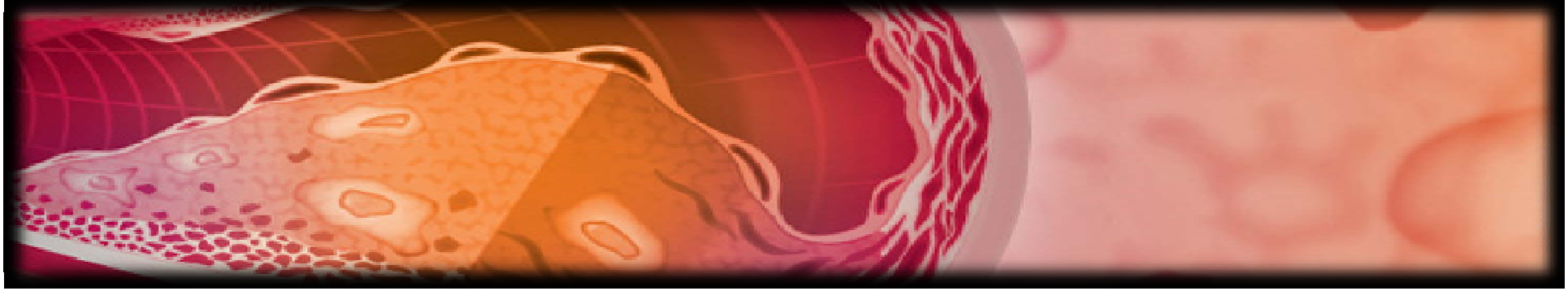
How To

Vascular Function in Animal and Clinical Study

Yeungnam Univ. Hospital

Jong-Seon Park, MD, PhD



Vascular Function Test, When ?

Potential Applications:

- Identification of cardiovascular risk (e.g. patient with intermediate risk)
- Evaluation of novel risk factors
- ***Evaluating patients for lifestyle, pharmacologic, and/or mechanical interventions***
- Investigation of mechanisms of atherosclerosis and vascular dysfunction
- Assess efficacy of therapy

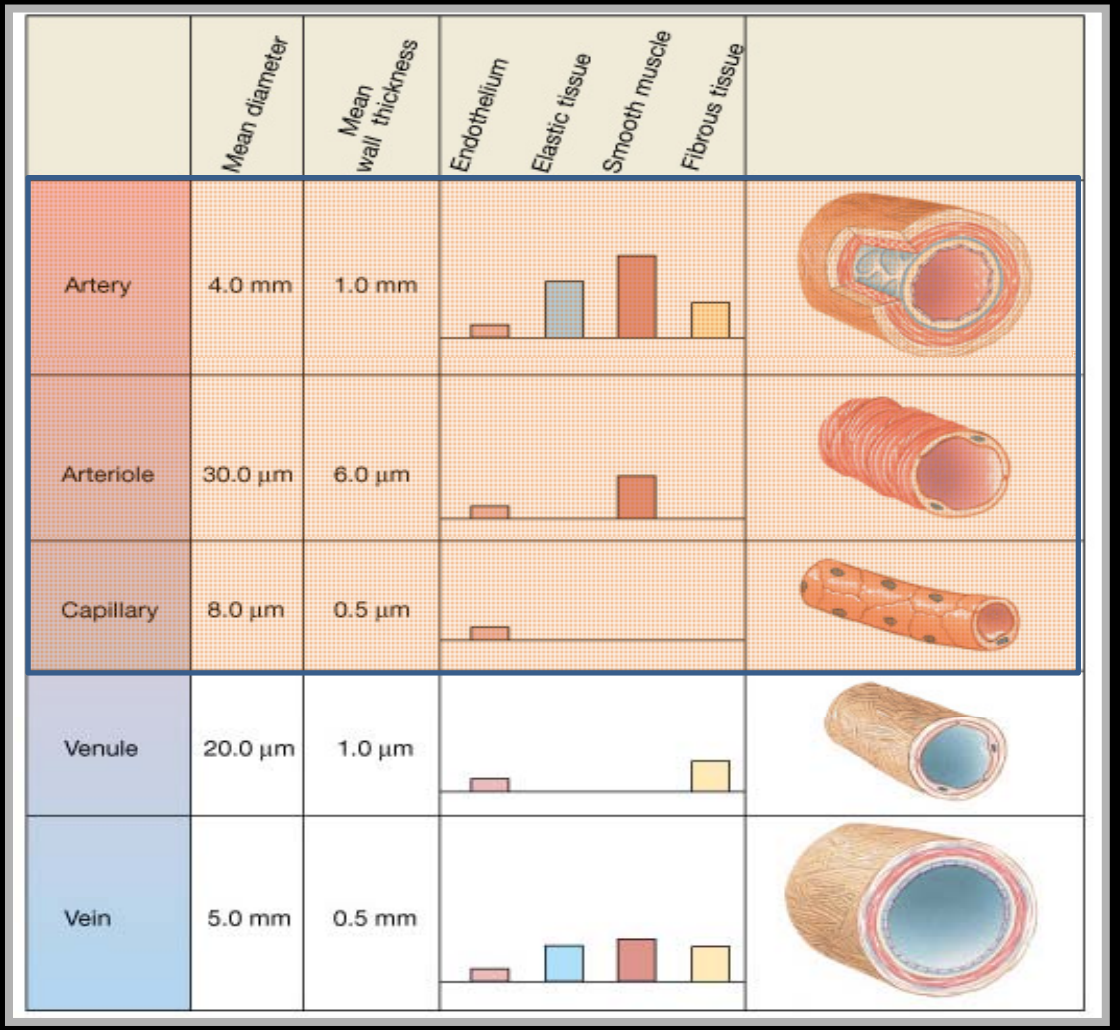
Contents

1. Vascular anatomy and physiology
2. Animal model
   a. Endothelial and vascular function
   b. Anatomical evaluation
3. Evaluation of vascular function in clinical studies

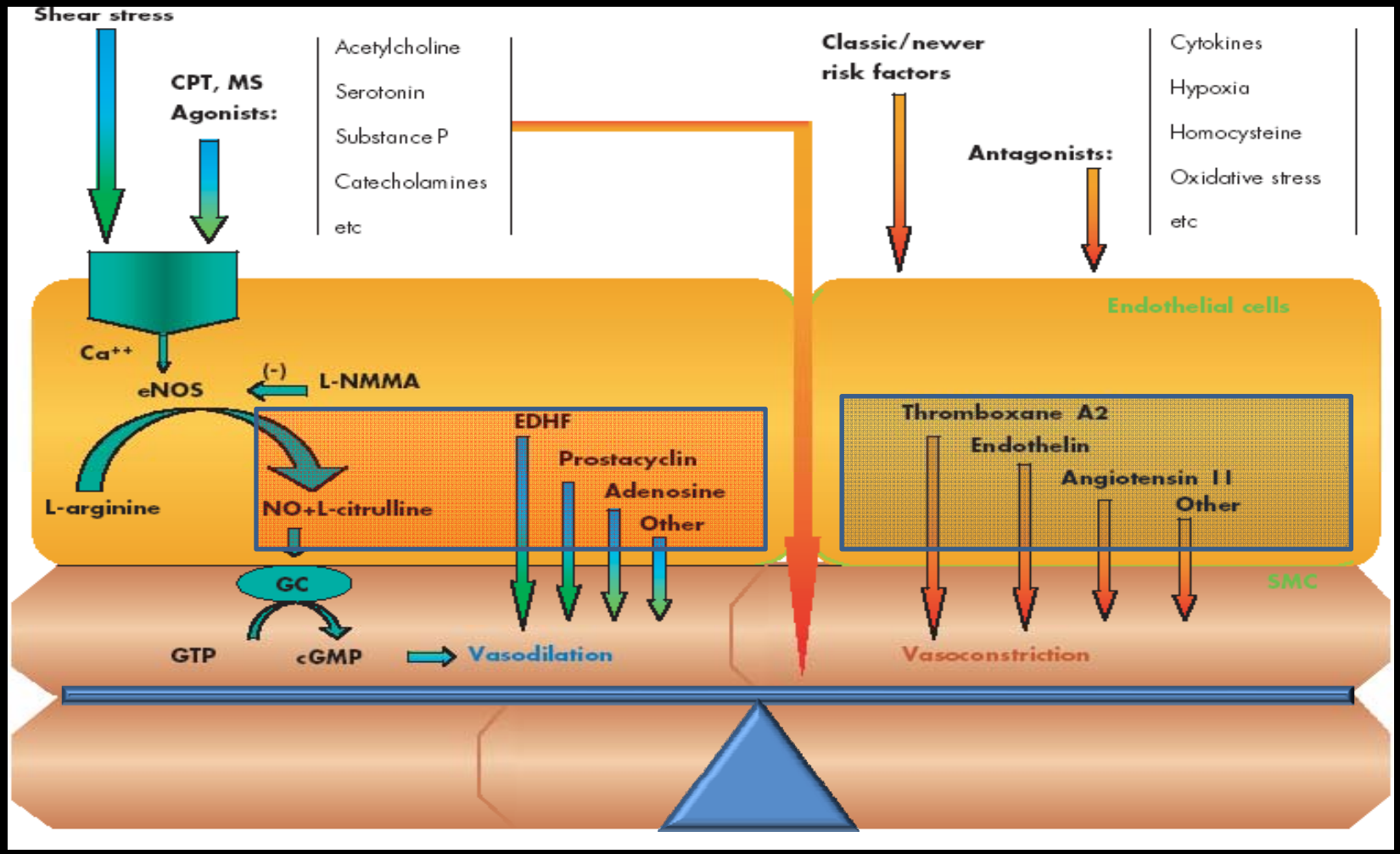
Make Up of Blood Vessels: Arteries and Arterioles

