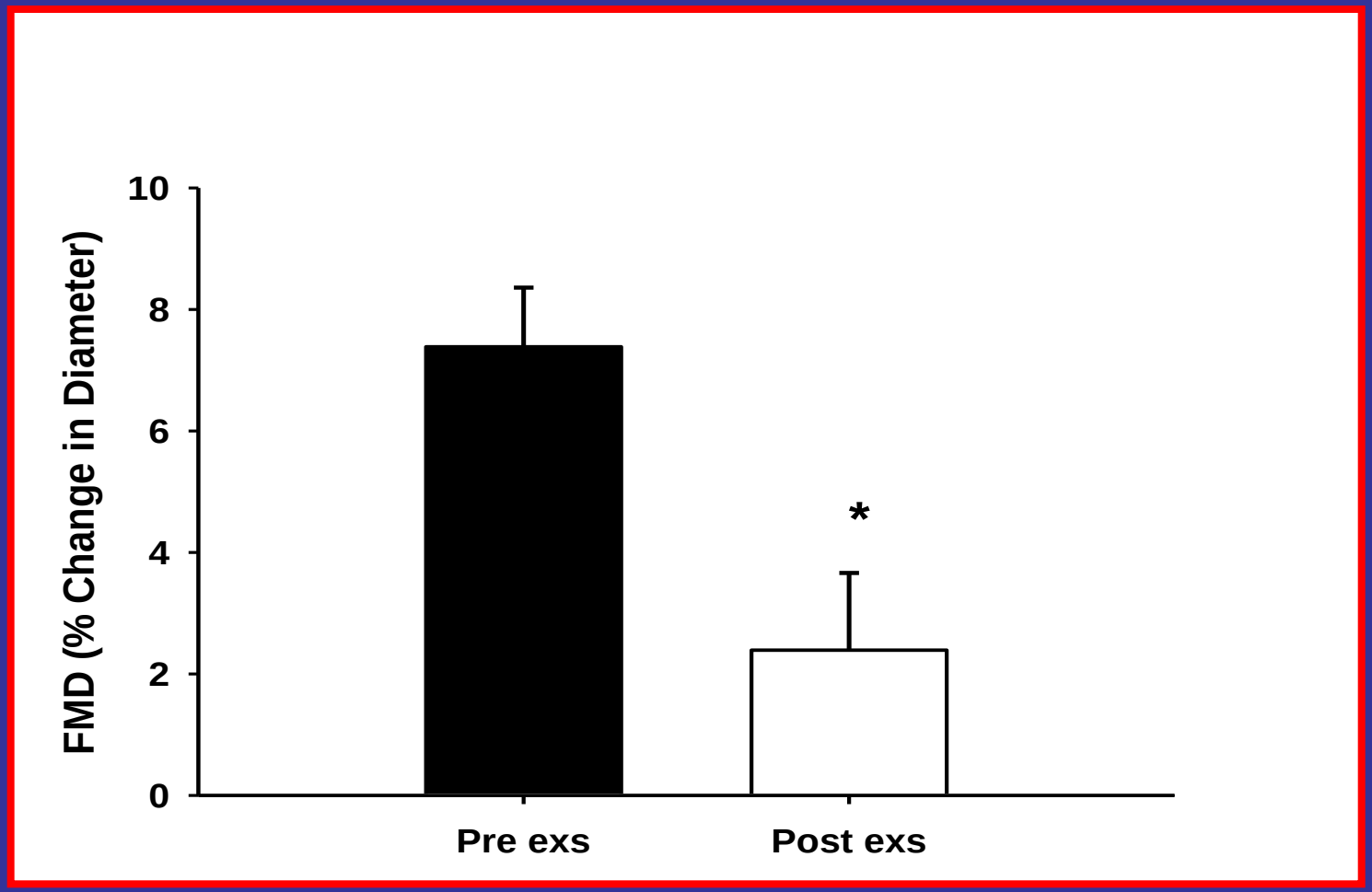
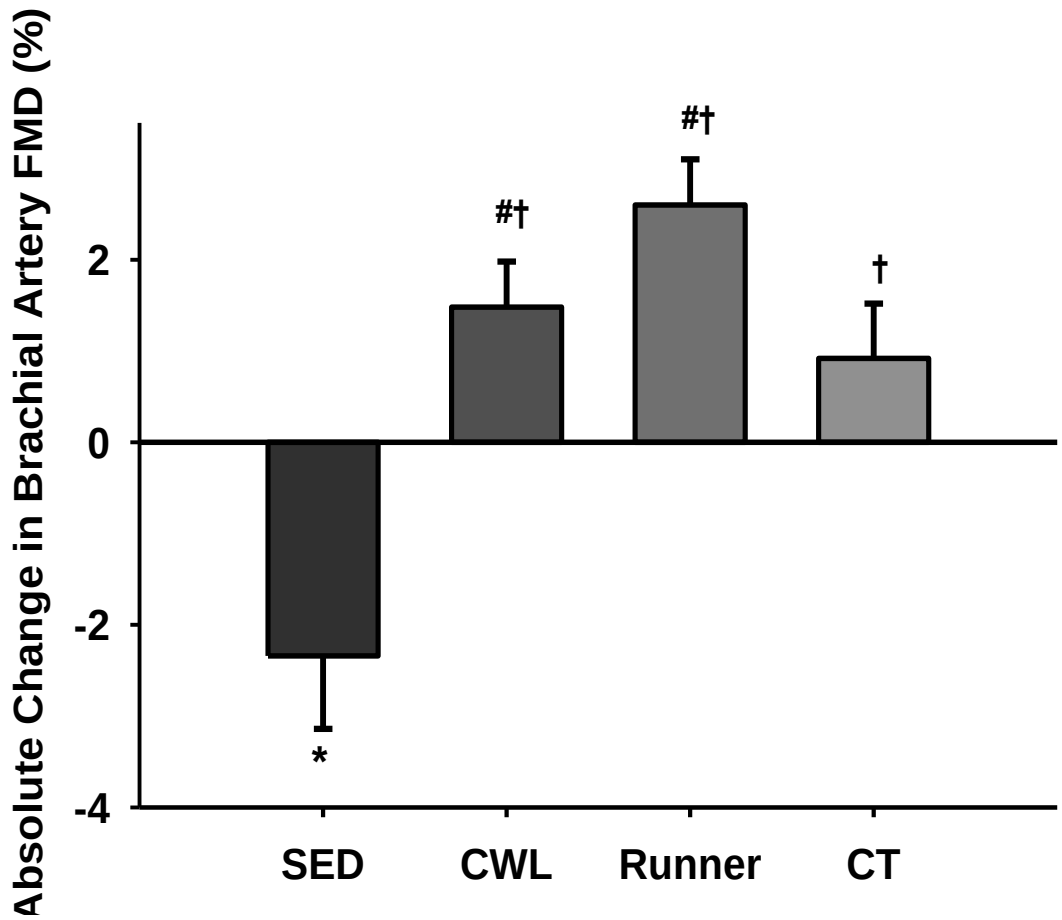
Effect of Weight Lifting on Flow Mediated Dilation in Sedentary Subjects



Jurva, JACC 2006

Effect of Different Modes of Exercise on Endothelial Response to Weight Lifting Stress



Benefit also seen with other stresses: hyperglycemia; lipid bolus

Assessment of endothelial function could help optimize the CV benefit of an exercise regimen.

Phillips, preliminary data 2013

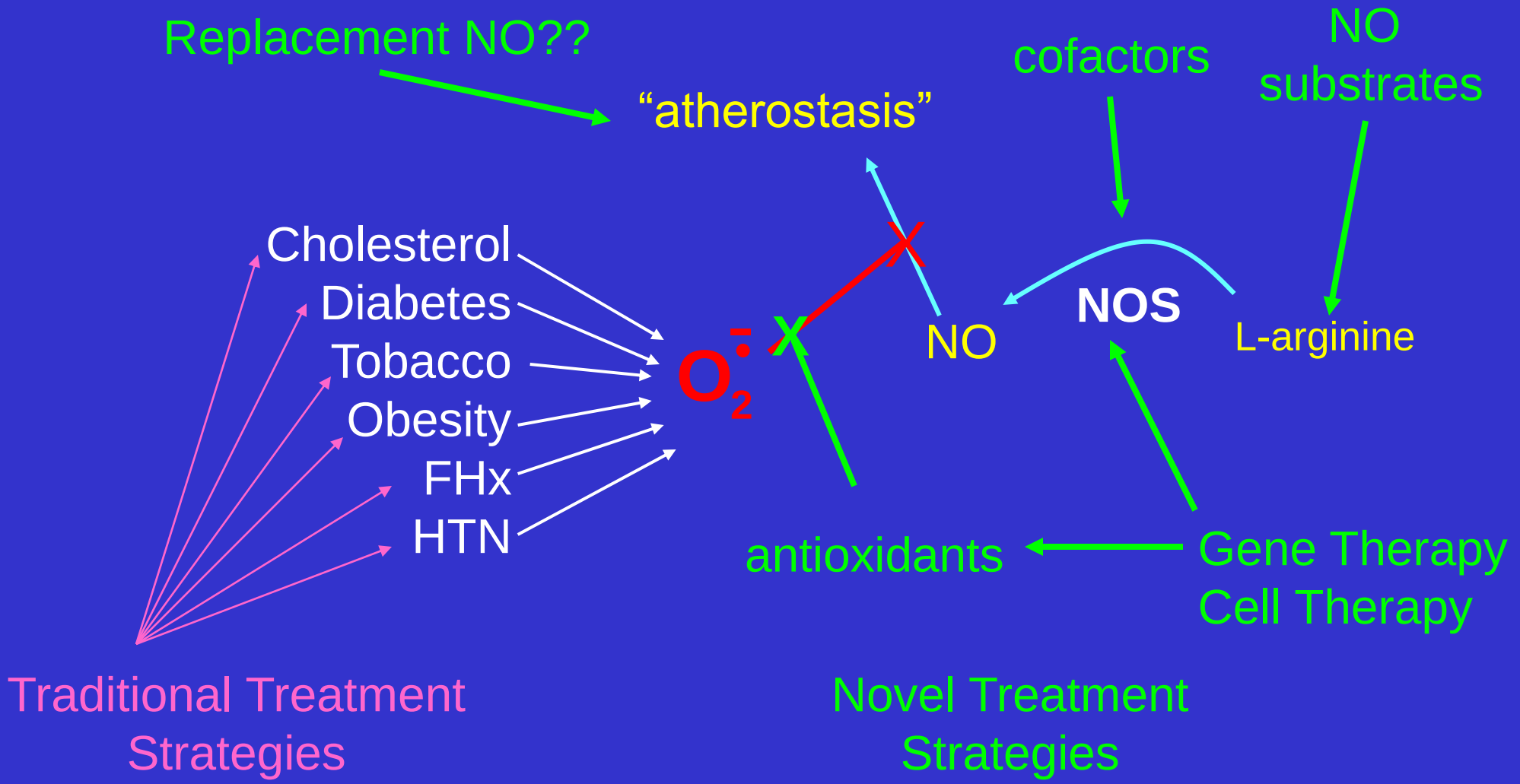
Methods to Improve Endothelial Function (NO release)

(? and reduce atherosclerosis?)

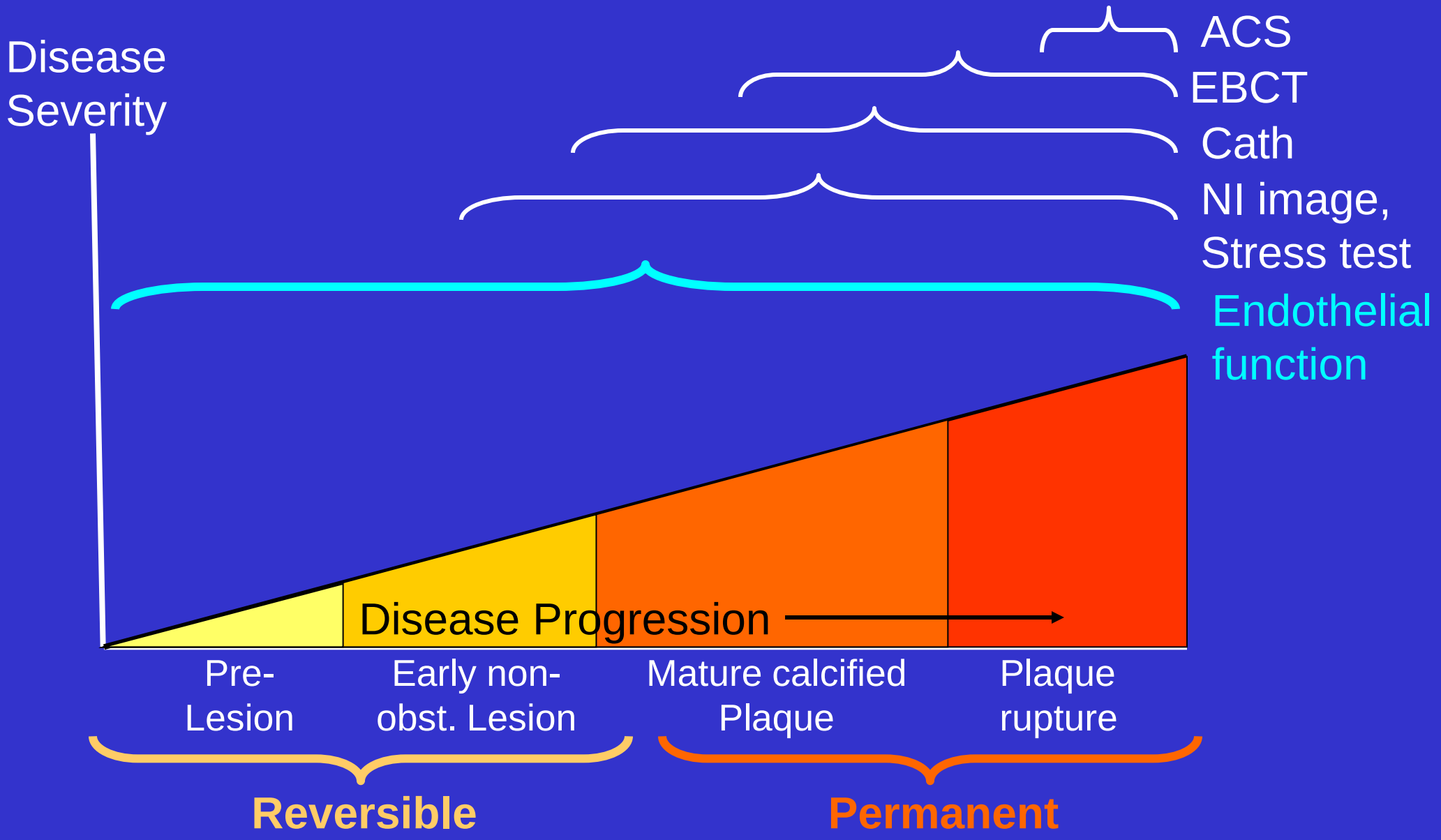
Exercise
Red Wine, other alcohol
Grape Juice
Estrogen Replacement
Statins
ACE-I
Arginine supplementation
Folic acid (high dose)
Vitamin C, E
Targeted Antioxidants
(SOD3, mitochondria)
Sildenafil

Magnesium supplementation
LDL apheresis
Nifedipine
Mediterranean diet
thiazolidinediones
fish oils, black tea
potassium channel antagonists
Bone-Marrow EPC
Dialysis
Probucol
Apo-A1 mimetics (D-4F)
Caveolin disruption