- **Endothelium**
- **Elastic tissues**
  - Rebounds
  - Evens flow
- **Smooth muscles**
- **Fibrous tissue**
  - Tough
  - Resists stretch

***** RBC : 7 µm***

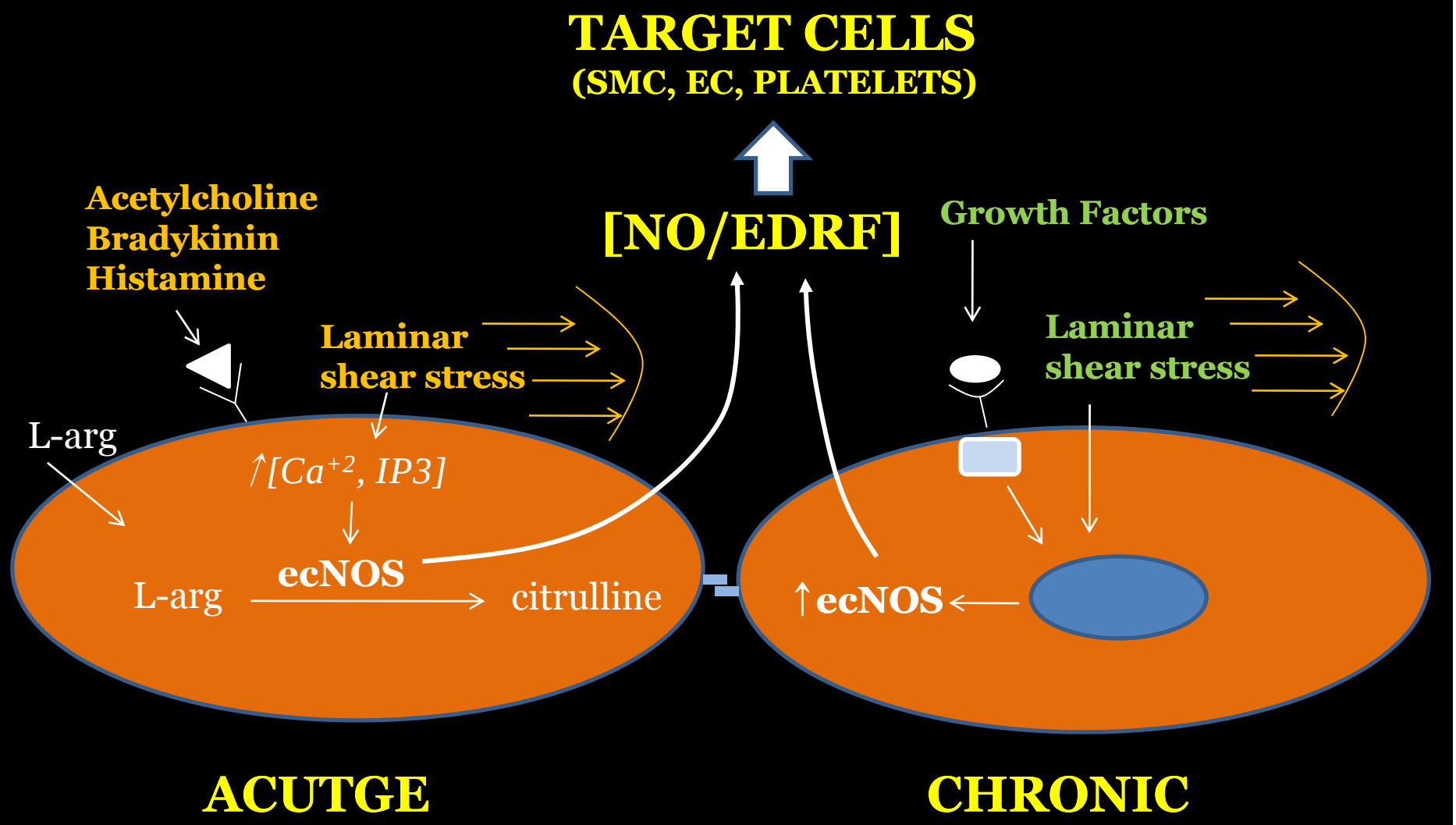


Maintaining the vascular tone: Vasodilation and Vasoconstriction



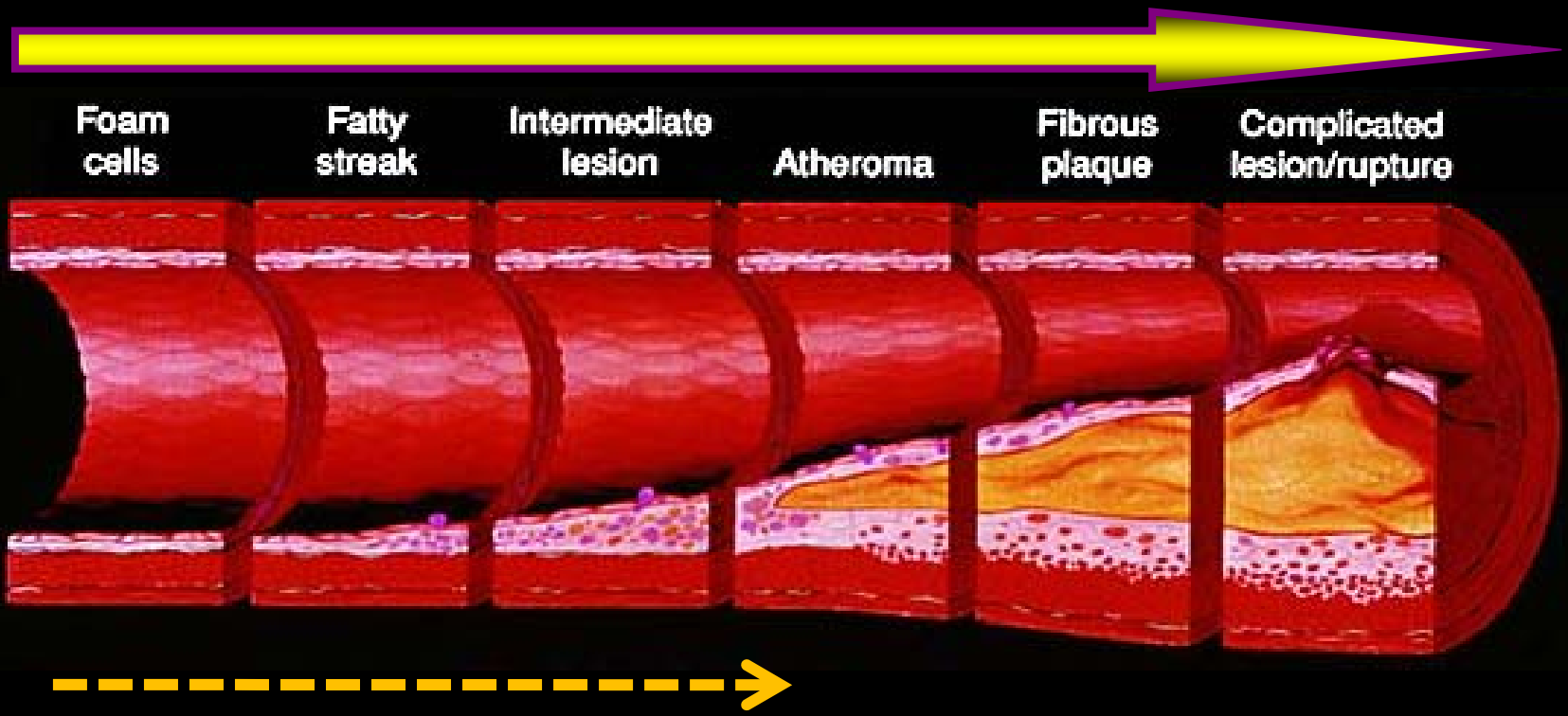
Tousoulis, et al., Heart 2005; 91: 353-358.

Endothelial Nitric Oxide (NO) Production



SMC, smooth muscle cell; EC, endothelial cell, IP, phosphoinositide metabolites

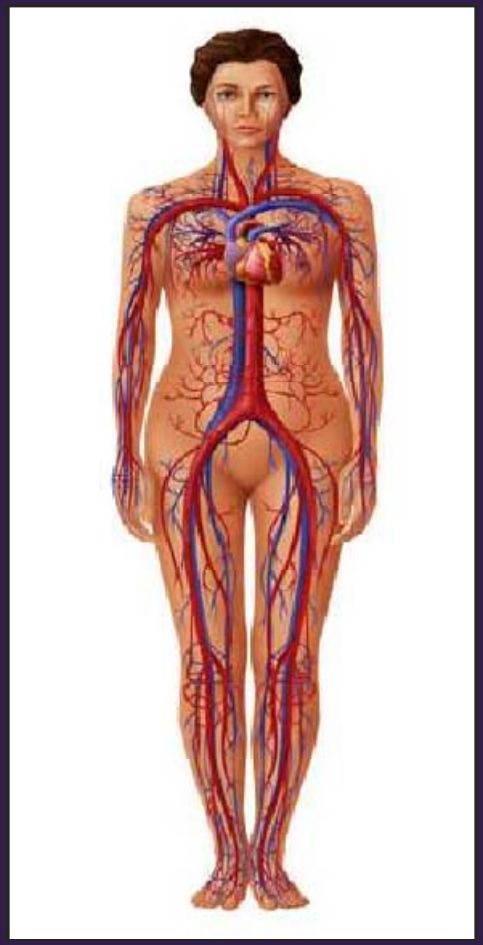
Stages of Atherosclerosis and Evaluation

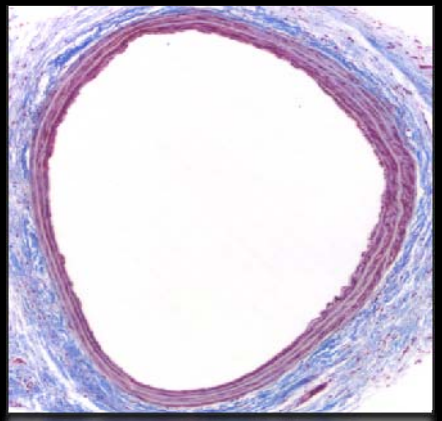


Functional evaluation

Anatomical evaluation (Imaging tools)

How can we evaluate vascular function ?





Pathologic state
Hormone
Cytokine
Nervous system
Volume state

1. **Regional response to drug or flow**

2. **Systemic hemodynamic change**

Methods for exploring endothelial function.

(Guerci et al., Diabetes Metab 2001; 27: 425-434.)

Type	Stimulus	Parameters measured	Evaluation techniques
Biochemical in vivo indirect methods	Pharmacological (L-arginine)	Plasma NO and PGI_2	Biochemical assays
	Physiological (ADP, 5-HT, histamine)	Urinary nitrate, nitrites	
		Adhesion molecules	
		Coagulation factors	
In vitro direct methods	Pharmacological(L-NAME, phenylephrine, noradrenaline, inhibitors of NOS and COX)	Flow	Cell culture
	Physiological (shear stress)	Shear rate	Shear rate
Invasive in vivo direct methods	L-NMMA (intra arterial)	Arterial diameter	Ultrasonography
	Acetylcholine, serotonin, bradykinin, substance P (intra arterial)	Arterial flow	Plethysmography
Non-invasive in vivo direct methods	Shear stress (post-ischemic dilation)	Arterial diameter	Ultrasonography
		Arterial flow	Plethysmography
			Echotracking
	Dipyridamole (intra venous)	Arterial flow	Positron emission tomography

Multi Chamber Myograph; Vascular Response to Drugs

Harvest Vascular Tissue

Preconditioning (vasoconstriction)

- Norepinephrine
- Phenylephrine

1. **Endothelium dependent response**

-Acetylcholine

- N[ω]-nitro-L-arginine

(L-NNA, 100 μM).; inhibitor

2. **Endothelium independent response**

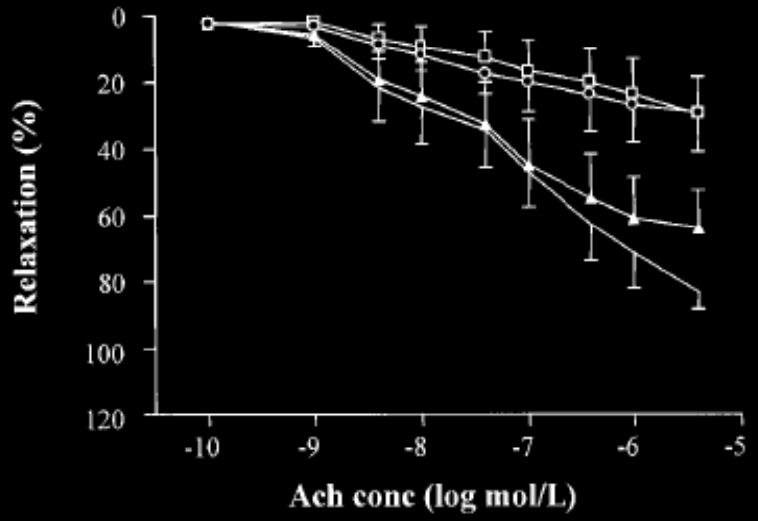
- Sodium nitroprusside

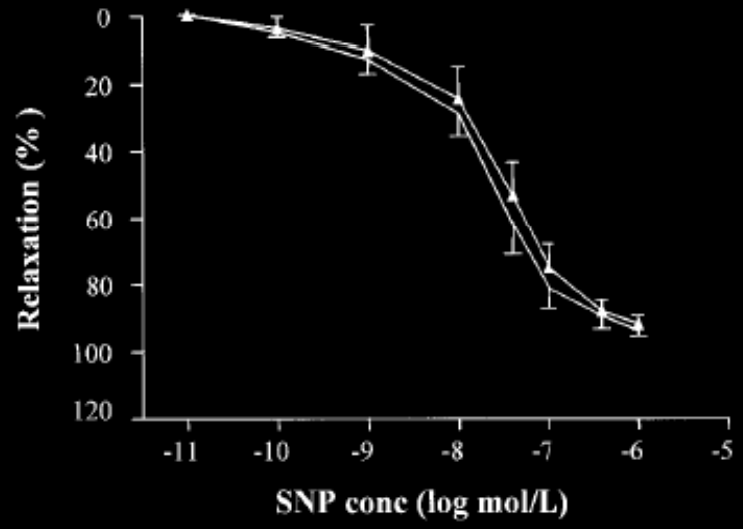
Multi Chamber Myograph from Danish Myo Technology

multiple testing of vessel reactivity with the chambers (8 ml volume)