Potential Clinical Utility of Measuring Endothelial Function



Implications for Detection of CAD




Questions?
Dubrovnik, September 26, 2013

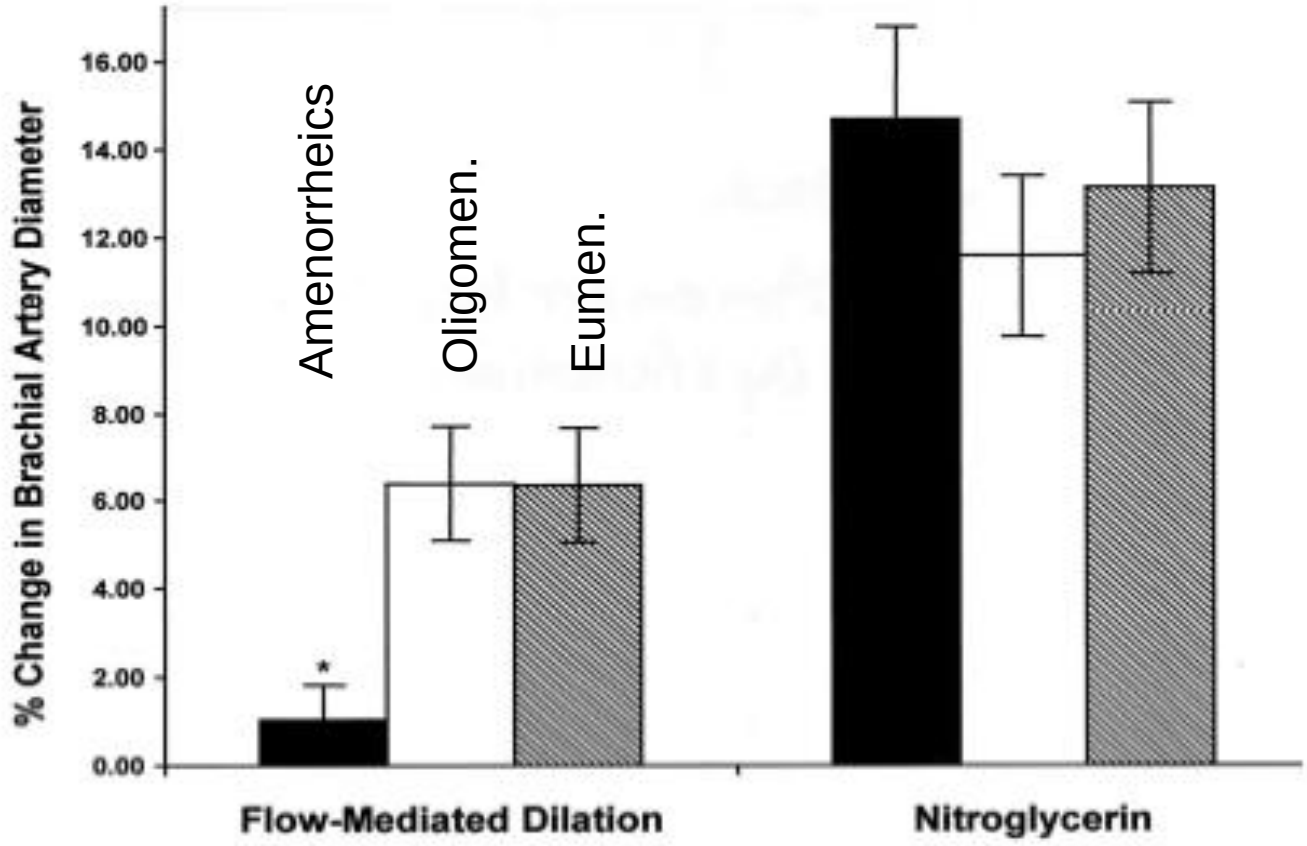
Endothelial Dysfunction Leads to Atherosclerosis
(Shear and Atherosclerosis)

Shear Stress **NO** **Arteriosclerosis**

The Female Triad: A common finding in elite women athletes

- Athletic amenorrhea is a component of the “Female Athlete Triad” which consists of *disordered eating*, *amenorrhea of hypothalamic origin*, and *osteoporosis*.
- The hormone profile of amenorrheic athletes is similar to that of postmenopausal women.
- Menopause is accompanied by endothelial dysfunction and accelerated atherosclerotic cardiovascular disease.
- **Could amenorrheic athletes be at higher risk for vascular disease?**

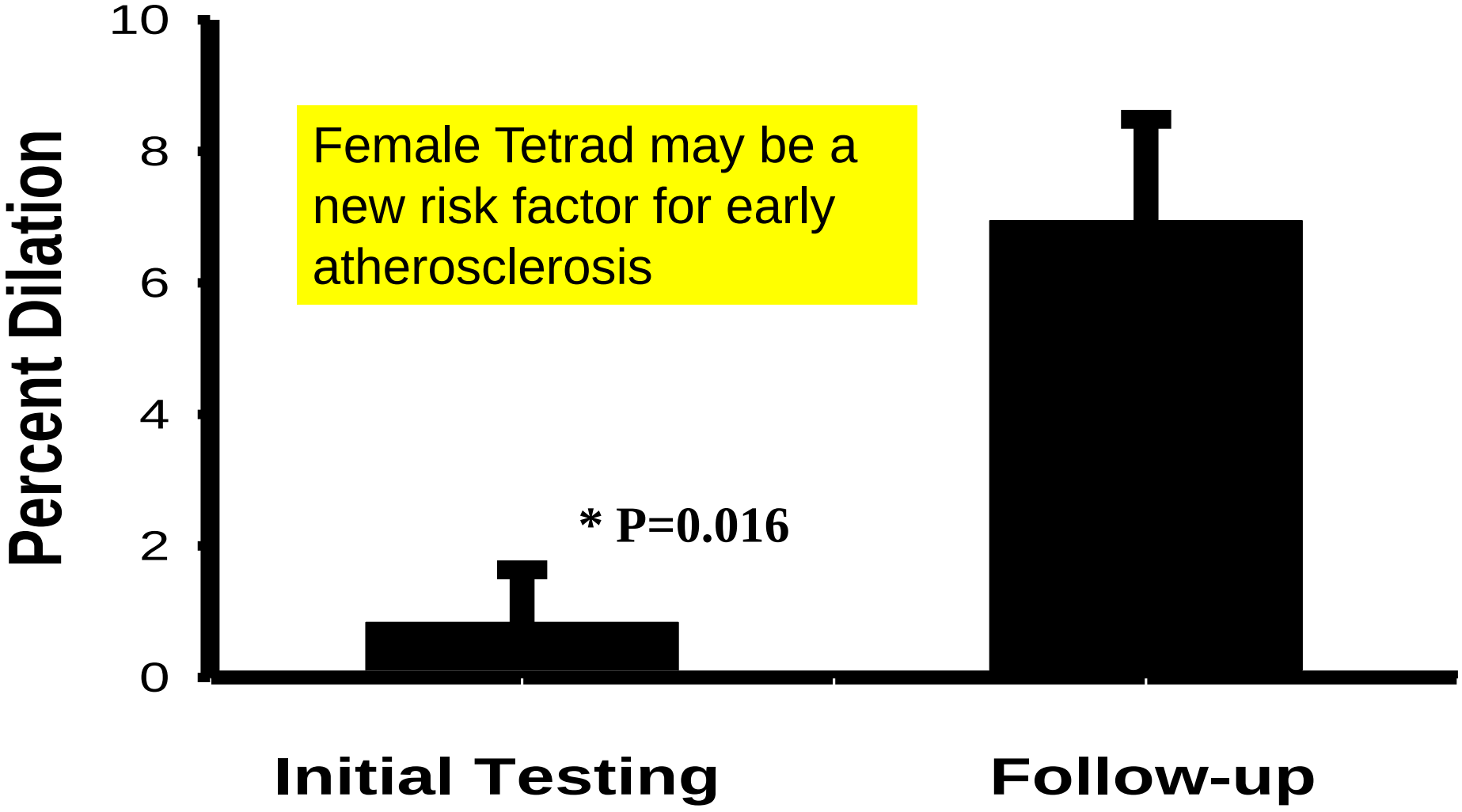
Effect of Amenorrhea on Endothelial Function in Women Runners



15 women in each group
All ran 25 mi/week
No other baseline differences

Hoch et al. MSSE 2003

Effect of Return of Menses on FMD
- 2 year follow up



Dieting

	AHA Diet	Atkins Diet
Fruits	High	Low
Vegetables	High	Low
Carbohydrates	High	Low
Fats & Oils	Low	High
Salt	Low	High
Meat	Low	High

Low Carbohydrate Diet

- Previous studies have shown…
  - Similar or better weight reduction vs. low fat diets
  - Improvement or no change in lipid profiles
  - Similar reduction in blood pressure
- Paradox
  - Weight loss is good for your health
  - Acute excessive intake of high-fat foods is bad for health. (High fat intake is a risk factor for CAD)

Hypothesis

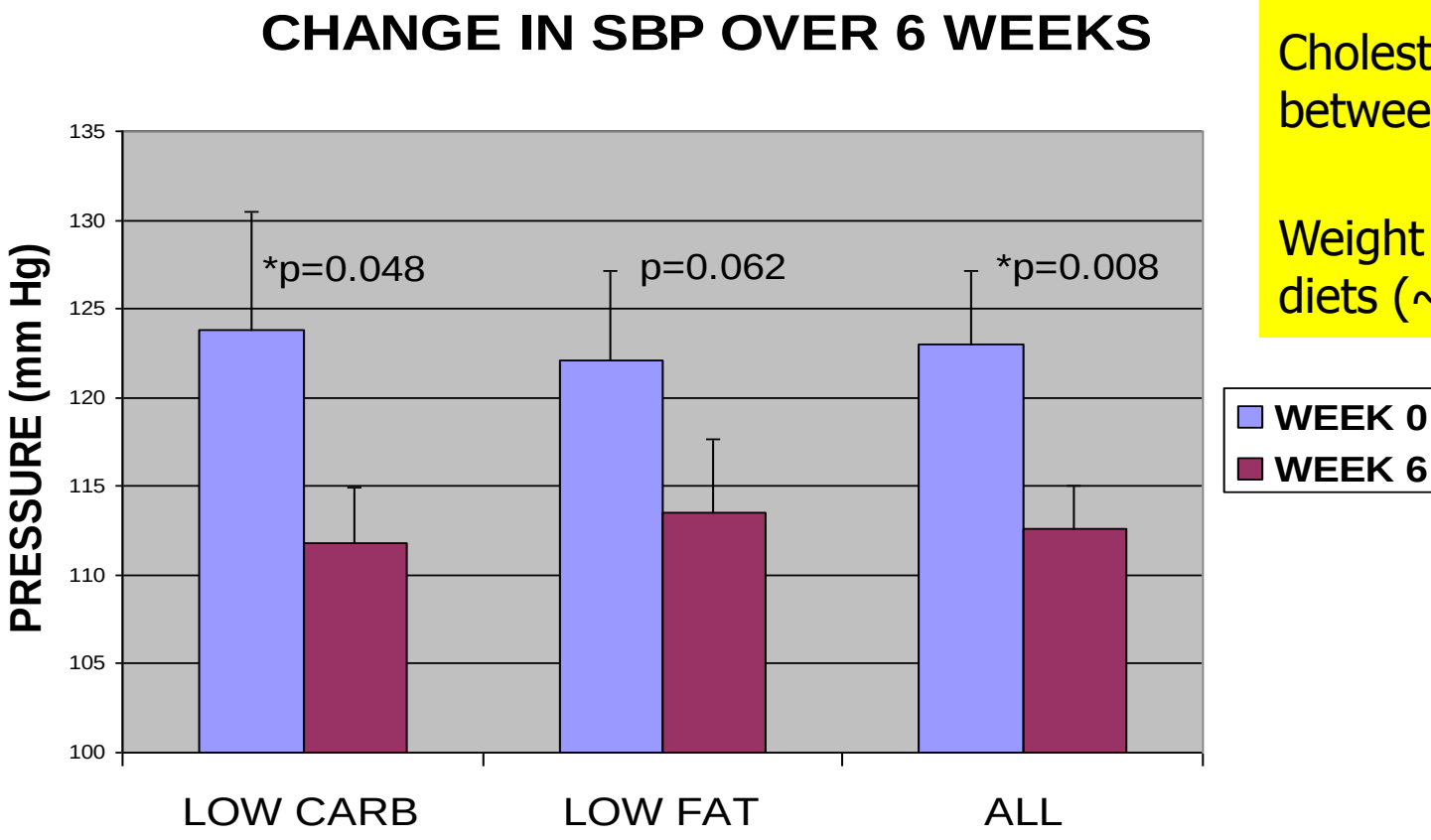
- By using an equally effective weight reduction regimen, a traditional low-fat diet will improve vascular endothelial function while a low-carbohydrate diet will not.

Materials and Methods

- Inclusion Criteria
  - Male or female, ages of 18 & 50 years, BMI of 29-39
  - Healthy subjects not currently on a diet
- Exclusion Criteria
  - Any known health problem that impairs endothelial function (history of CV disease, HTN, elevated cholesterol, etc.)
  - For the 11 subjects that have completed the diet, 40+ subjects were screened and eliminated because of exclusion criteria!
- Randomized 6 week diet

- Phillips et al. Hypertension, 2008

Results

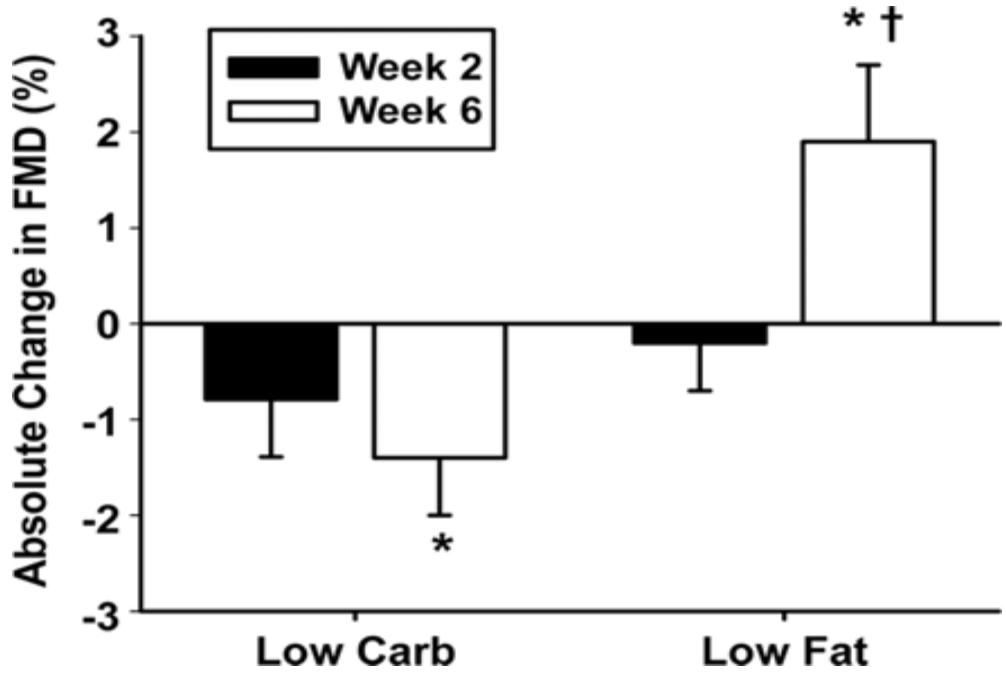


Systolic blood pressure decreased similarly with both diets

Cholesterol did not differ between diets.

Weight loss was similar between diets (~4-5 kg).

Results



Not all diets are the same. Endothelial function may help identify what works best.

- Phillips et al. Hypertension, 2008

Summary

- Nitric oxide, derived from the endothelial enzyme NOS, is a potent regulator of vasomotor tone in the normal human heart.
- Risk factors enhance oxidative stress, impair conduit vessel dilation and NO responses, and initiate a pro-atherogenic state.
- Loss of NO appears to be the final common pathway leading to development of atherosclerosis.
- Novel therapies designed to improve or restore NO bioavailability should be effective in preventing and reversing atherosclerosis and its complications.

Antioxidants and Atherosclerosis

Secondary Prevention: Clinical Trials

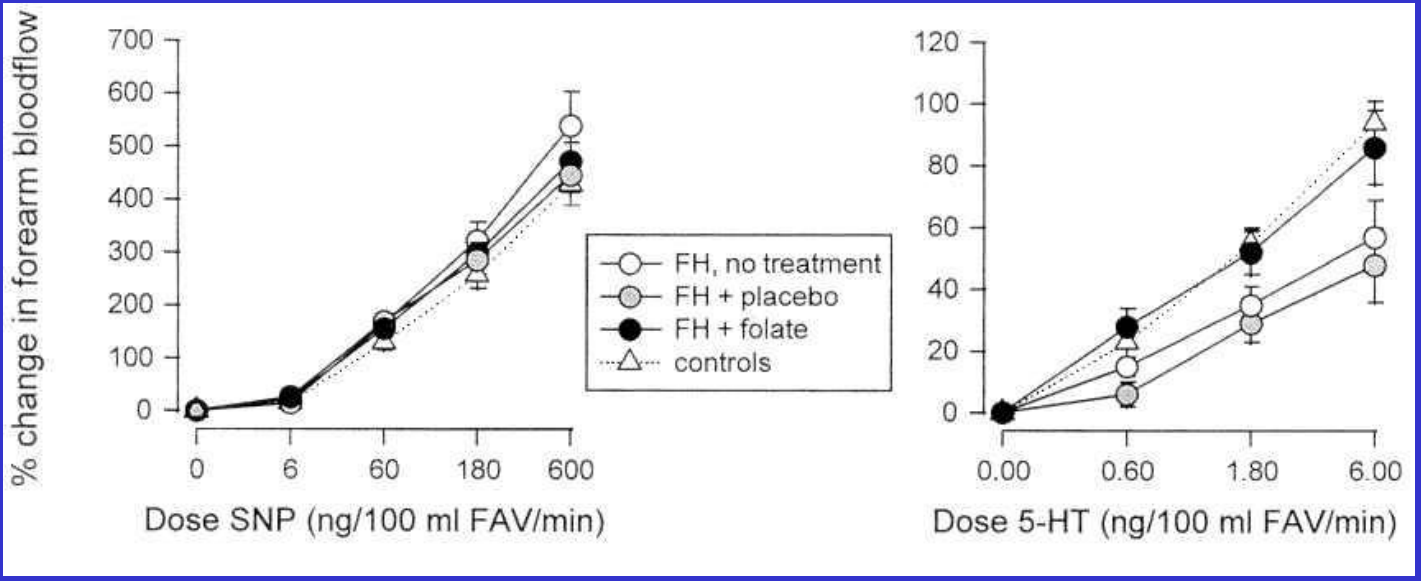
Study	Year	Subjects (n)	F/U (yrs)	Tx	Result
CHAOS	1996	2002	2	Vit E	+
Azen et al.	1996	146		Vit E	+
CARET	1996	18314	12	ß-carotene	-
MPV	1997	317	0.6	Vit E+C	-
PART	1997	101	0.6	Probucol	+
ATBC	1997	1862	3-5	ß-carotene	-

Antioxidants and Atherosclerosis

Primary Prevention: Epidemiological

Study	Year	Subjects (n)	F/U (yrs)	Tx	Result
NHANES	1992	11348	10	Vit C	+
Nurse Health Study	1993	87245	8	Vit C	-
Health Prof. Study	1993	39910	4	Vit E	+
LRC-CPPT	1994	1899	13	ß-carotene	+
Gale et al.	1995	730	20	Vit C	+
Iowa Women Health	1996	34486	7	Vit E	+
Rotterdam Study	1999	4802	4	Vit C	-
Knekt et al.	1994	5000	14	Vit E	+

Folate and Endothelial Function



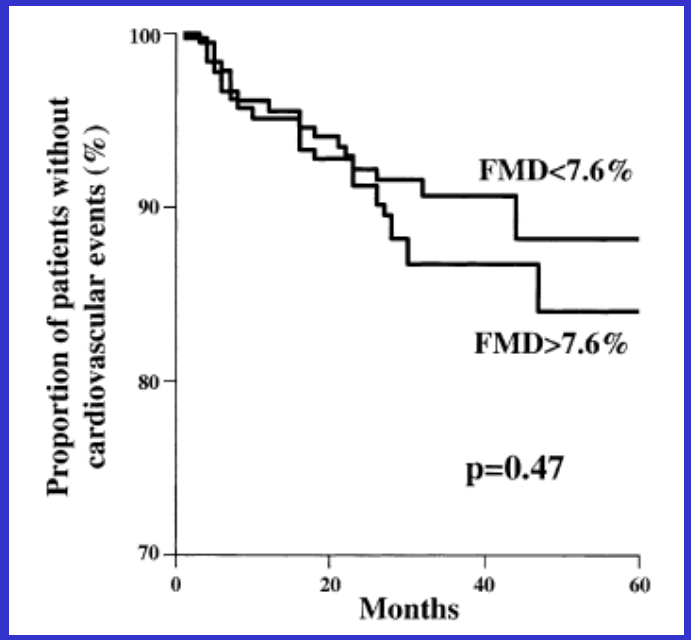
20 subjects with FH
20 controls
Treat with folate for one month
Assess endothelial function

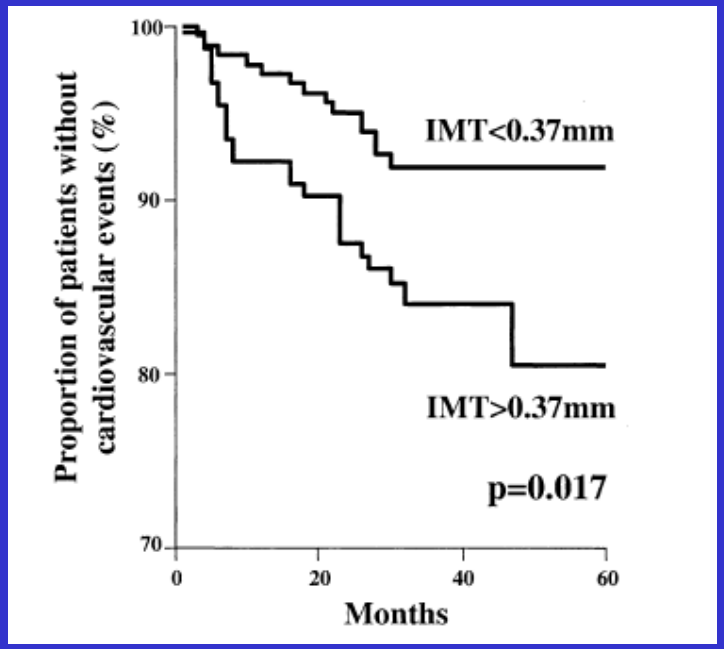
	FH			
	Baseline	+Placebo	+Folic acid	Controls
Sex, male/female	14/6			15/5
Age, y	35±3			32±2
Body mass index, kg/m^2	23.4±0.7			23.1±0.5
Smoking, yes/no	7/13			6/14
Mean arterial pressure, mm Hg	81±2	83±2	79±2	83±2
Heart rate, bpm	65±2	66±2	65±2	63±2
Baseline FBF, mL · dL FAV^{-1} · min^{-1}	2.8±0.2	3.2±0.3	2.9±0.2	3.2±0.3
Total cholesterol, mmol/L	8.0±0.3*	8.9±0.4†	8.5±0.4†	4.6±0.2
HDL-cholesterol, mmol/L	0.96±0.05*	0.97±0.05	0.96±0.05	1.31±0.09
Triglycerides, mmol/L	1.55±0.18*	1.73±0.24	1.61±0.17	1.02±0.08
Apo-B, g/L	1.61±0.07*	1.79±0.09†	1.74±0.09†	0.80±0.05
Folate, nmol/L	12.8±1.0	18.3±2.1	151±20.5‡	(>6.8)

Improving the Risk without changing the risk factor

- Verhaar, et al. Circ., 1999

Prognosis: FMD vs. IMT





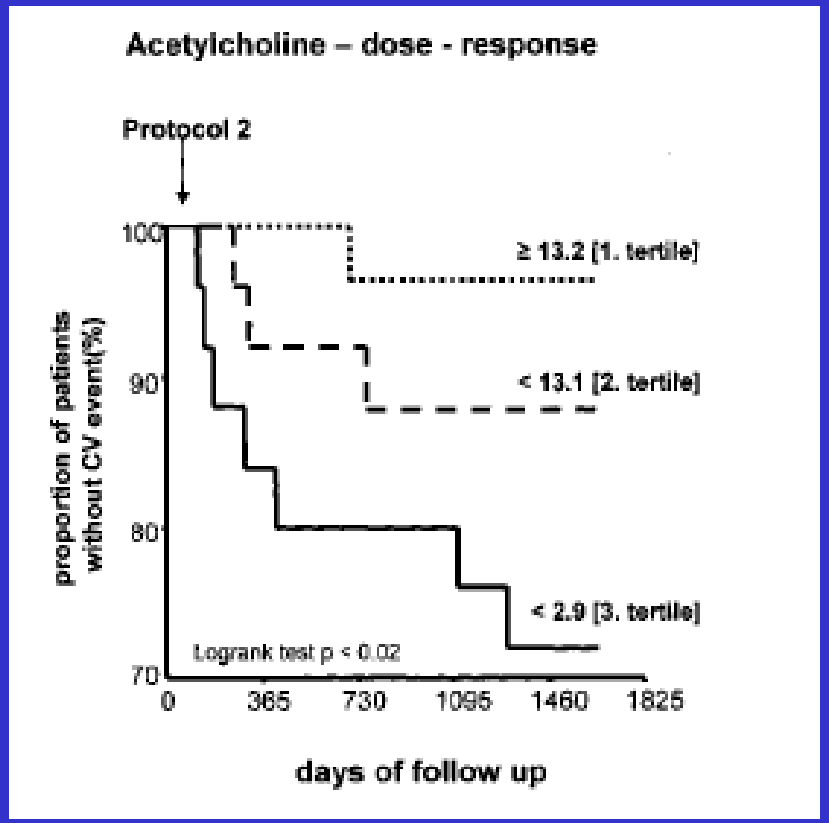
- 398 consecutive patients undergoing cath
- Stratified by FMD >7.6% or <7.6%
- Followed 5 years for patients with CAD
- Events: MI, Death, hospitalization for worsening angina, repeat angiography for angina

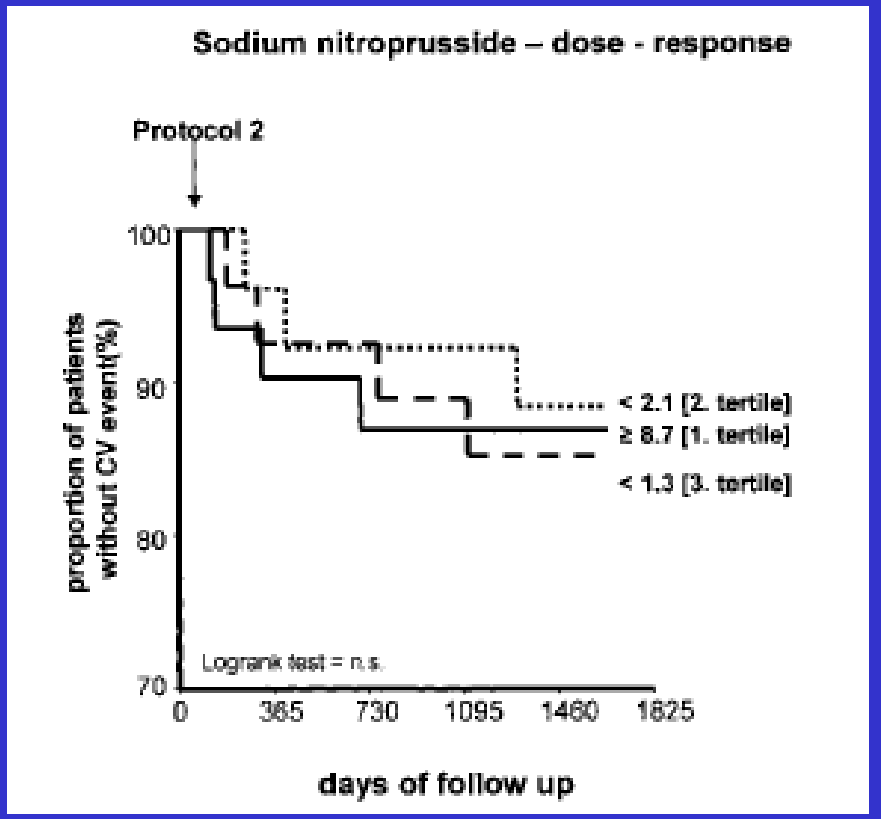
Single measure of FMD vs. sequential

Frick et al. JACC 2005

Prognosis and Endothelial Function

(outcomes (death, MI, CVA) following an improvement in endothelial function)



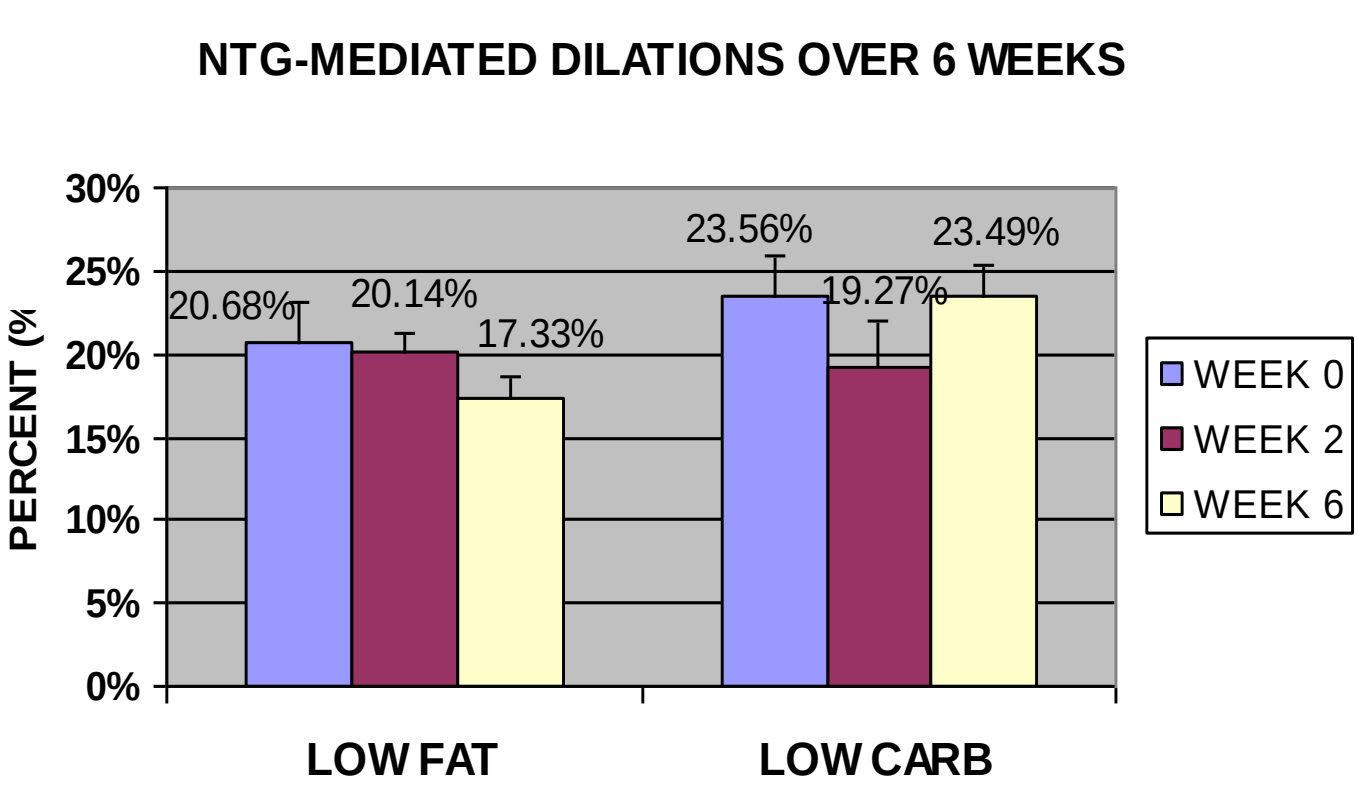


Ach (FBF)

SNP (FBF)

- Fichtlscherer, et al. Circ, 2004

Results: Post-Nitroglycerin Administration



There were no differences in NTG-induced dilation for subjects on both diets over 6 weeks.