Endocrinology 2001 142(8) 3317-3323

Multi Chamber Myograph; Vascular Response to Drugs

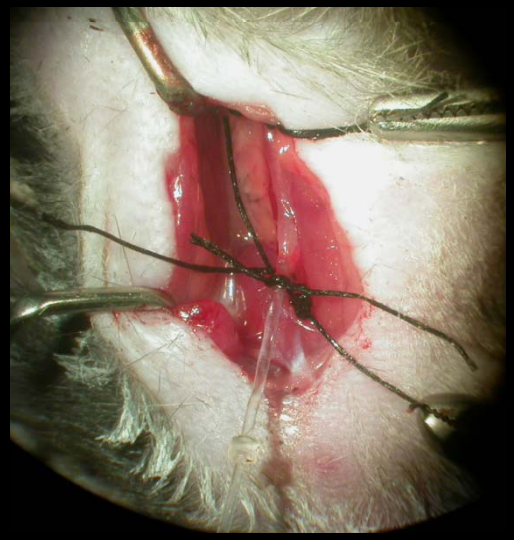


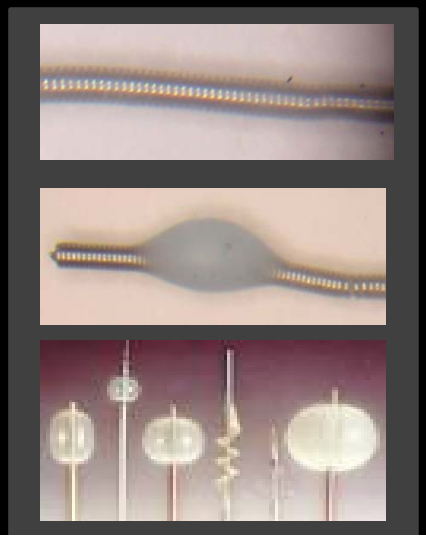


WT (L-NNA) n=7
bGH (L-NNA) n=8
WT n=8
bGH n=8

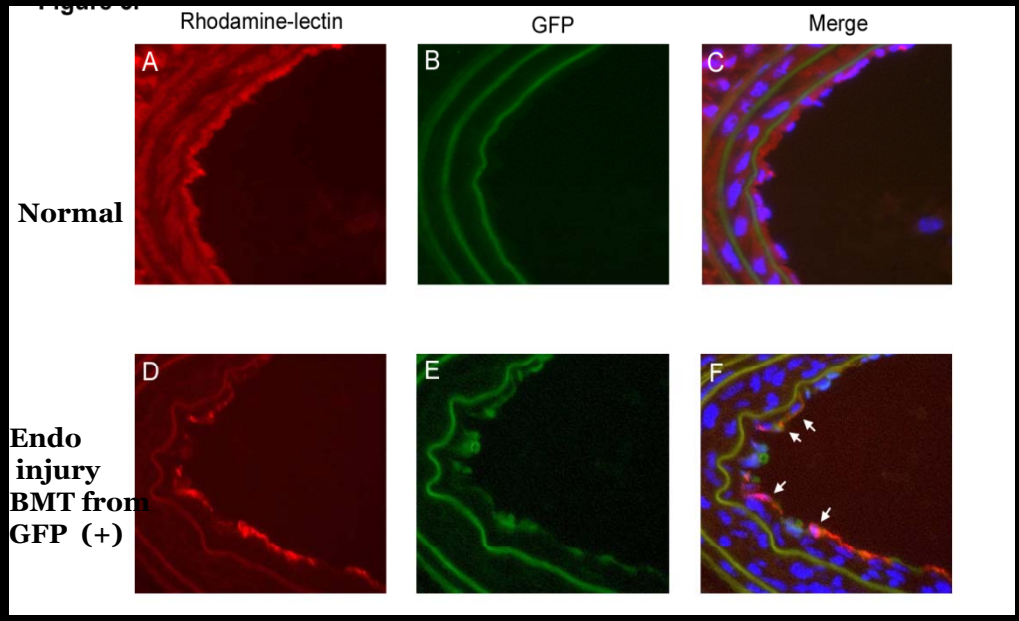
Endocrinology 2001 142(8) 3317-3323

Carotid A. Injury ; Endothelial Denudation Model in Mice and Rats



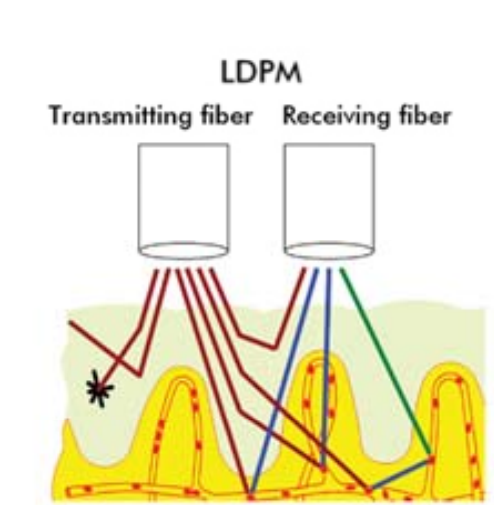


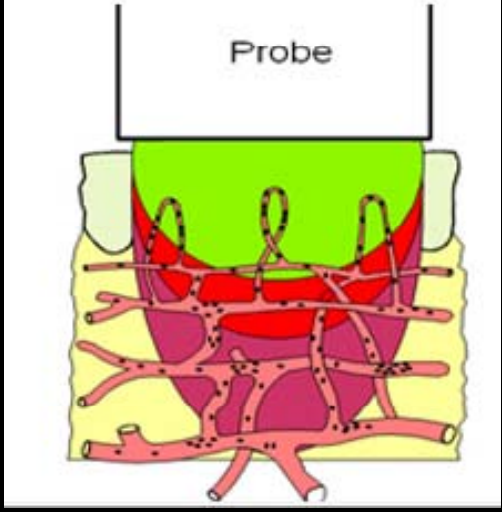
Immunofluorescence stain



EB stain

Laser-Doppler Instruments

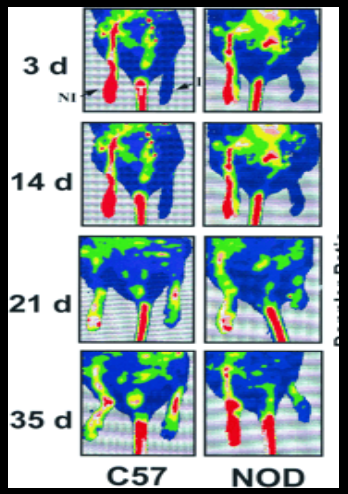




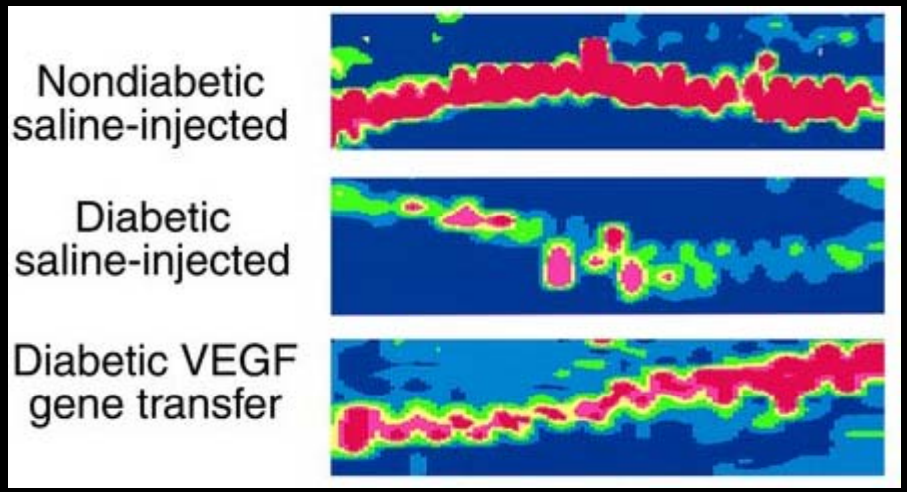
- ✓ **Measuring depth : 0.5-1 millimeter**
- ✓ **No current laser Doppler instrument can provide absolute perfusion values (e.g. ml/min/100 gram tissue).**
- ✓ **Measurements are expressed as Perfusion Units (PU), which are arbitrary**

Laser-Doppler Instruments

Laser-Doppler fluximetry (LDF) and Laser-Doppler imaging (LDI)