AHA 2007 Goals and Results

Goal: Reduce death rate from coronary heart disease and stroke by 25%

- 23.1% reduction in death rate from coronary heart disease
- 19.1% reduction in death rate from stroke

Goal: Reduce prevalence of tobacco use, high blood cholesterol and physical inactivity by 25%

- 20.0% reduction in prevalence of high cholesterol
- 12.9% reduction in prevalence of tobacco use
- 2.5% reduction in those not engaged in moderate or vigorous physical activity

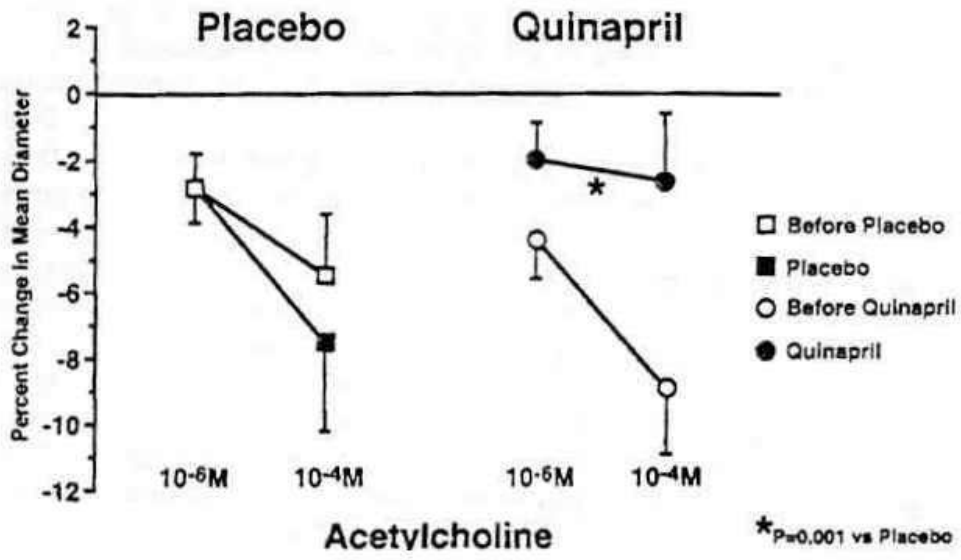
Goal: Reduce rate of uncontrolled high blood pressure by 25%

- 8.5% reduction in uncontrolled high blood pressure

Goal: Eliminate the growth of obesity and diabetes (Goal: 0% rate of growth)

- 1.39% rate of growth in obesity
- .28% rate of growth in diabetes (no new data since baseline)

Quinapril and Endothelial Function



Effects of chronic treatment with the angiotensin converting enzyme (ACE) inhibitor quinapril on endothelium-dependent vasoconstriction of epicardial coronary segments (as assessed by quantative angiography) in the placebo and quinapril patients. After 6 months the vasoconstriction to acetylcholine was markedly blunted in the quinapril group (40 mg/day) only. Modified from [75].

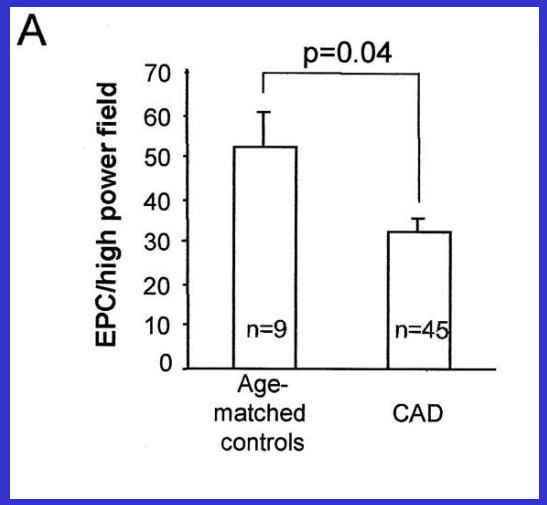
-TREND (Trial on Endothelial Dysfunction)

-Pts. with CAD but without HTN, CHF, or high Chol.

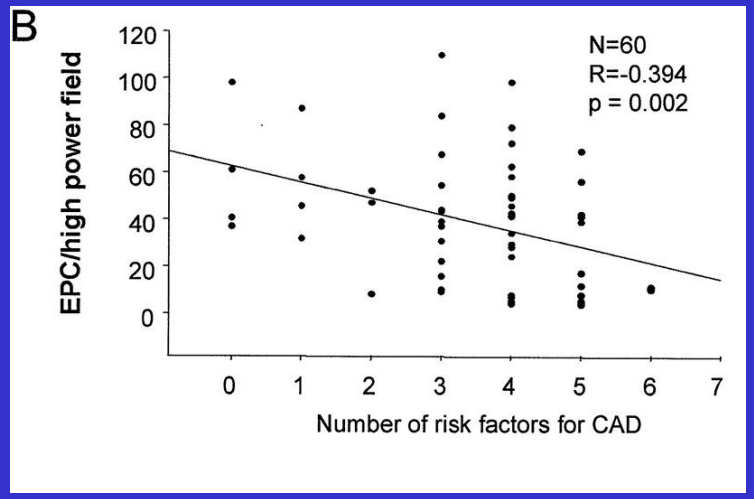
-6 month f/u cath

- Treasure, NEJM 1995

Stem Cells and Endothelial Function

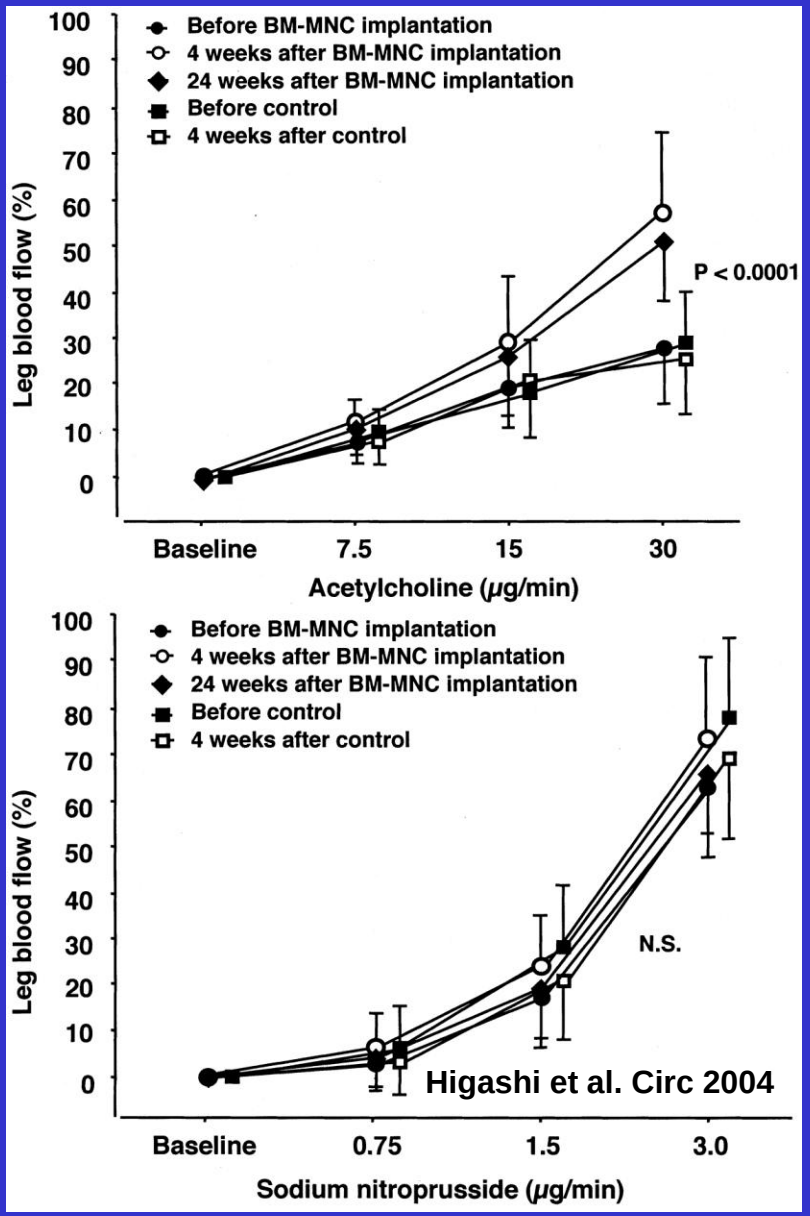


45 patients with CAD; 15 controls

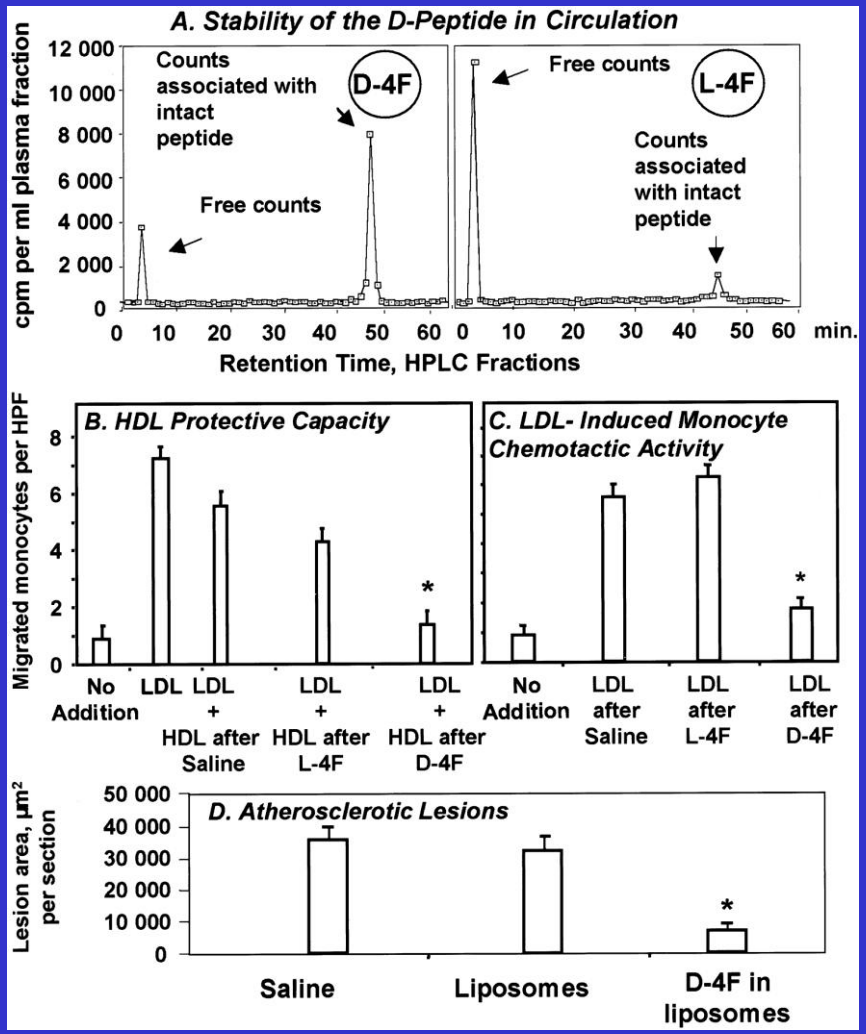


Vasa et al. Circ Res 2001

7 patients with PVD; autologous BM stem cells

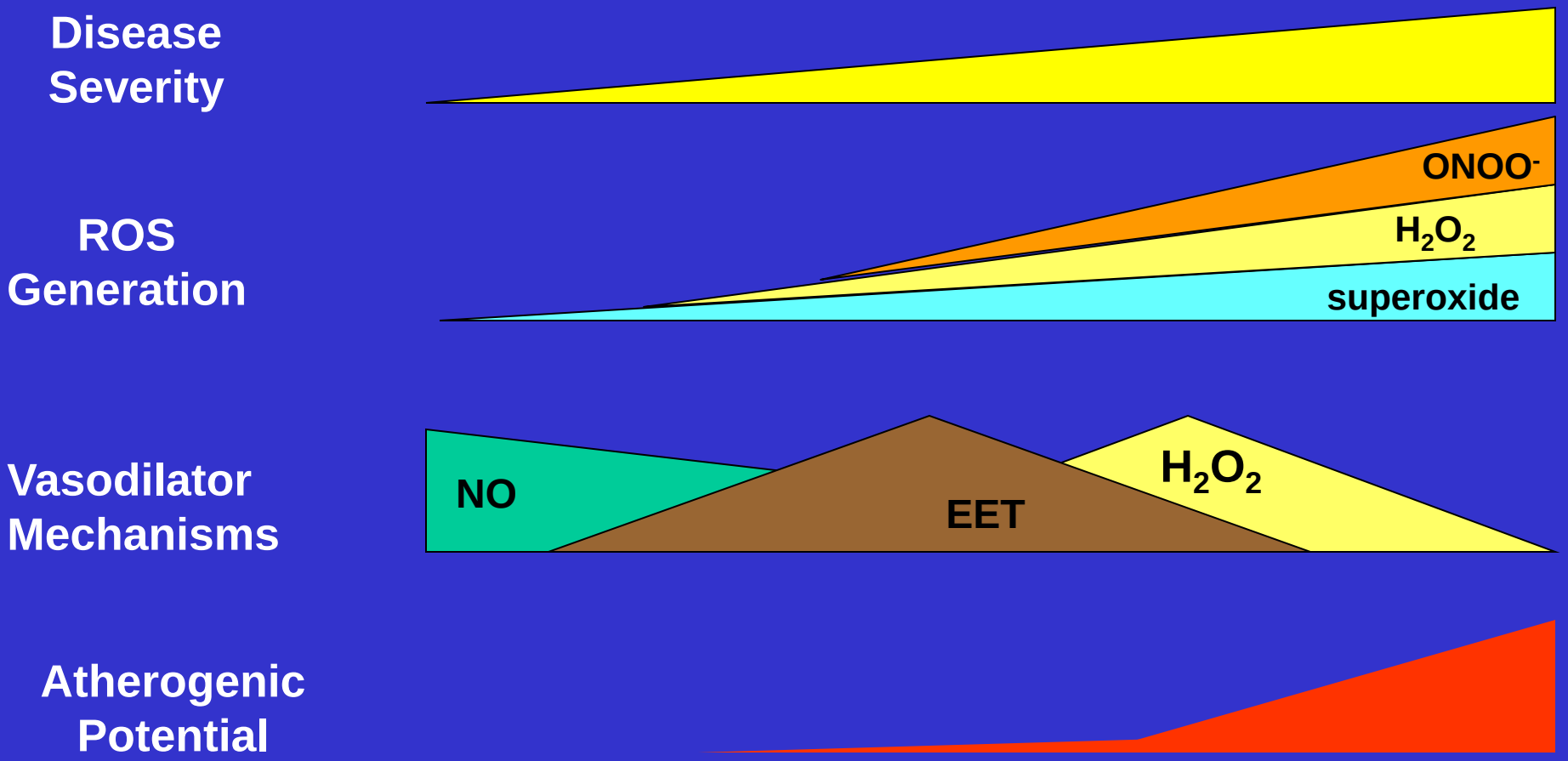


Studies in LDL receptor-null mice (LDL R-/-)

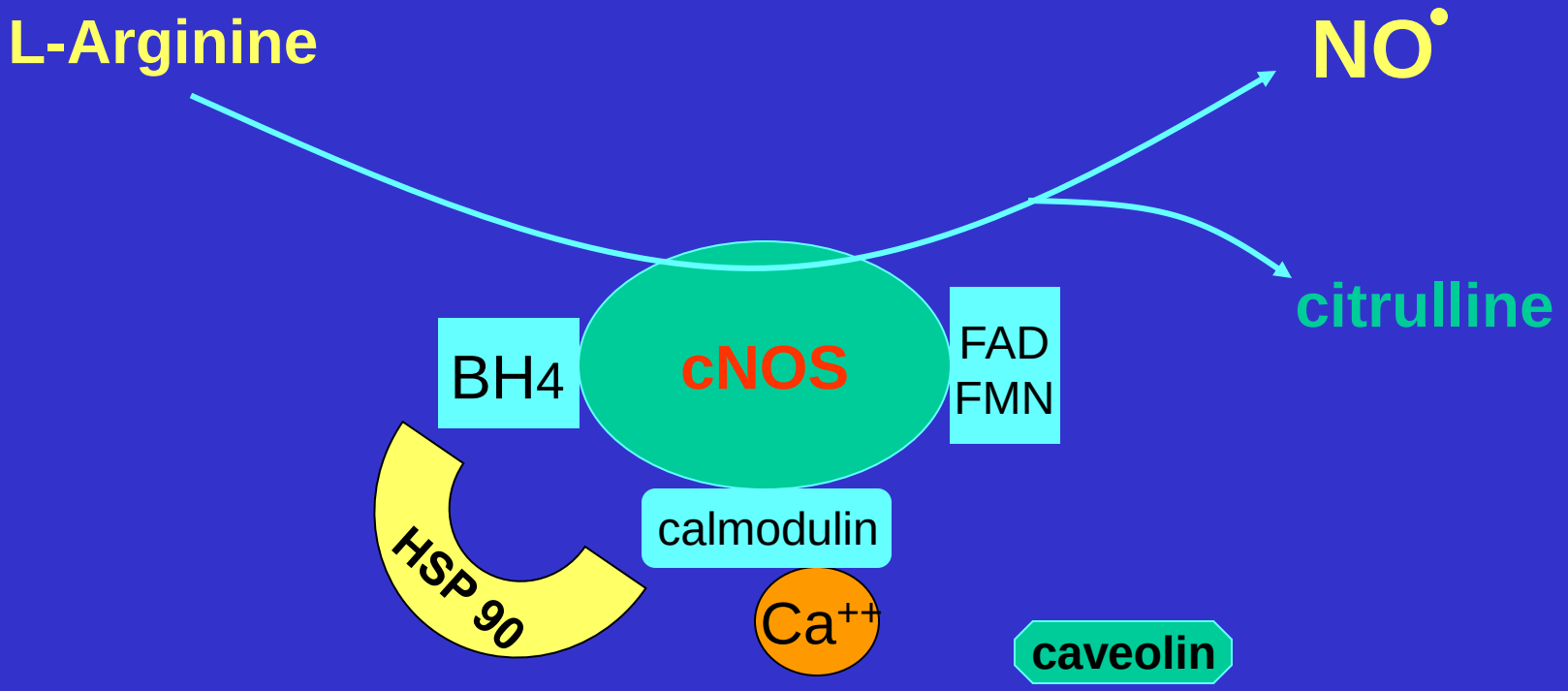


Navab, M. et al. Circulation 2002;105:290-292

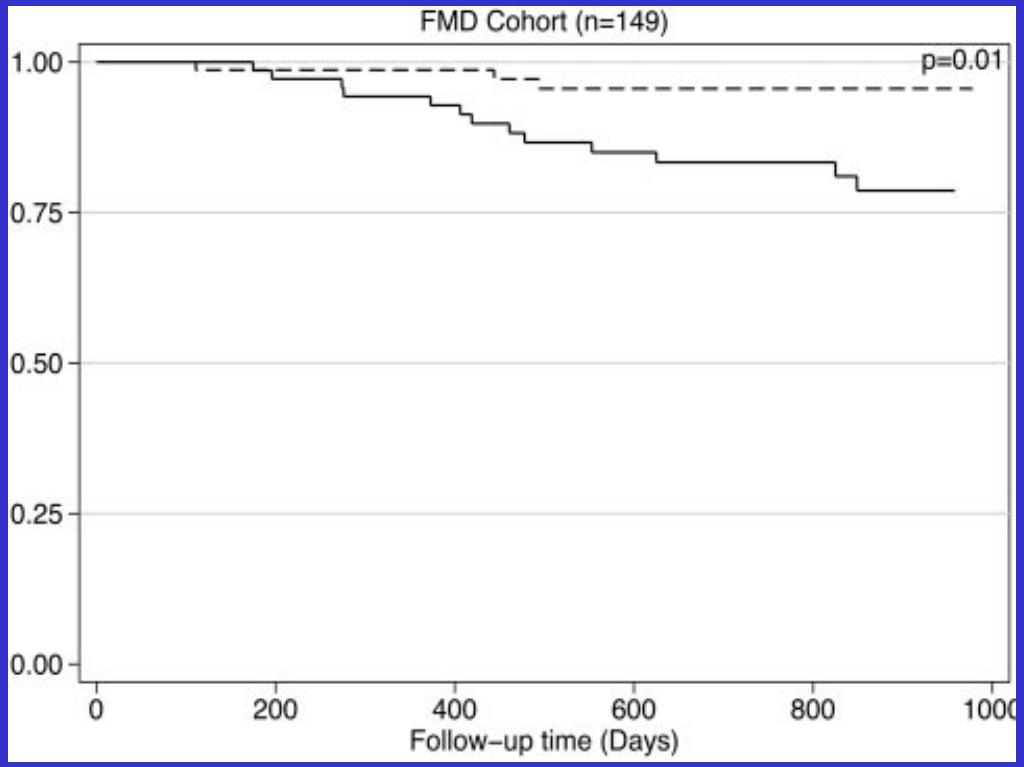
ROS and Vasomotor Function



Endothelial Nitric Oxide Synthase



Endothelial Function and Prognosis in CHF



Kaplan-Meier plot of survival over time

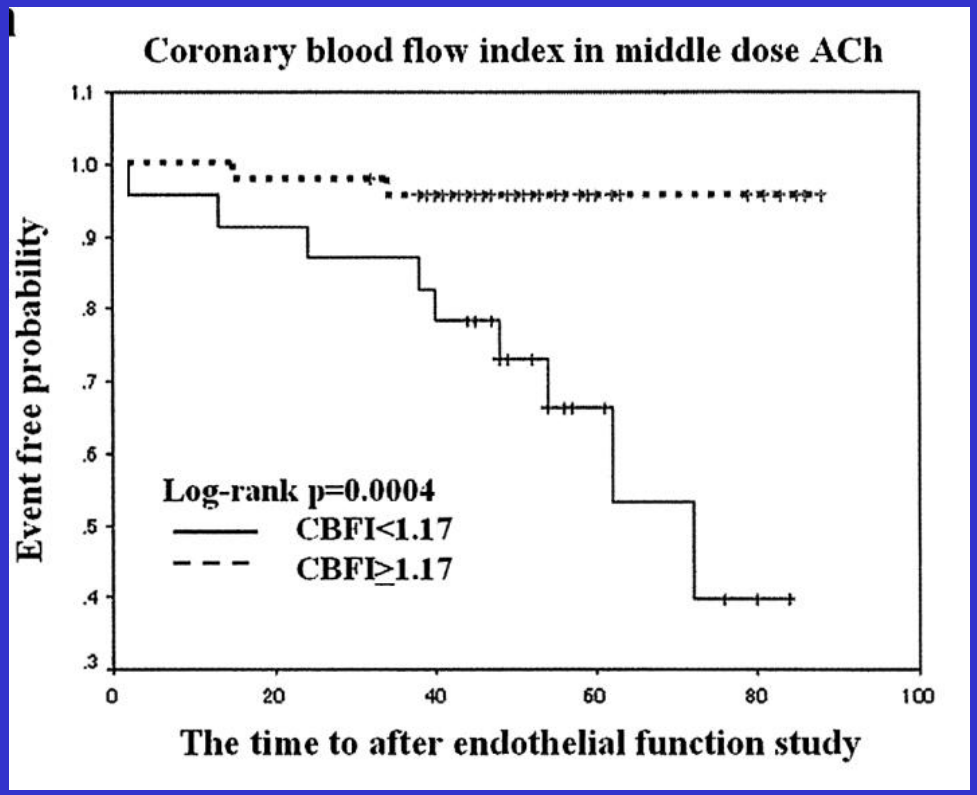
> median FMD

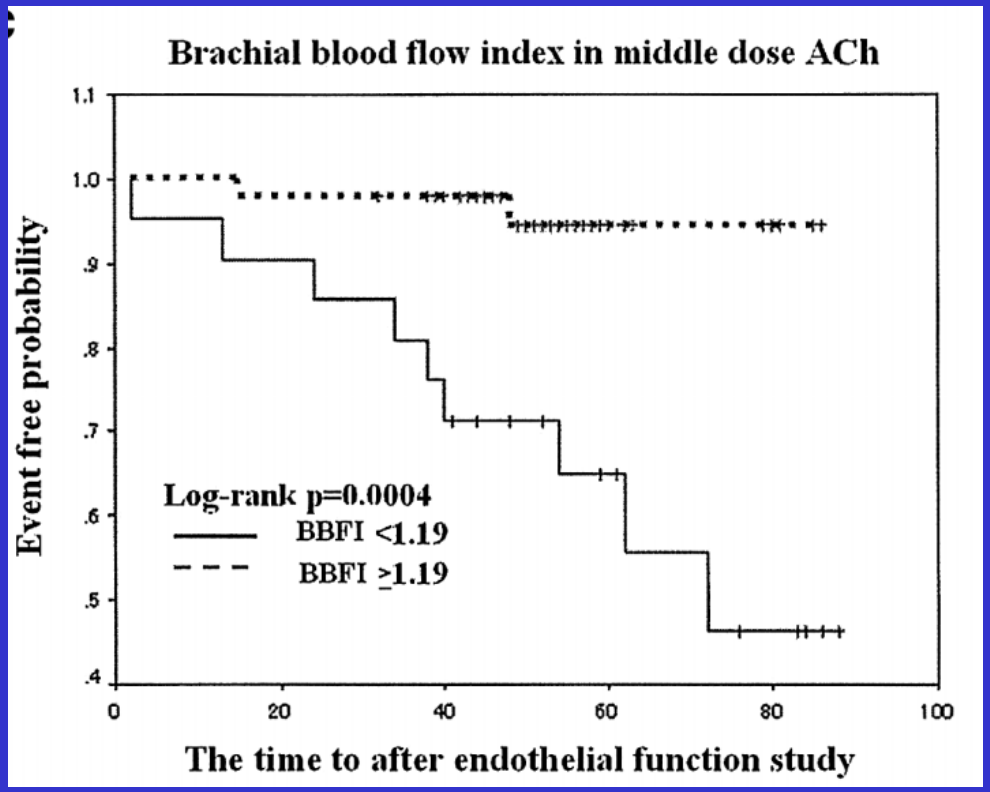
< median FMD

149 FC II-III CHF subjects, EF<40%.
Meds stopped the day of FMD testing.

Katz, Circulation 2005

Prognostic Equivalence Between Coronary and Brachial Endothelial Function

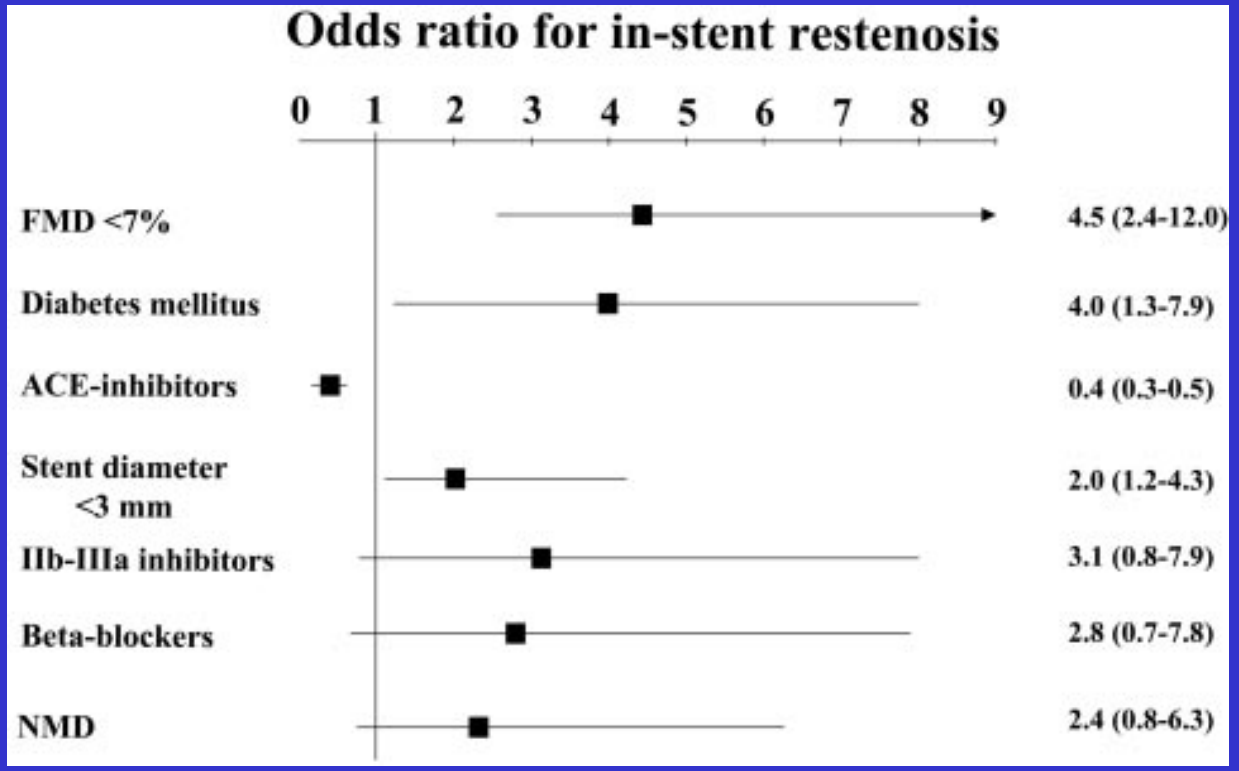




Patients: 70 with suspected CAD, no known PVD
Event: MI, Death, readmission for UA.

Takase et al. Circ. J. 2006

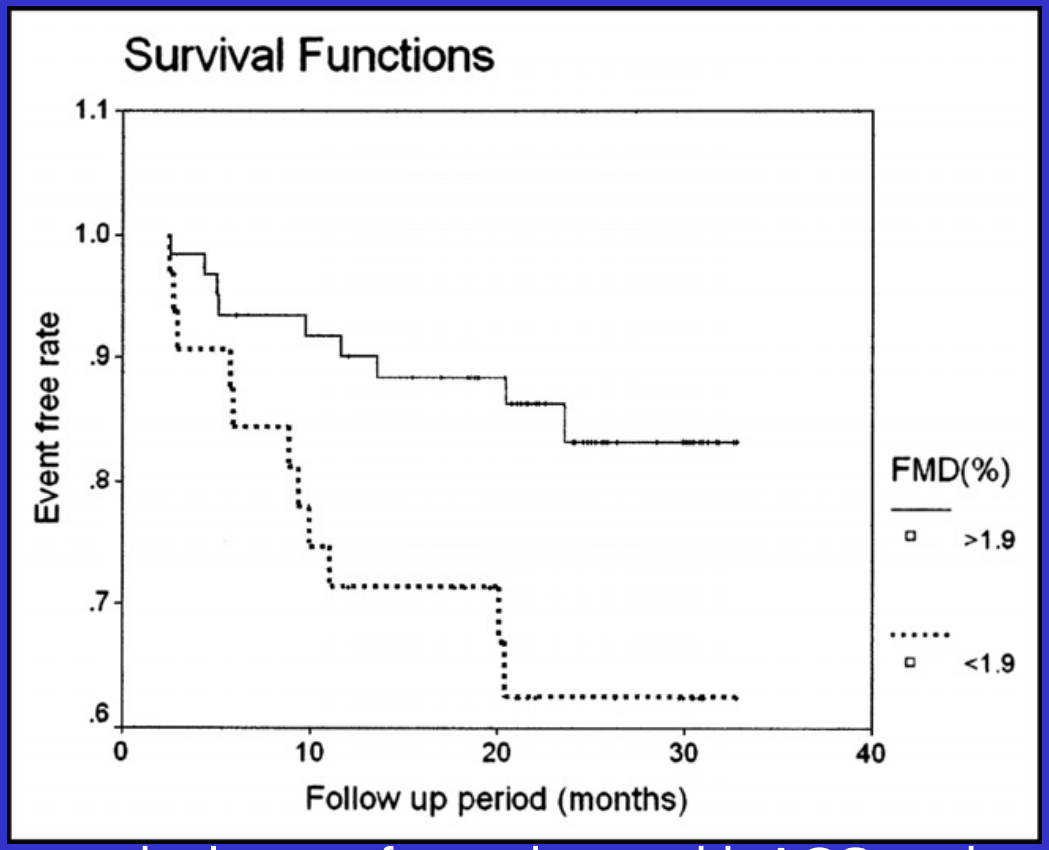
Prognostic Importance of FMD in PCI Patients



Multivariate logistic regression analysis showing that FMD, diabetes mellitus, and stent diameter <3 mm are independently associated with significantly increased risk of in-stent restenosis.