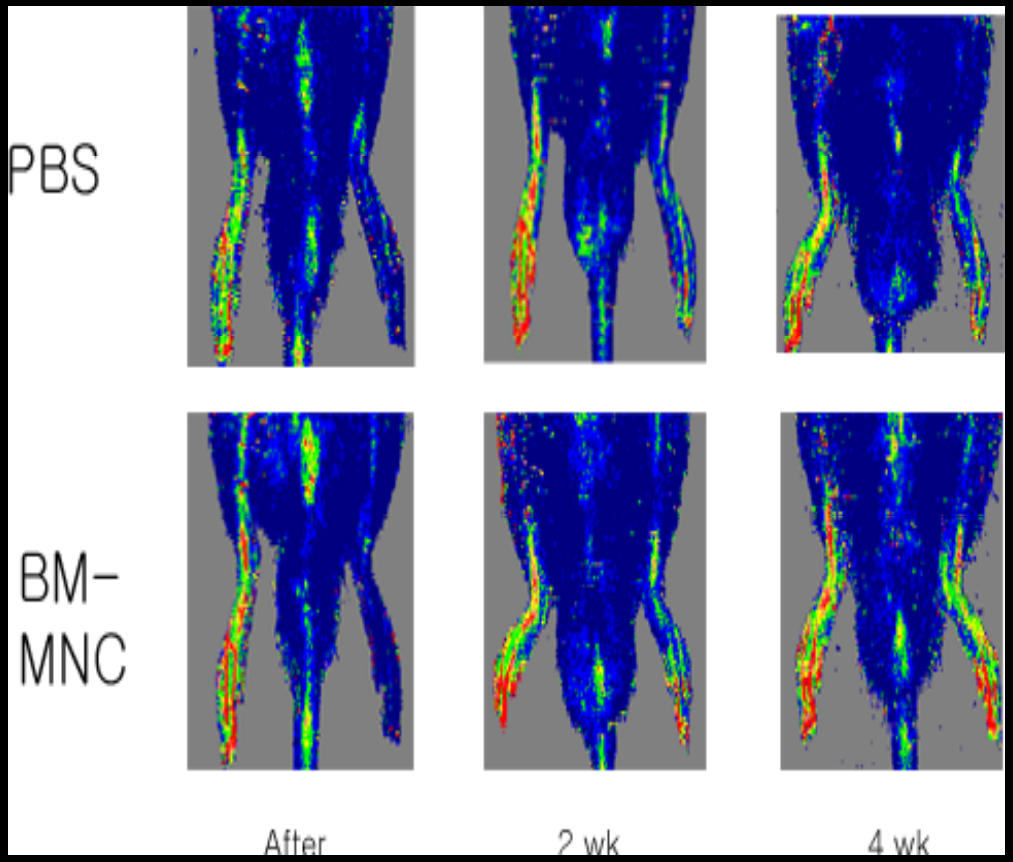
Circulation. 2009 Feb 10;119(5):699-708

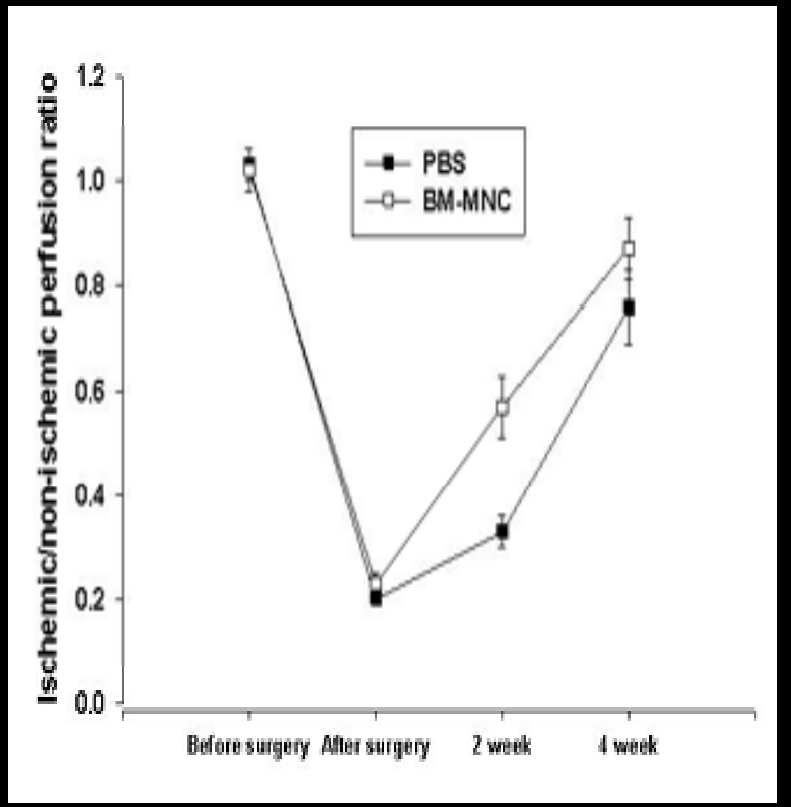


J. Clin. Invest. ***107****(9): 1083-1092 (2001).*

Laser-Doppler Instruments

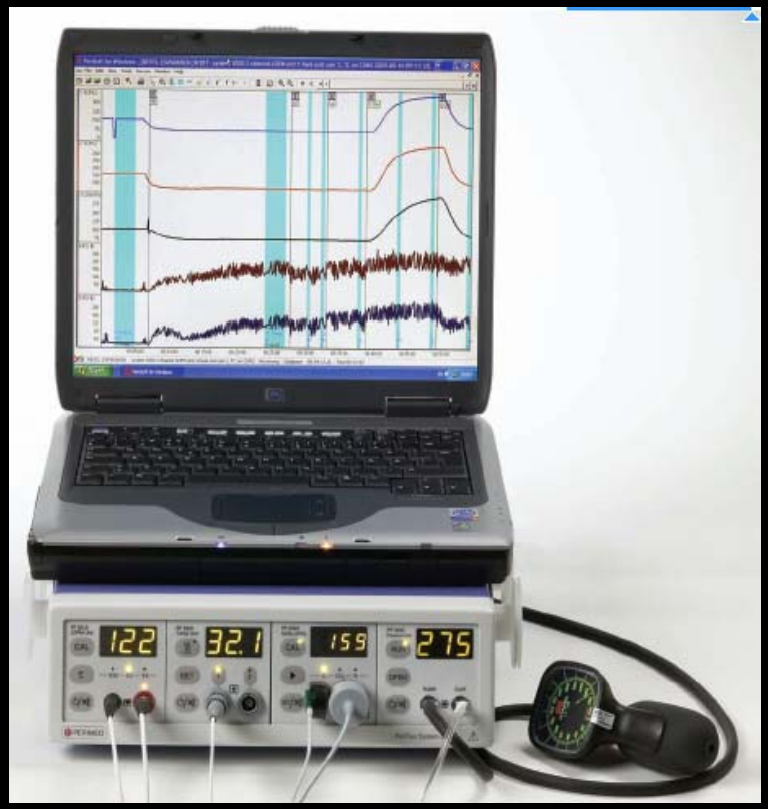
BM stem cell therapy in ischemic leg model of rats





Laser-Doppler Instruments

Laser Doppler Blood Perfusion Monitoring and t*cpO2/tcpCO2*



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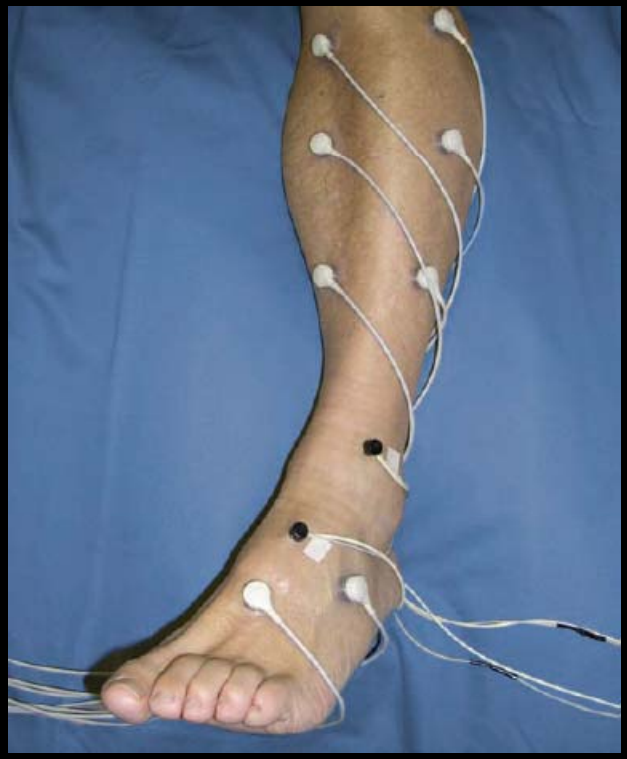
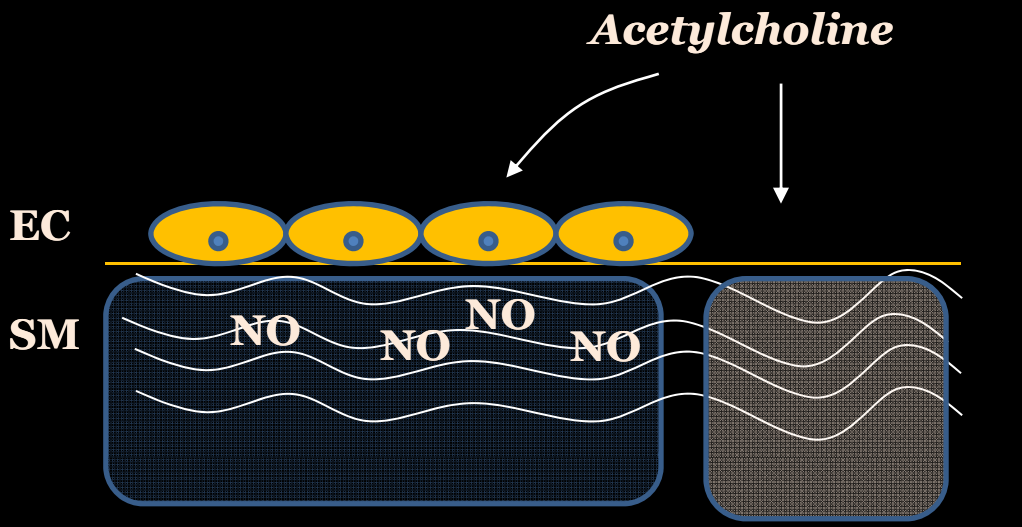
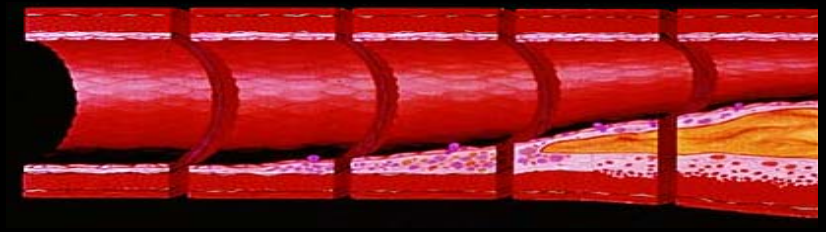