Patti et al. Circ. 2005

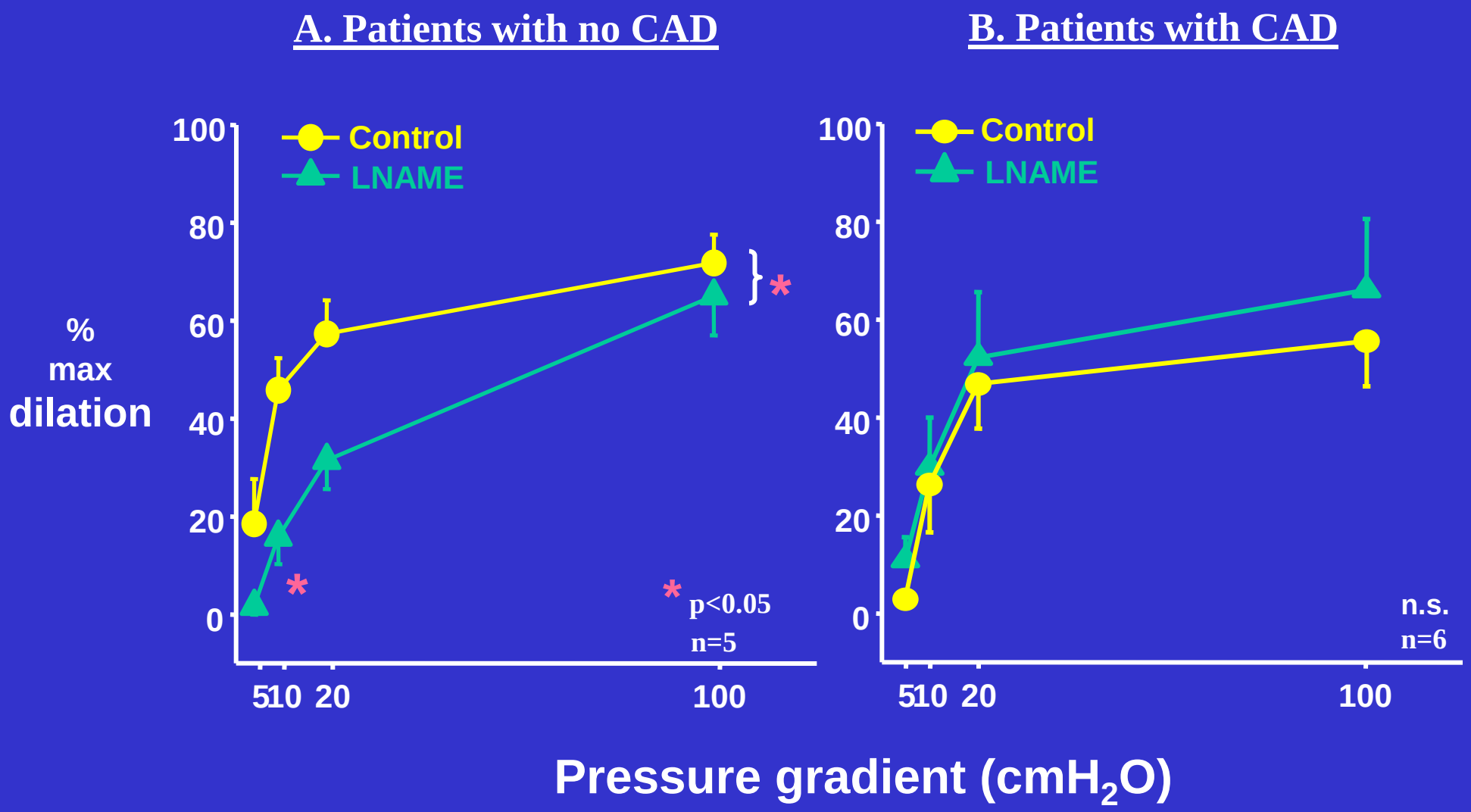
Prognostic Importance of FMD in CAD Patients



Kaplan-Meier survival curve for patients with ACS and no ST-segment elevation. FMD was the strongest independent predictor of events (CV death, MI, CVA, UA) in multivariate analysis.

Karatzis et al. AJC 2006

Flow-induced Dilation (CAD vs. no CAD)



Miura et al. Circulation 2001

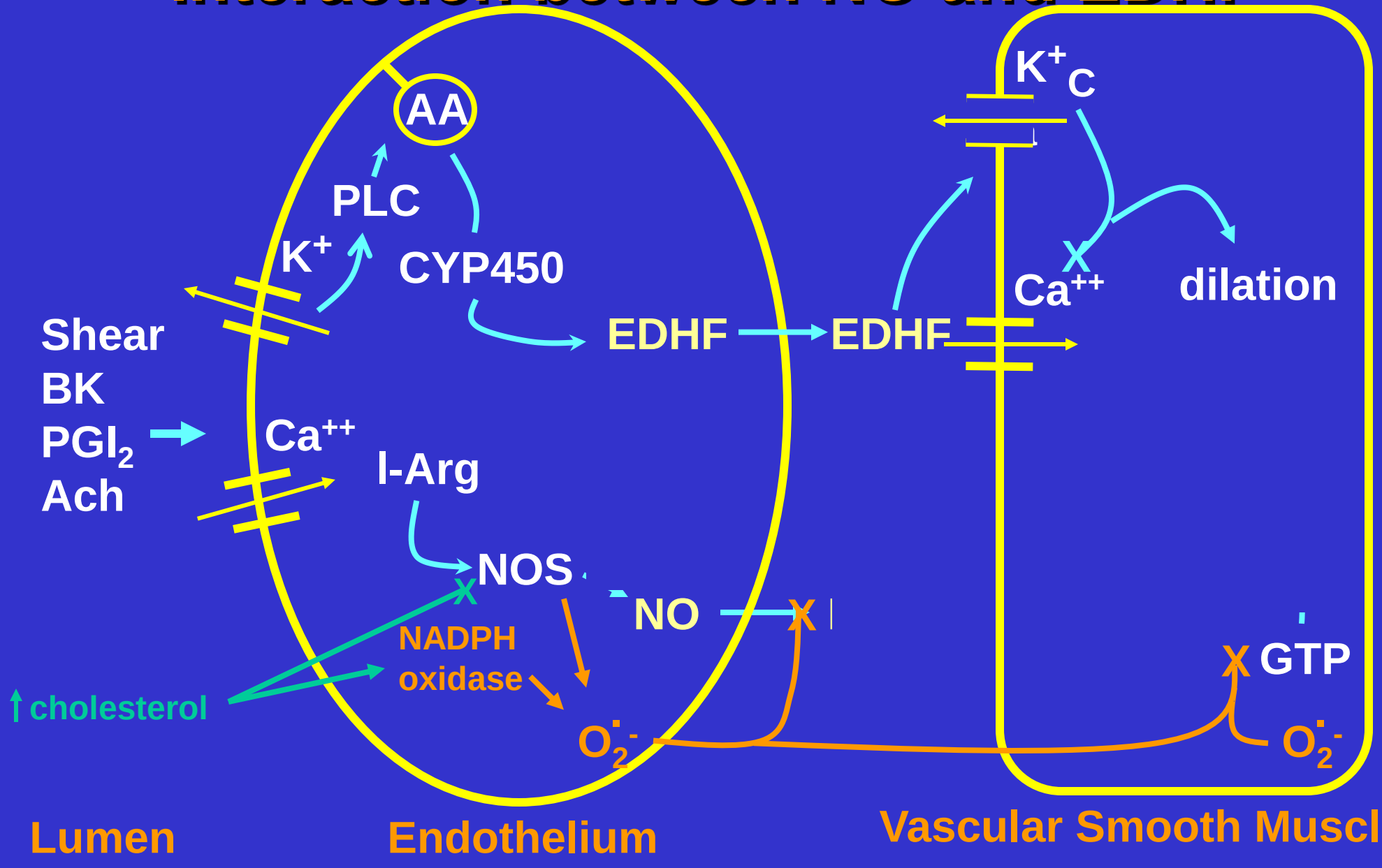
Therapeutic Improvement in IMT

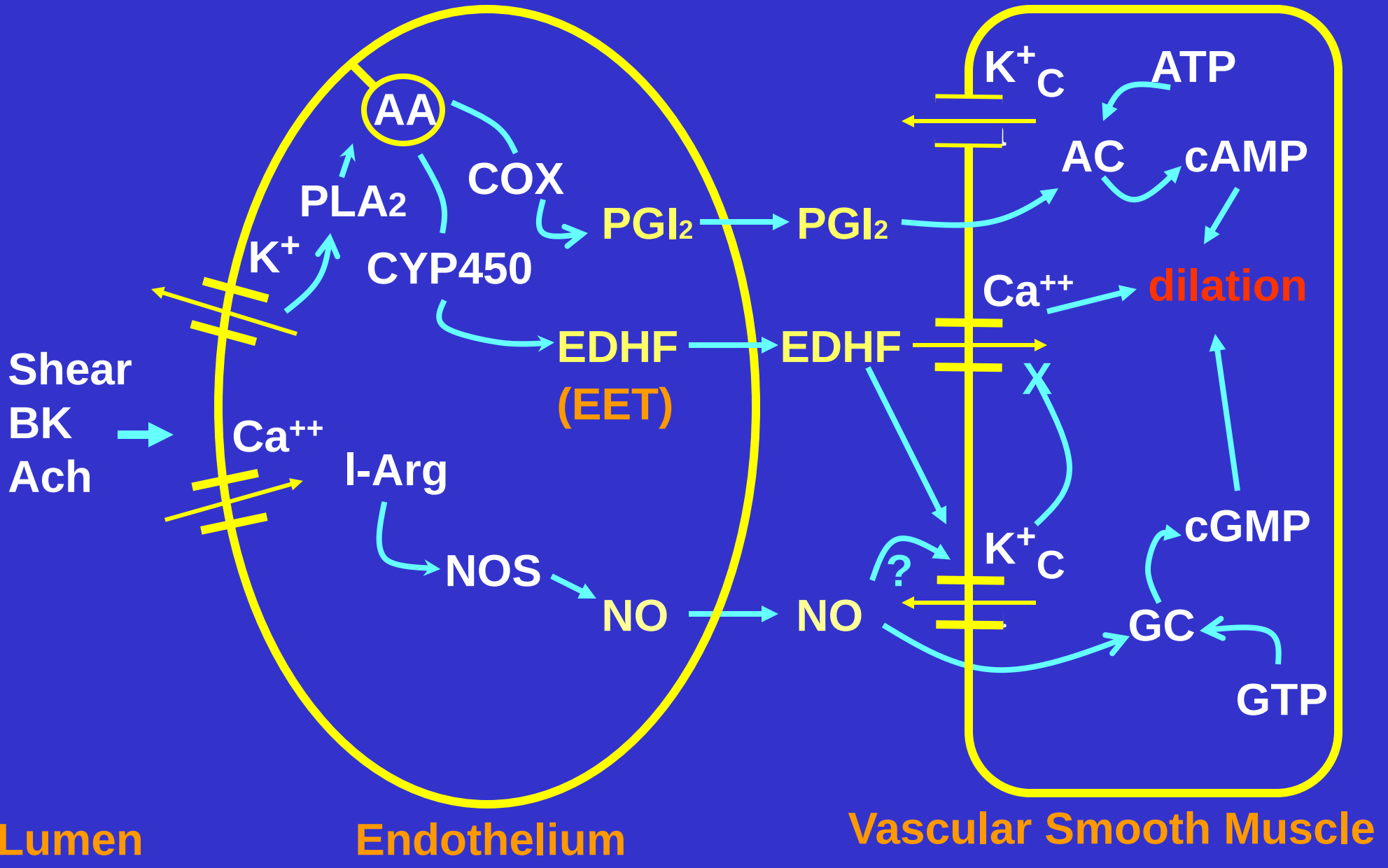
Hodis et al. (Ann Int Med) studied 94 adult subjects with CAD
Lovastatin vs. control
F/u at 1 year revealed IMT reduction of 0.031 mm; $p<0.05$

Koshiyama et al. (J. Card. Pharmacol.) compared amlodipine to placebo (n=11 in each group) on IMT. In 6 months they saw a 0.052mm reduction in A group ($p<0.05$)

Spacil et al. (Angiology) studied 21 adults with FH treated with statins or fibrates.
F/u at 29 months showed decrease in IMT 0.83 to 0.68; $p<0.01$; greater effect with fibrates

Interaction between NO and EDHF




Shear
BK
Ach
Ca++
K+
PLA2
AA
COX
CYP450
PGI2
EDHF
(EET)
l-Arg
NOS
NO
PGI2
EDHF
NO
K+C
ATP
AC
cAMP
Ca++
dilation
K+C
?
cGMP
GC
GTP
Lumen
Endothelium
Vascular Smooth Muscle

Efficacy of Folic Acid as a Therapy for Impaired Endothelial Function

Verhaar et al. (Circ 1999) In patients with FH, folic acid restored normal endothelial function without lowering cholesterol

Wilmink et al. (ATVB, 2000) showed that folic acid prevented the acute reduction in FMD following a fat load (whipped cream) in normal subjects

Nitric Oxide Synthase Isoforms

	nNOS (NOSI)	iNOS (NOSII)	eNOS (NOSIII)
Calcium dependent	+	-	+
Calmodulin dependent	+	+	+
Constitutively active	+	-	+
Caveolin sensitive	+	-	+
NO Production	+	+++	+
Localization			
Endothelium	+	+	+++
Smooth muscle	+	+++	+
Neurons	+++		

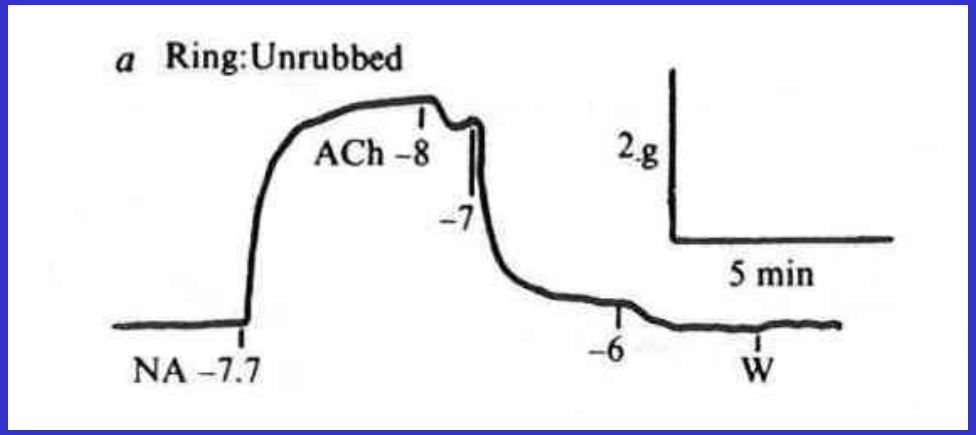
Potential Mechanism of Folic Acid-Improvement in Endothelial Function