Provocation methods

1. Local thermal hyperemia
2. Inotophoresis (acetyocholine)
3. Post-occlusive reactive hyperemia

Acetylcholine Provocation Test





RCA : 20-50-80 μg LCA : 20-50-100 μg

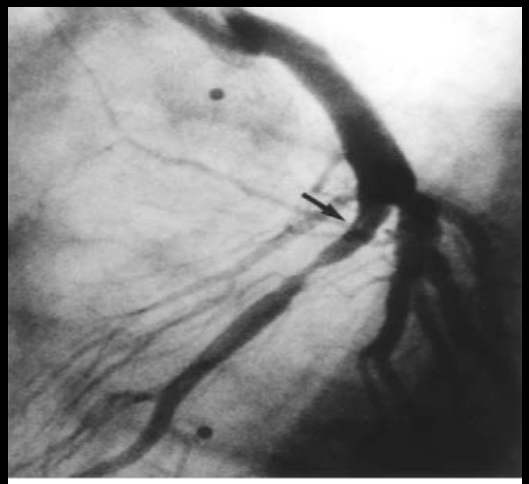
Complications

1. *VT 1.2%*
2. *VF 0.1%*
3. *Shock 0.3%*
4. *Paroxysmal A fib 17.1%*

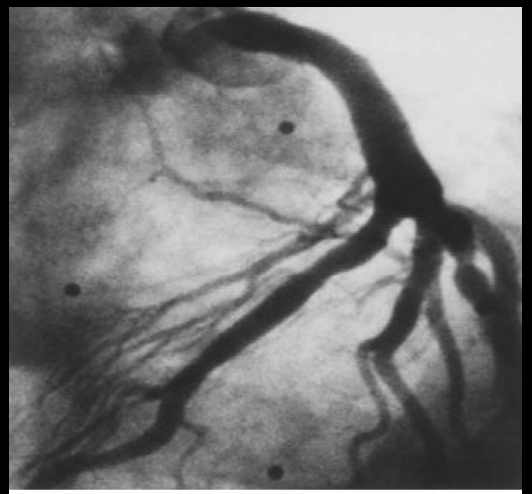
J of Cardiology 2008, 51, 131

Acetylcholine Provocation Test

Baseline



Acetylcholine

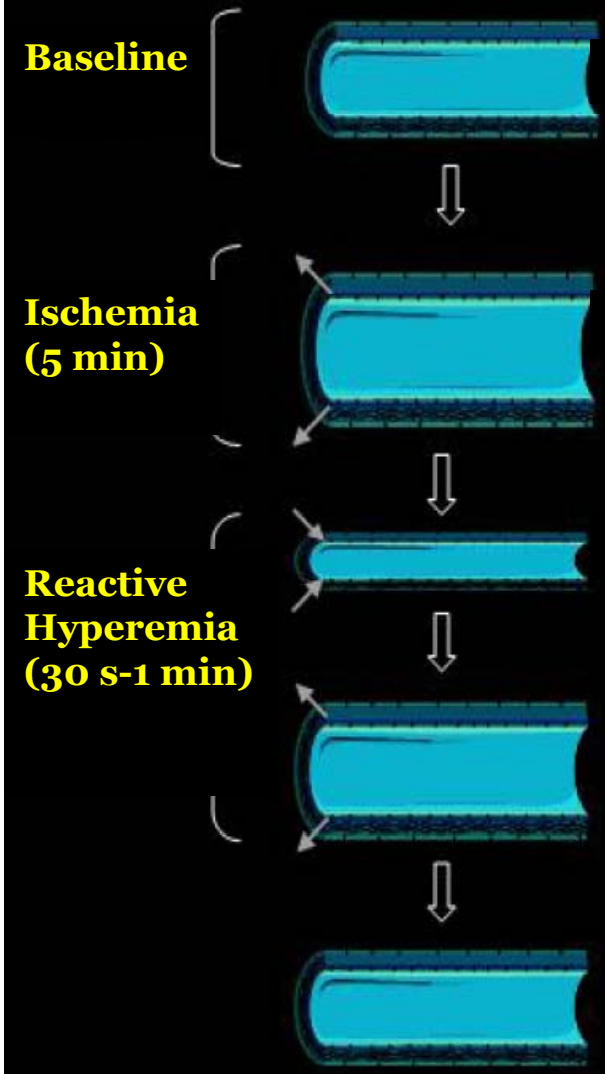


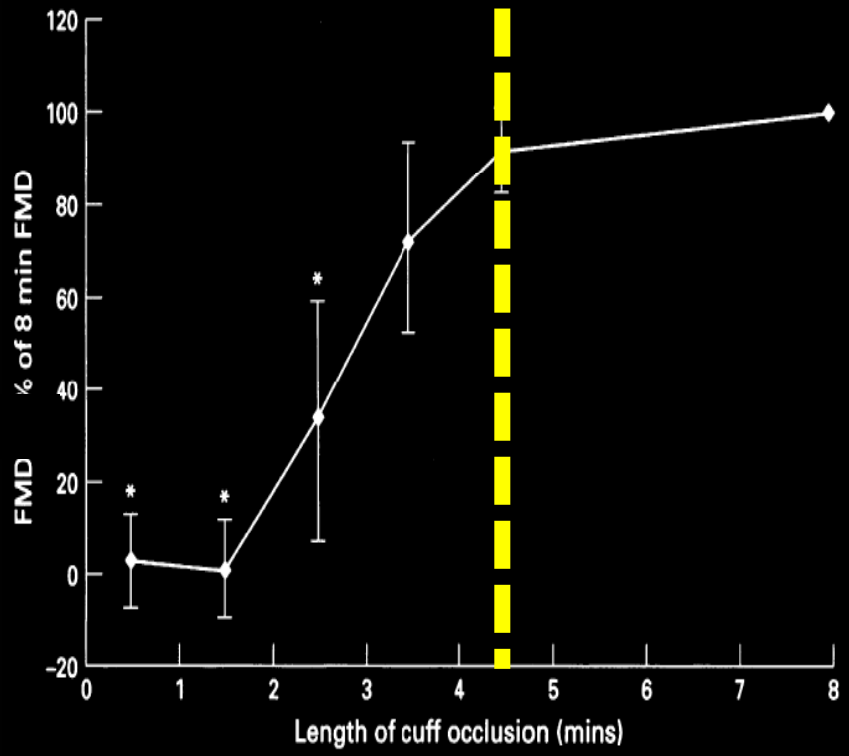
Nitroglycerin

Follow up (3.7 years)

Schachinger, et al. 2000. Circulation 101: 1899

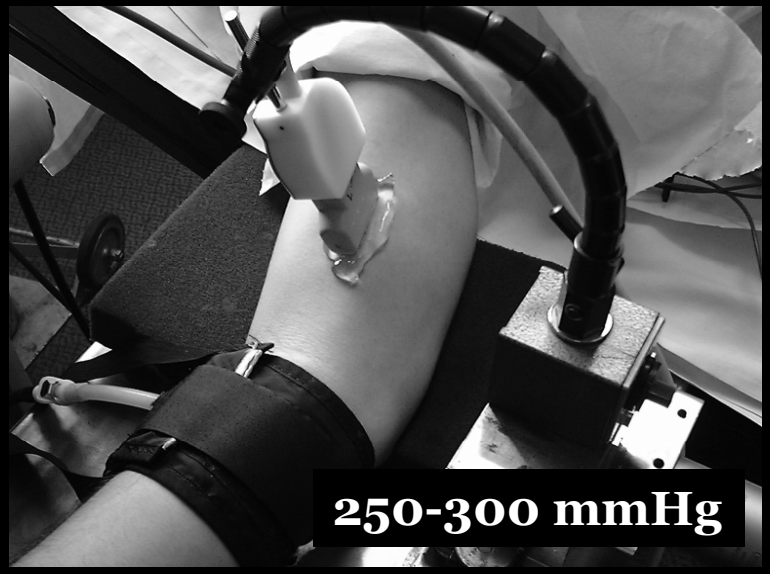
Flow Mediated Dilation

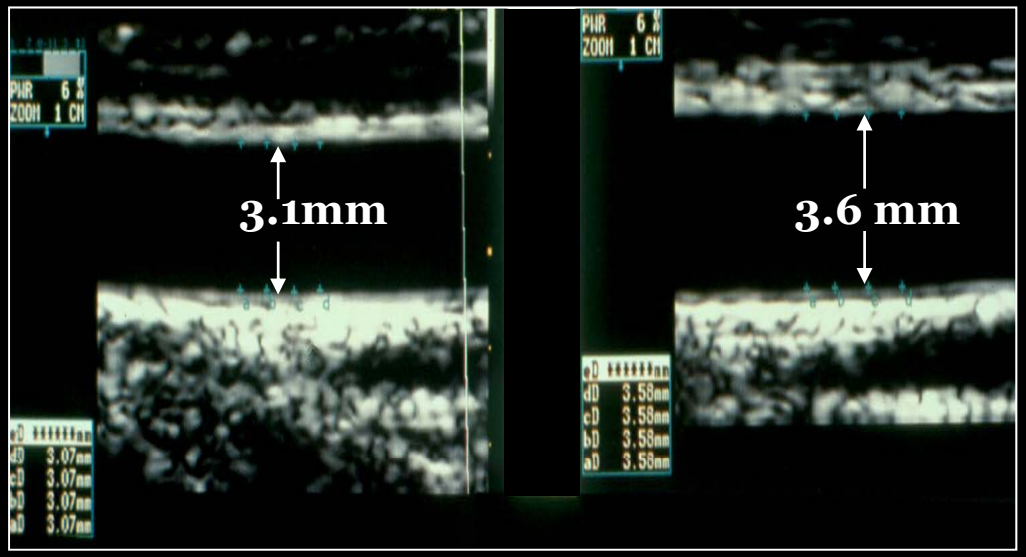




Heart. 1997 Jul;78(1):22-7.

Flow Mediated Dilation





Baseline Reactive hyperemia

Site

1. Brachial a
2. Radial a

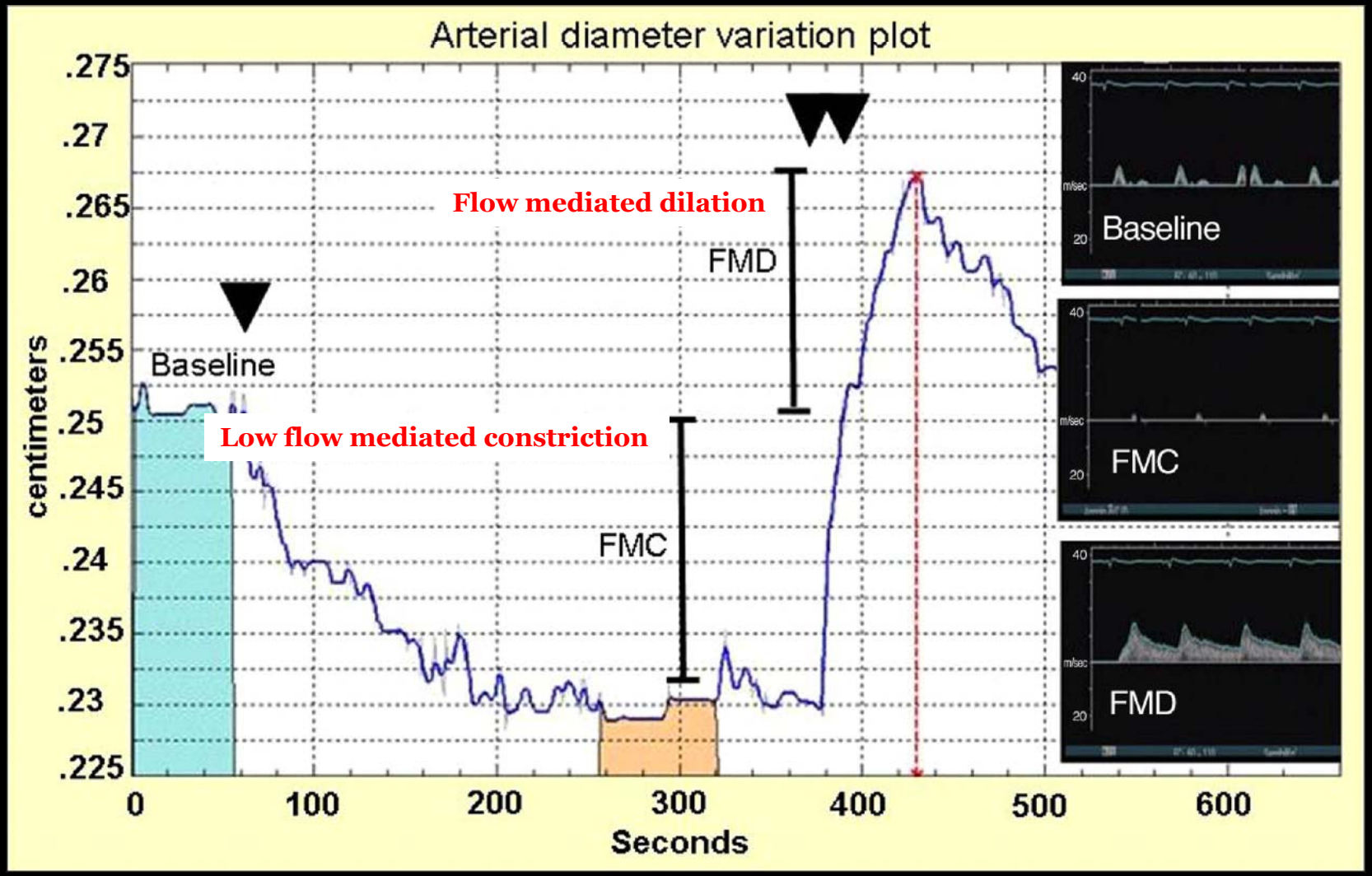
Measurement

$FMD\% = [D_P - D_B]/D_B \times 100$

Protocol

1. Baseline
2. Occlusion (5 min)
3. Reperfusion (1 min)
4. Drug response
   NTG (3 min)

Low Flow Mediated Constriction and Flow Mediated Dilation in Radial a.



J Am Coll Cardiol, 2008; 51:1953-1958

Flow Mediated Dilation

Advantages

Non-invasive

Safe and quick

Correlates with coronary vascular function

Flow is a physiological stimulus for vasodilation

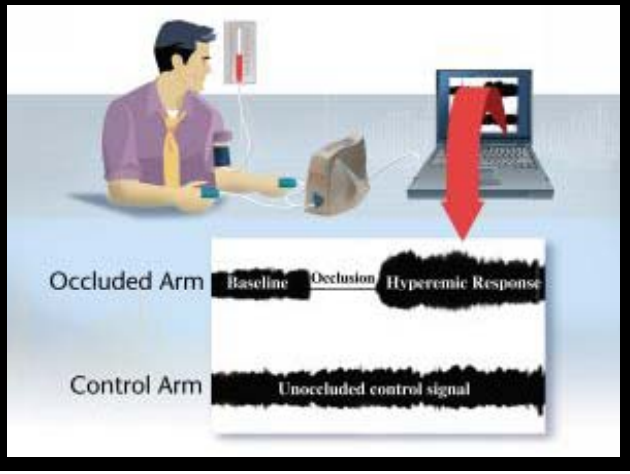
Disadvantages

Poor resolution relative to arterial size

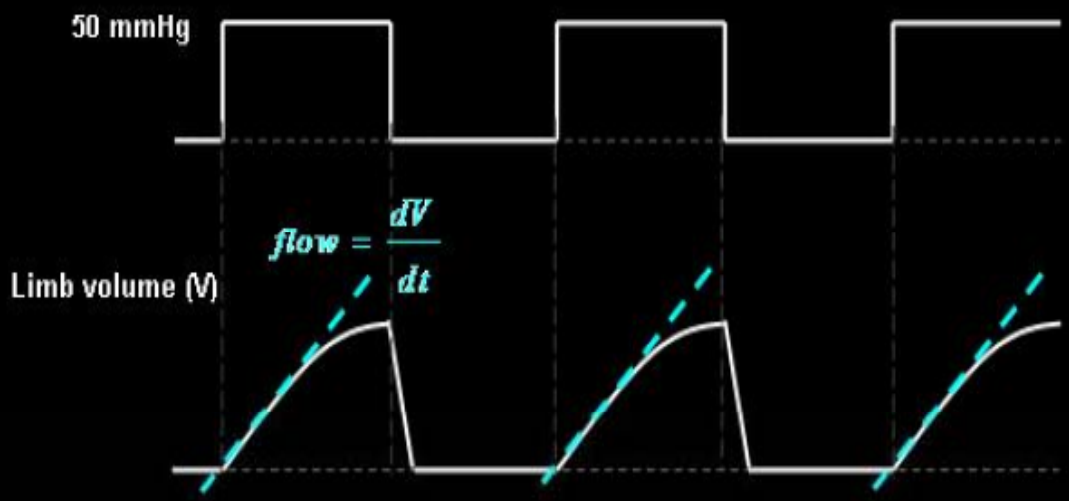
Variability

Lacks standardization

Operator-dependent

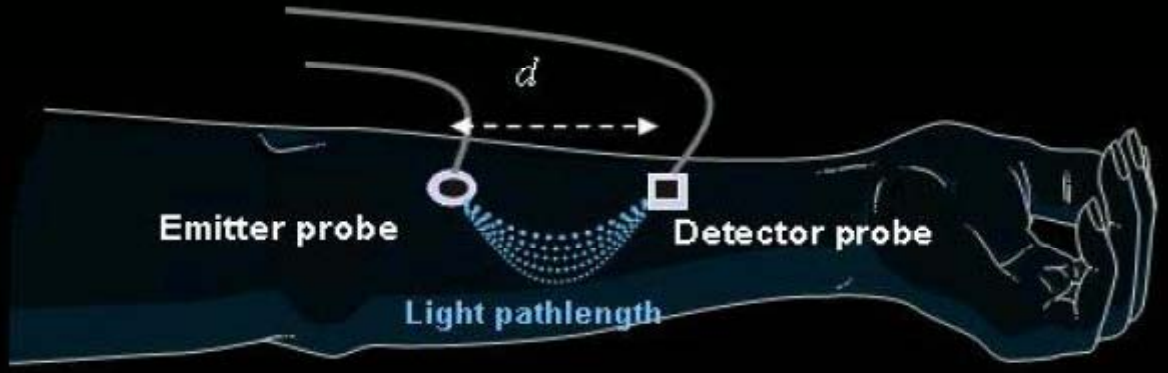


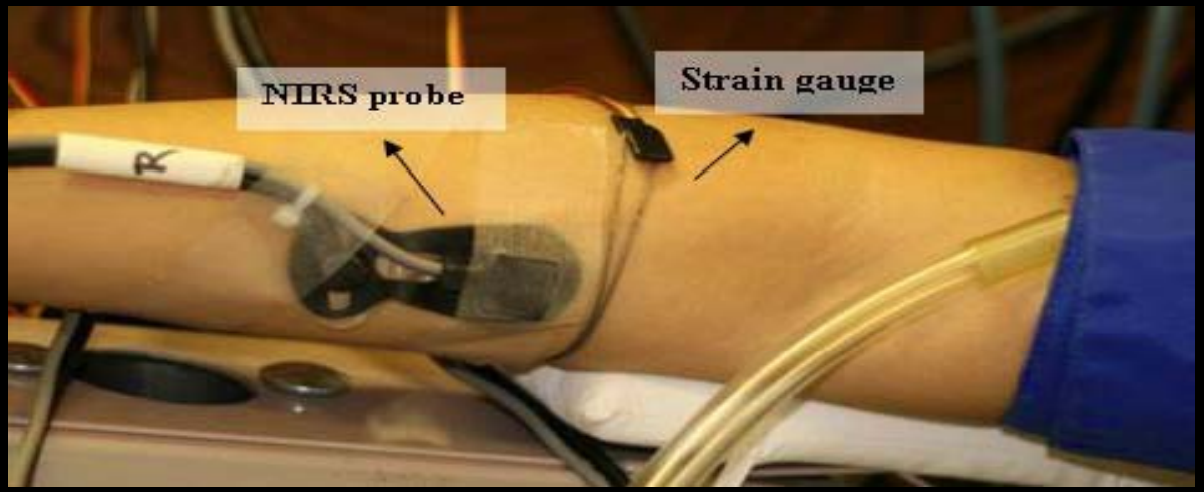
Strain Guage Plethysmography



- FBF is measured by temporarily occluding the venous return (by a cuff inflated to 50 mmHg) and measured the slight swelling of the distal portion of the limb due to continued arterial inflow.
- Result of forearm flow is expressed as ml/100 ml tiss/min or the percent changes in flow.