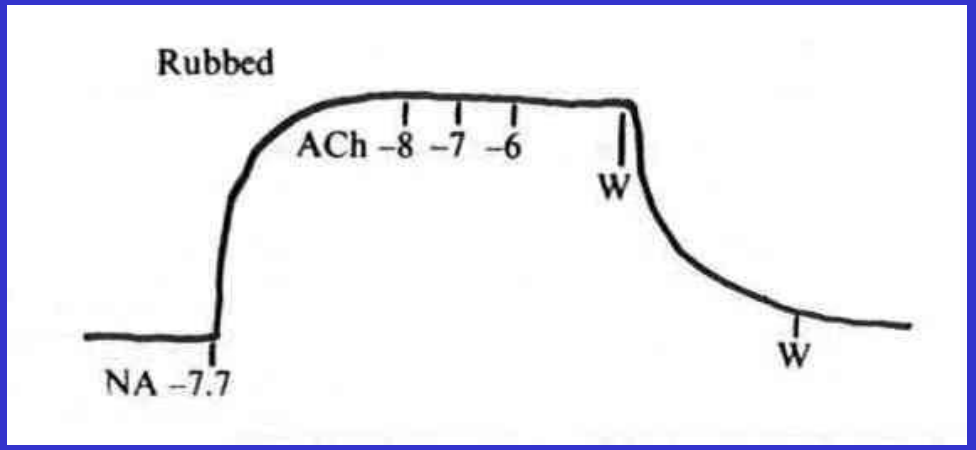
Reduction in homocysteine levels – cofactor for MTHFR (only in those with hyperhomocysteinemia)

Direct ROS scavenging effect

Increase BH4:BH2 ratio, thus increasing NO production

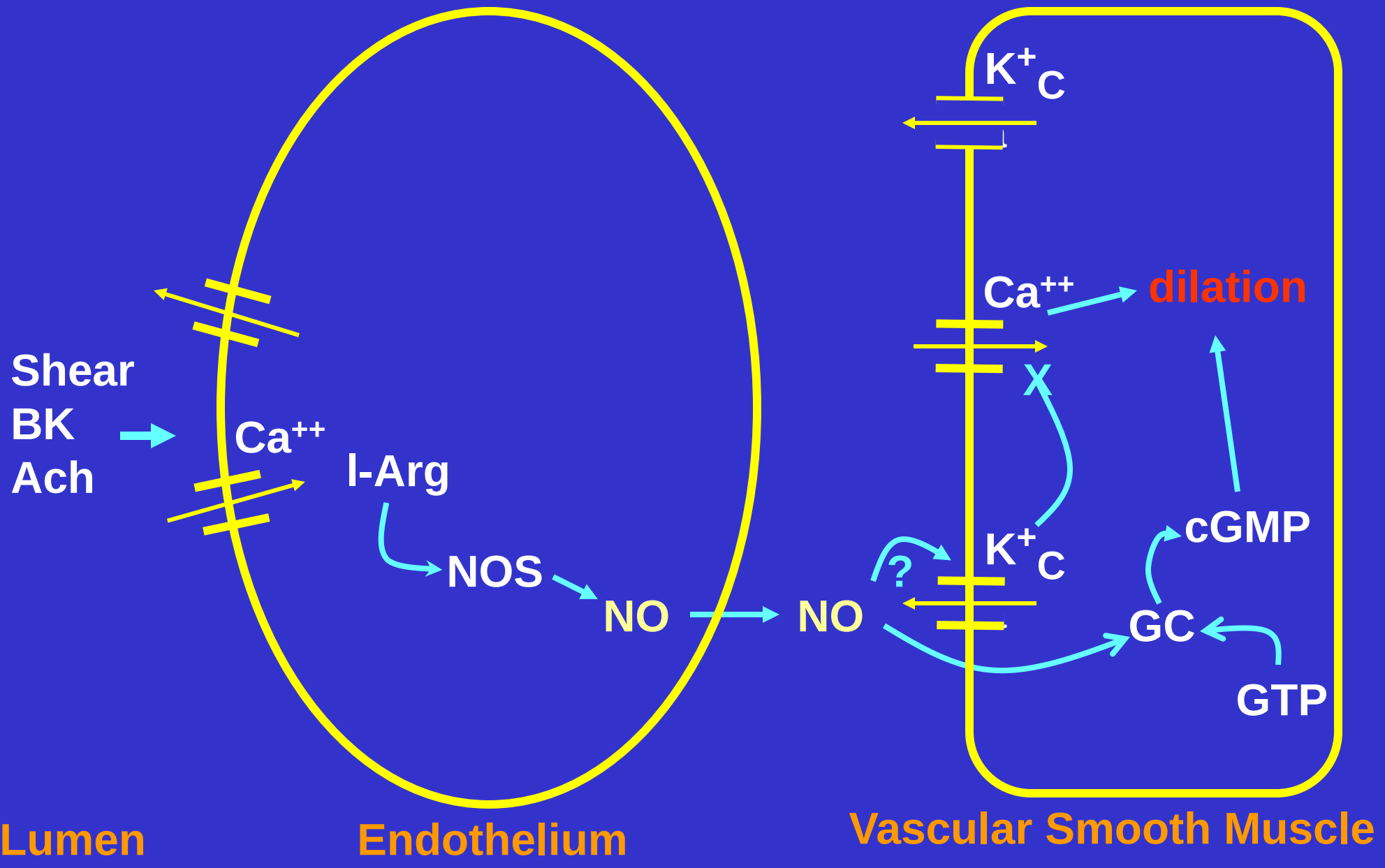
First Description of Endothelial-Dependent Vasodilation



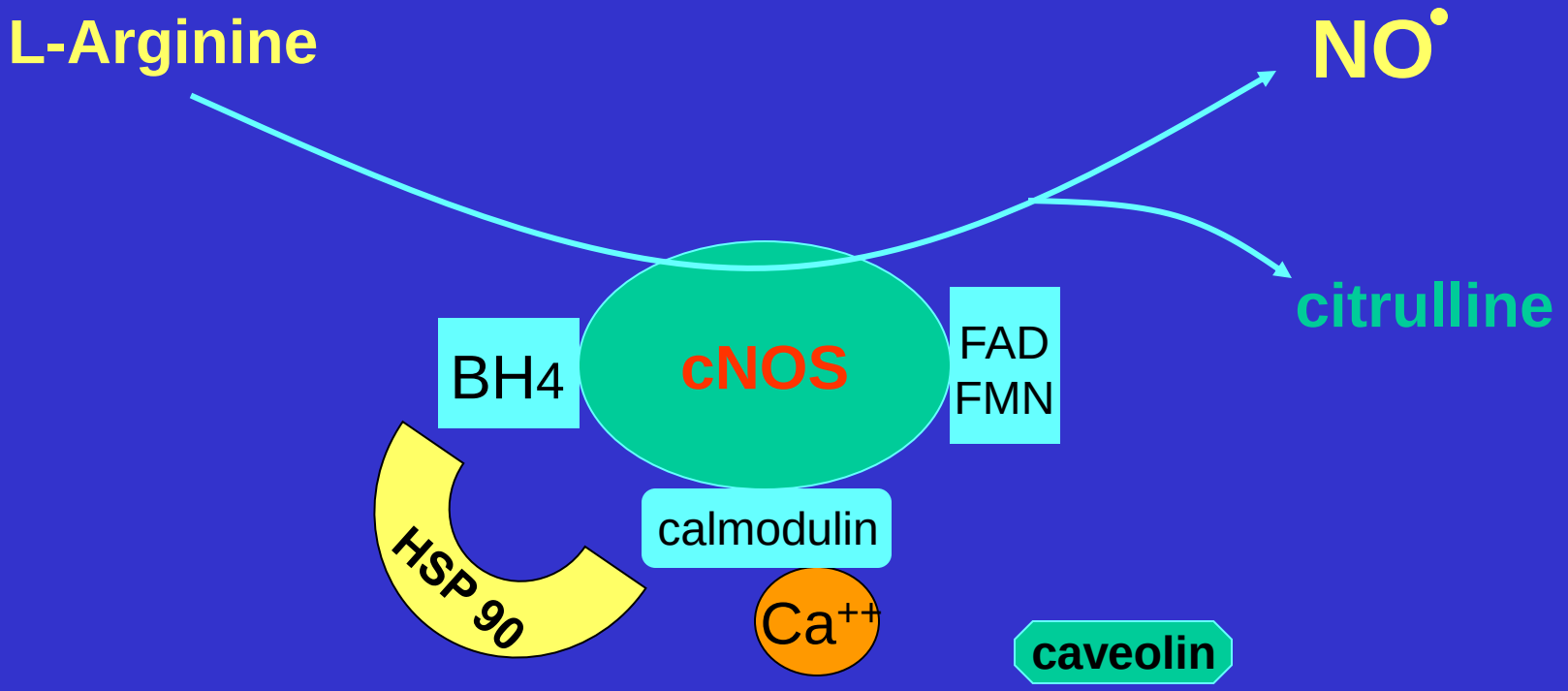


- Rabbit aorta
- NE constriction
- Ach dilation

- Furchgott and Zawadzki, Nature 1980


Shear
BK
Ach
Ca++
l-Arg
NOS
NO
NO
?
K+C
Ca++
dilation
X
K+C
cGMP
GC
GTP
Lumen
Endothelium
Vascular Smooth Muscle

Endothelial Nitric Oxide Synthase



Nitric Oxide Synthase Isoforms

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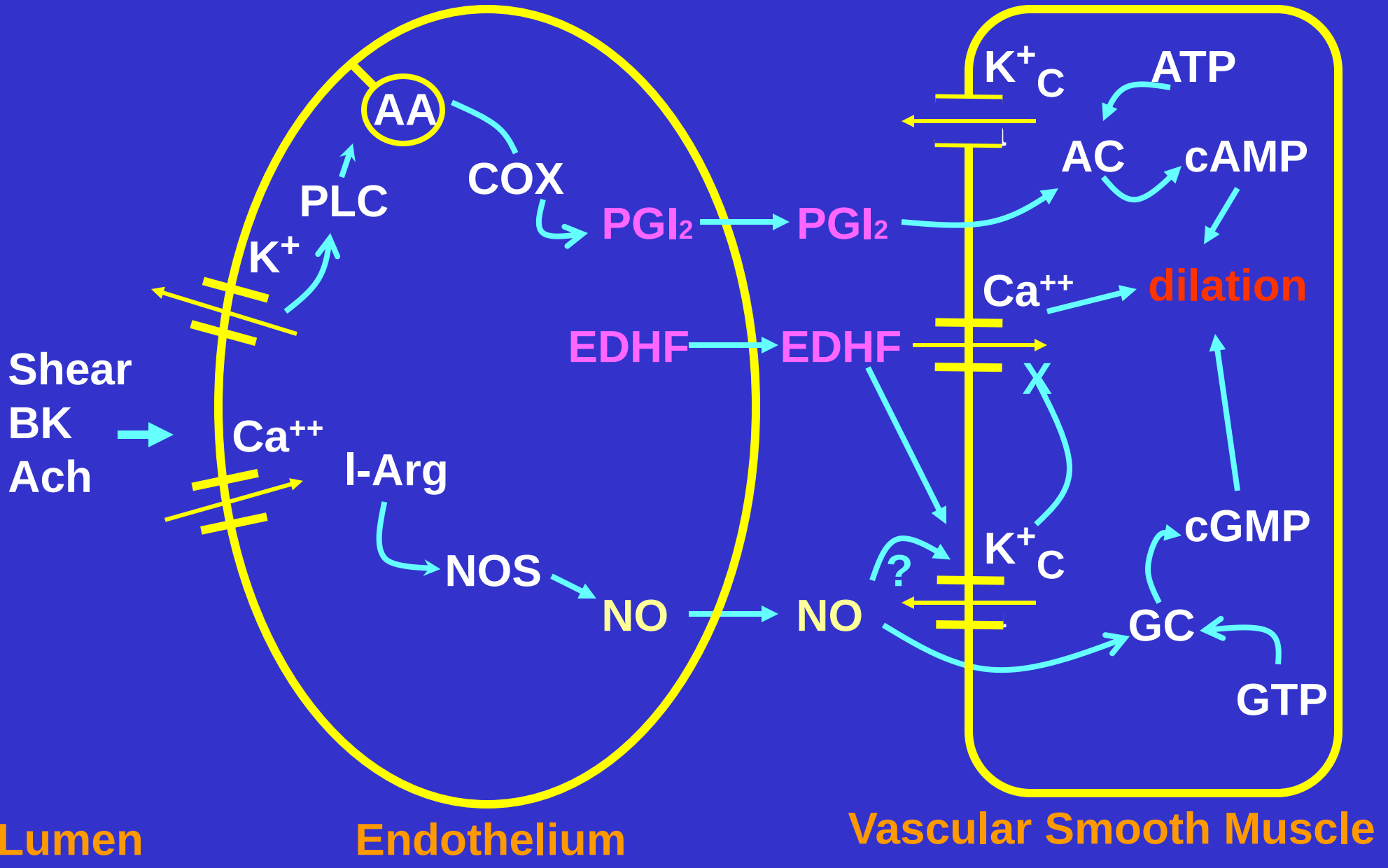
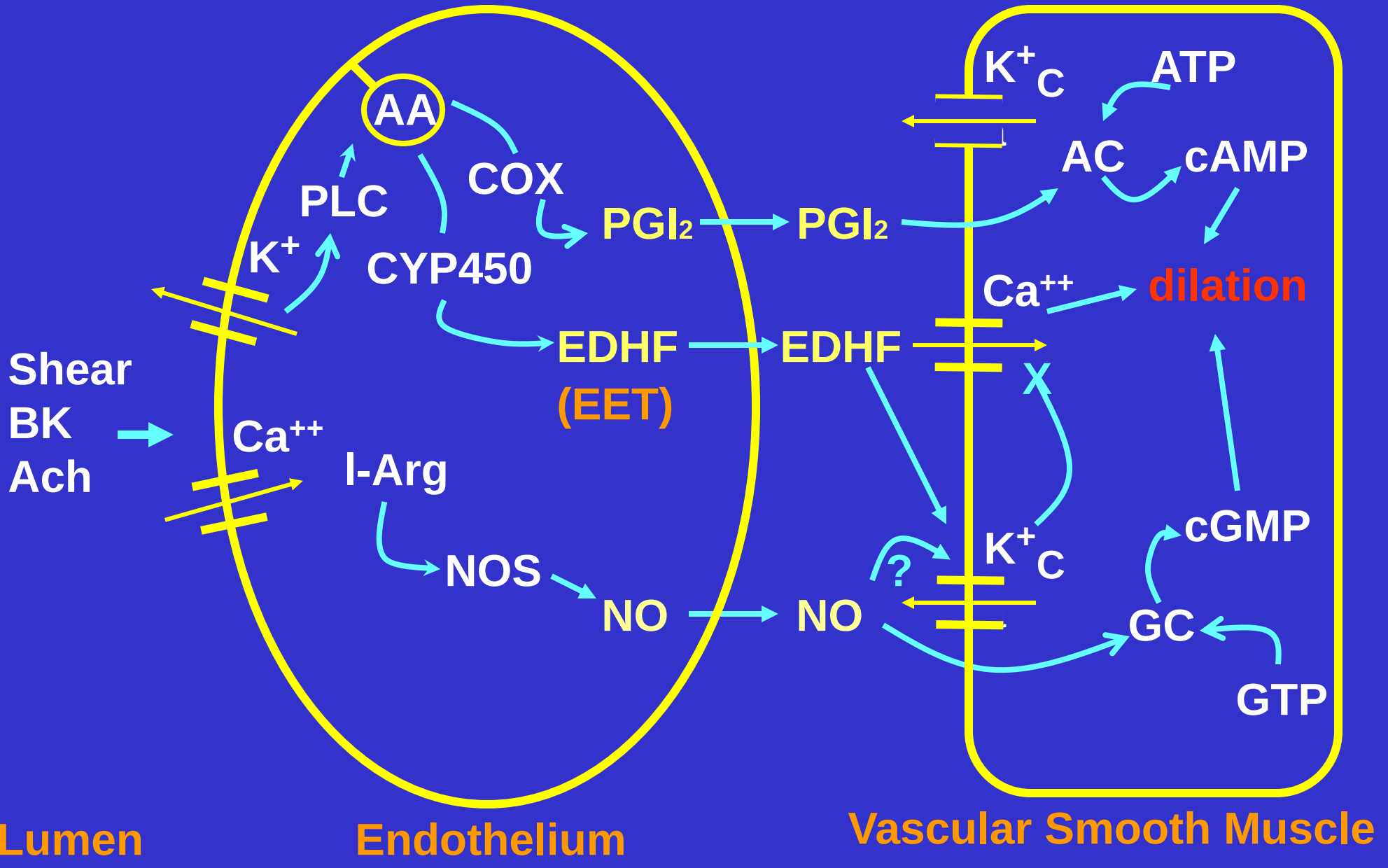
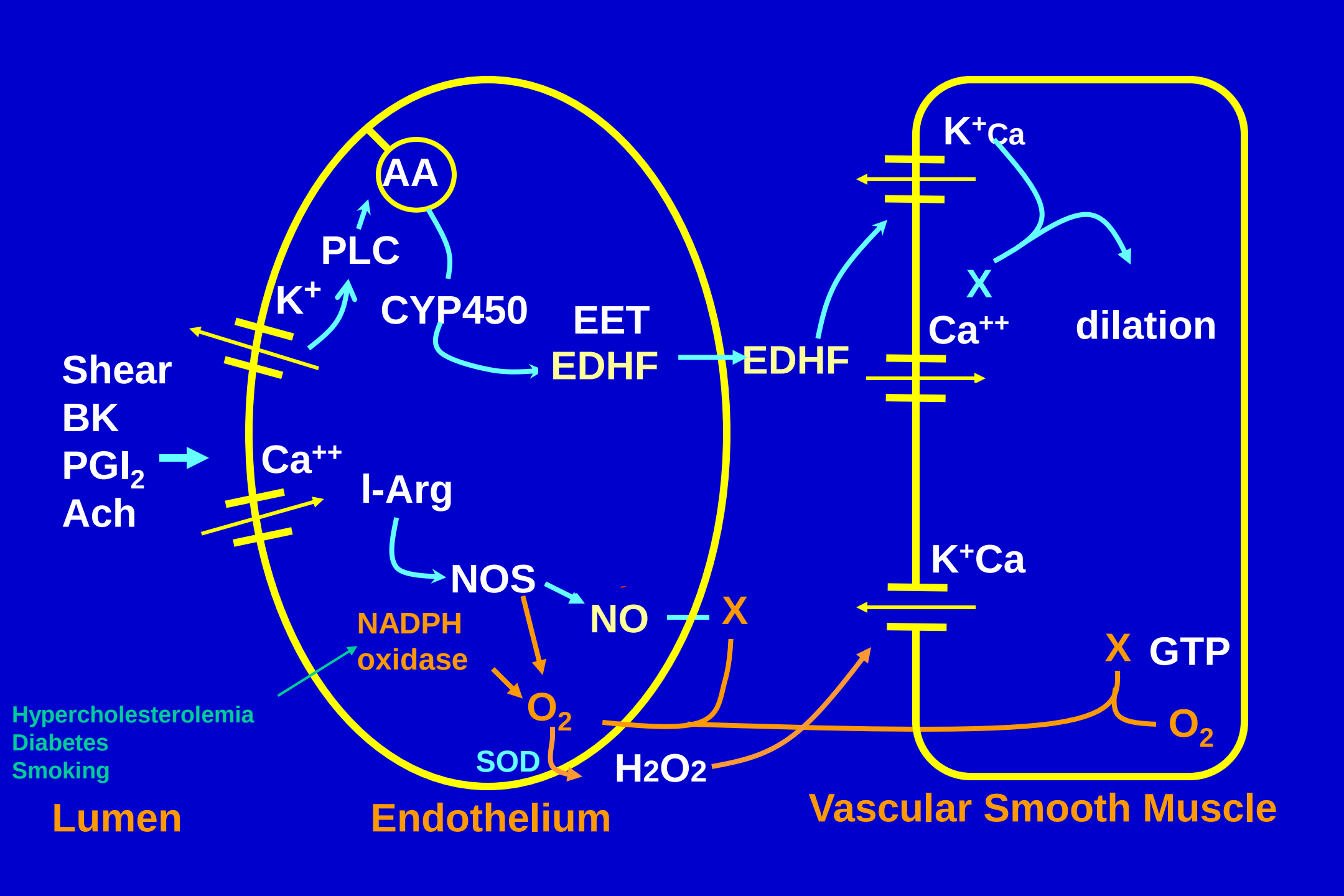
Caveolin sensitive	+	-	+
NO Production	+	+++	+
Localization			
Endothelium	+	+	+++
Smooth muscle	+	+++	+
Neurons	+++		


Shear
BK
Ach
Ca++
K+
PLC
AA
COX
PGI2
PGI2
EDHF
EDHF
l-Arg
NOS
NO
NO
?
K+C
K+C
ATP
AC
cAMP
Ca++
dilation
cGMP
GC
GTP
Lumen
Endothelium
Vascular Smooth Muscle

Possible Etiologies of EDHF

- Potassium ions
- Epoxyeicosatrienoic Acid
- Hydrogen Peroxide
- Nitric Oxide
- Prostacyclin
- Gap Junctions

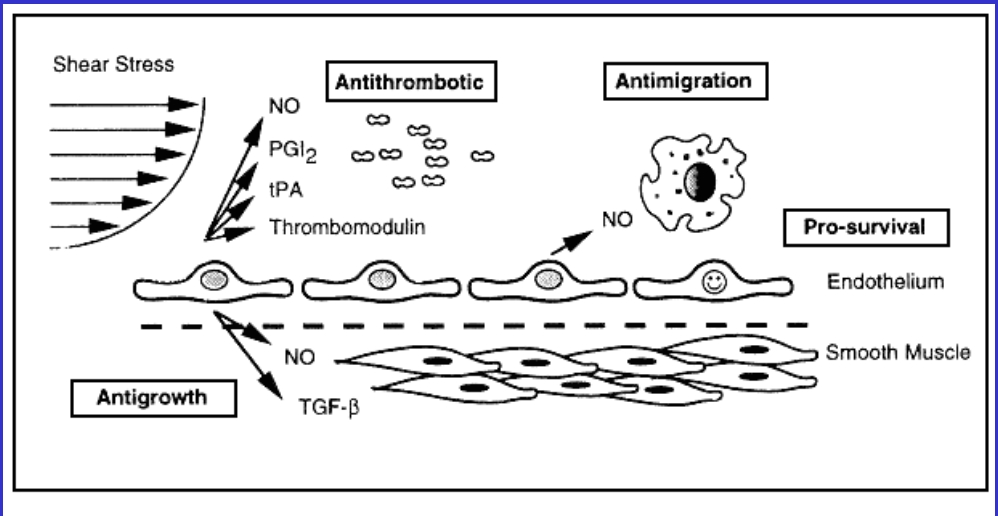

Shear
BK
Ach
Ca++
K+
PLC
AA
COX
CYP450
PGI2
EDHF
(EET)
l-Arg
NOS
NO
PGI2
EDHF
NO
K+C
ATP
AC
cAMP
Ca++
dilation
?
K+C
cGMP
GC
GTP
Lumen
Endothelium
Vascular Smooth Muscle


AA
PLC
K+
CYP450
EET
EDHF
EDHF
K+Ca
X
Ca++
dilation
Shear
BK
PGI2
Ach
Ca++
l-Arg
NOS
NO
X
K+Ca
NADPH
oxidase
X
GTP
O2
O2
SOD
H2O2
Hypercholesterolemia
Diabetes
Smoking
Lumen
Endothelium
Vascular Smooth Muscle

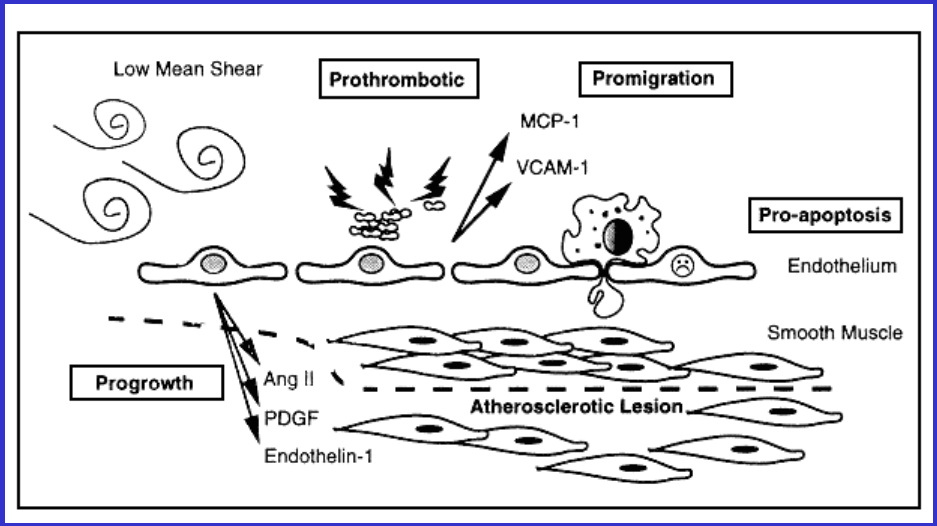
Is NO-mediated Vasodilation Really Important?

Endothelial Dysfunction Leads to Atherosclerosis (Shear and Atherosclerosis)

Laminar shear



Flow reversals

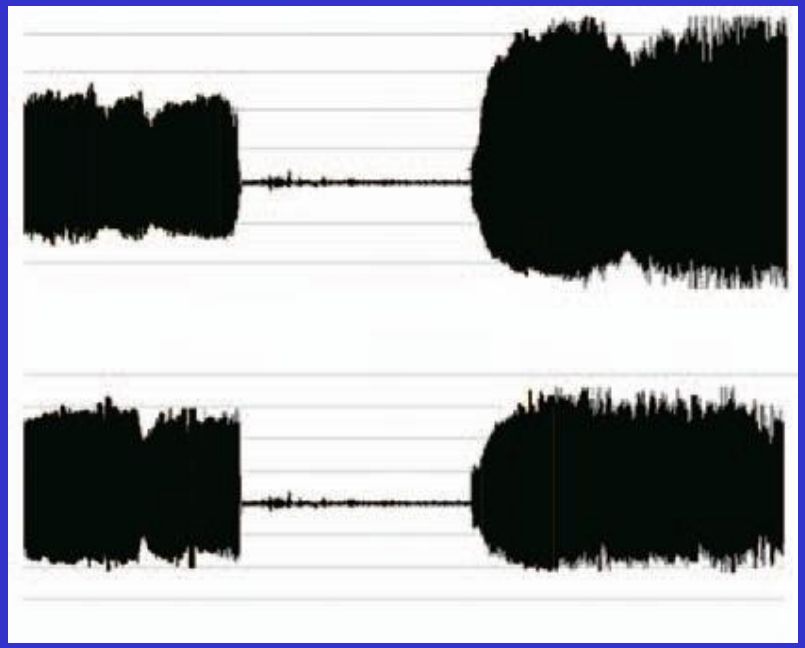


- Traub et al. ATVB 1998

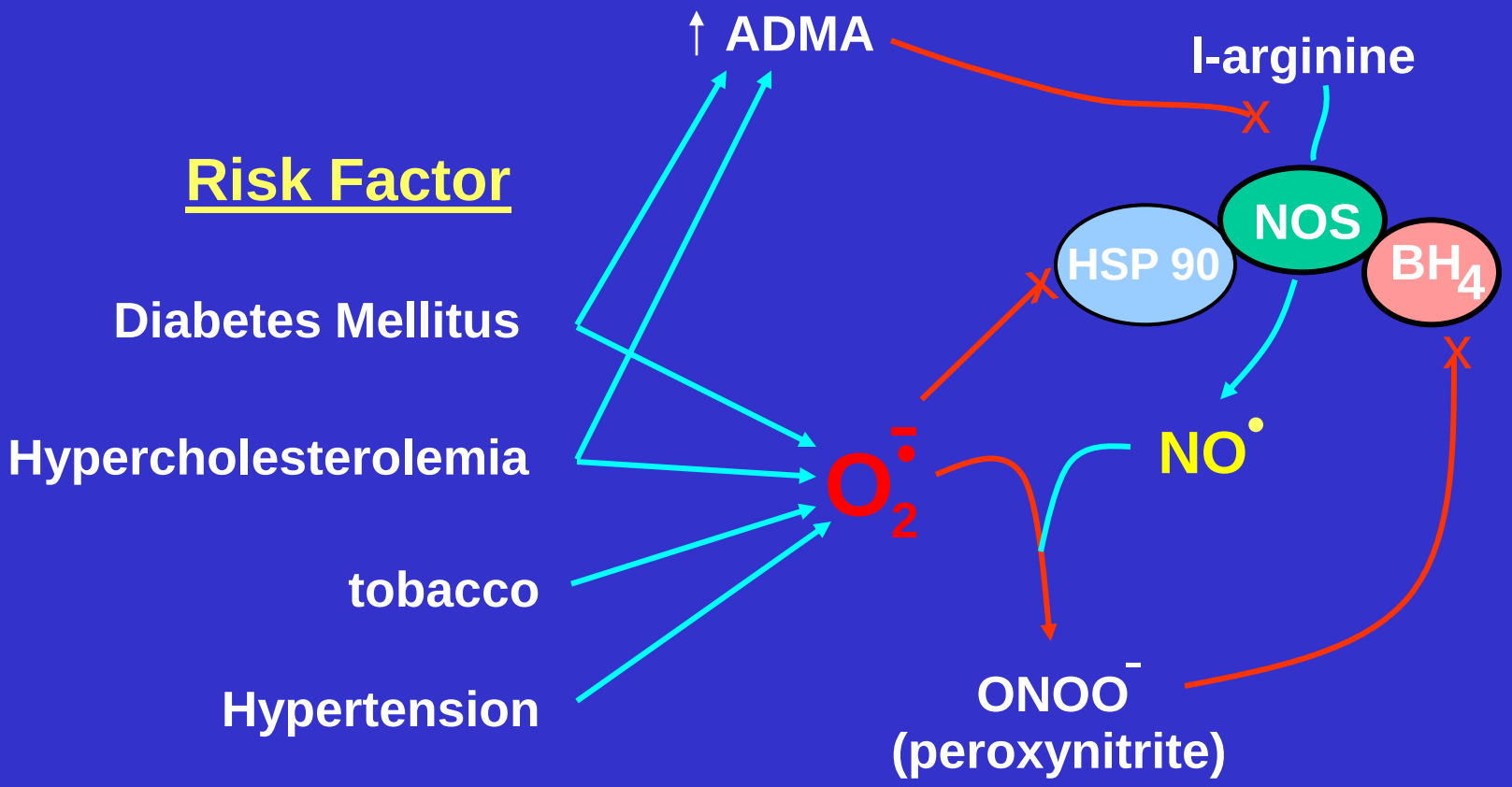
In Vivo Measurement of Endothelial Function (Endo-PAT 2000)

Normal Endothelial Function

Abnormal Endothelial Function



Mechanism of Reduced Vascular NO



Development of Atherosclerosis