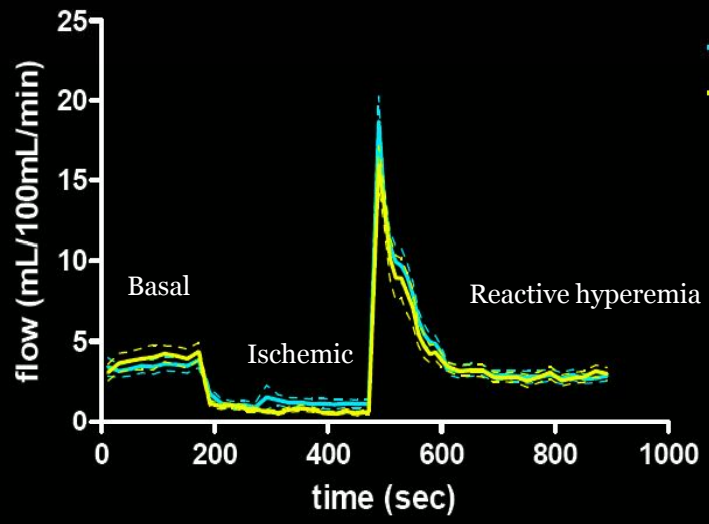
Near infrared spectroscopy (NIRS)

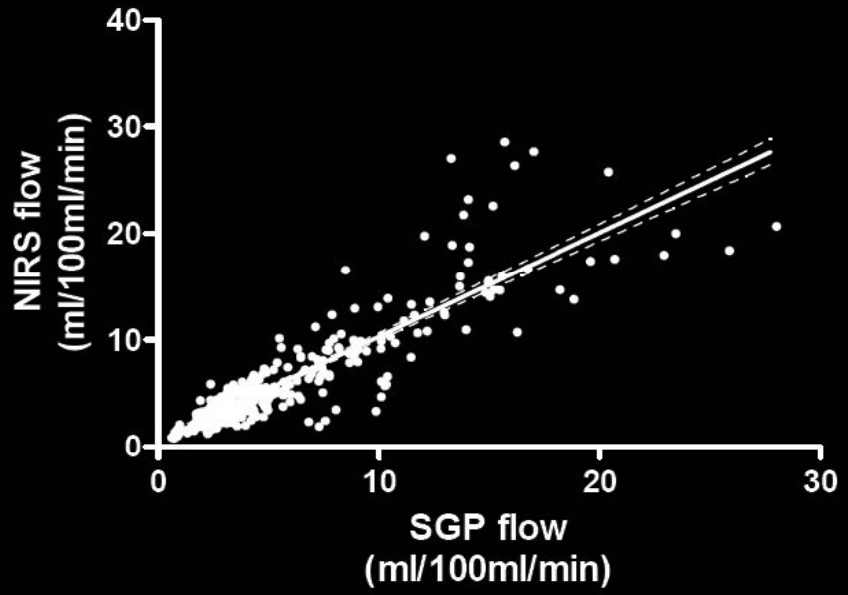




- wavelength of **805 nm** that provides a measurement independent of the degree of hemoglobin oxygenation allowing total hemoglobin concentration measurement

Near infrared spectroscopy (NIRS)





Montreal University
Nina Olamaei

Brachial Artery Catheterization with Venous Occlusive Plethysmography

Advantages

Accessible circulation

Mapping dose-response relationships of endothelial agonists / antagonists

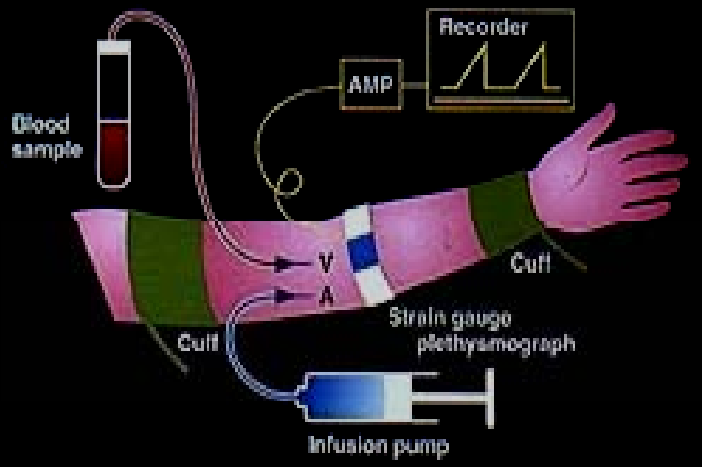
Examination of basal endothelial function (with NOS antagonist infusion)

Disadvantages

Invasive

Median nerve injury, infection, vascular injury

Inappropriate for large population studies



Aortic stiffness

Independent predictor of

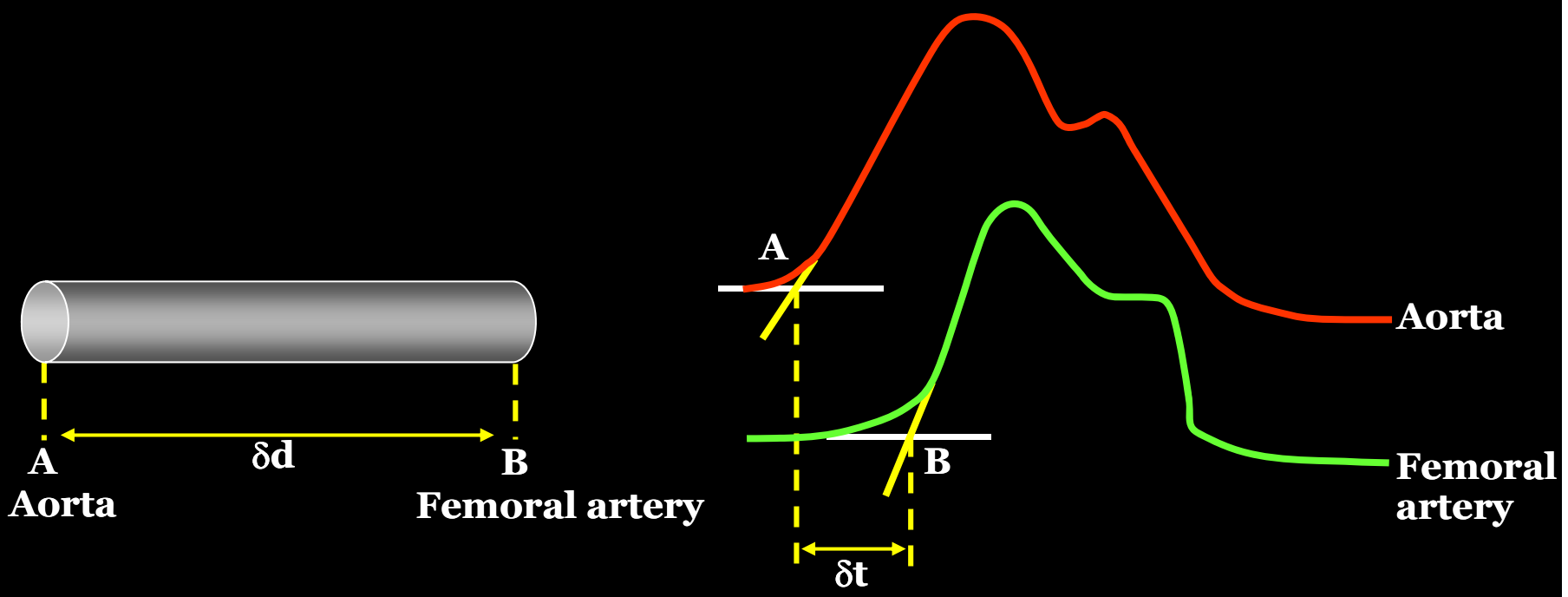
- All-cause and CV mortality in patients with essential HTN

Markers of aortic stiffness:

- Aortic pulse wave velocity (PWV)

- Augmentation index (AIx)

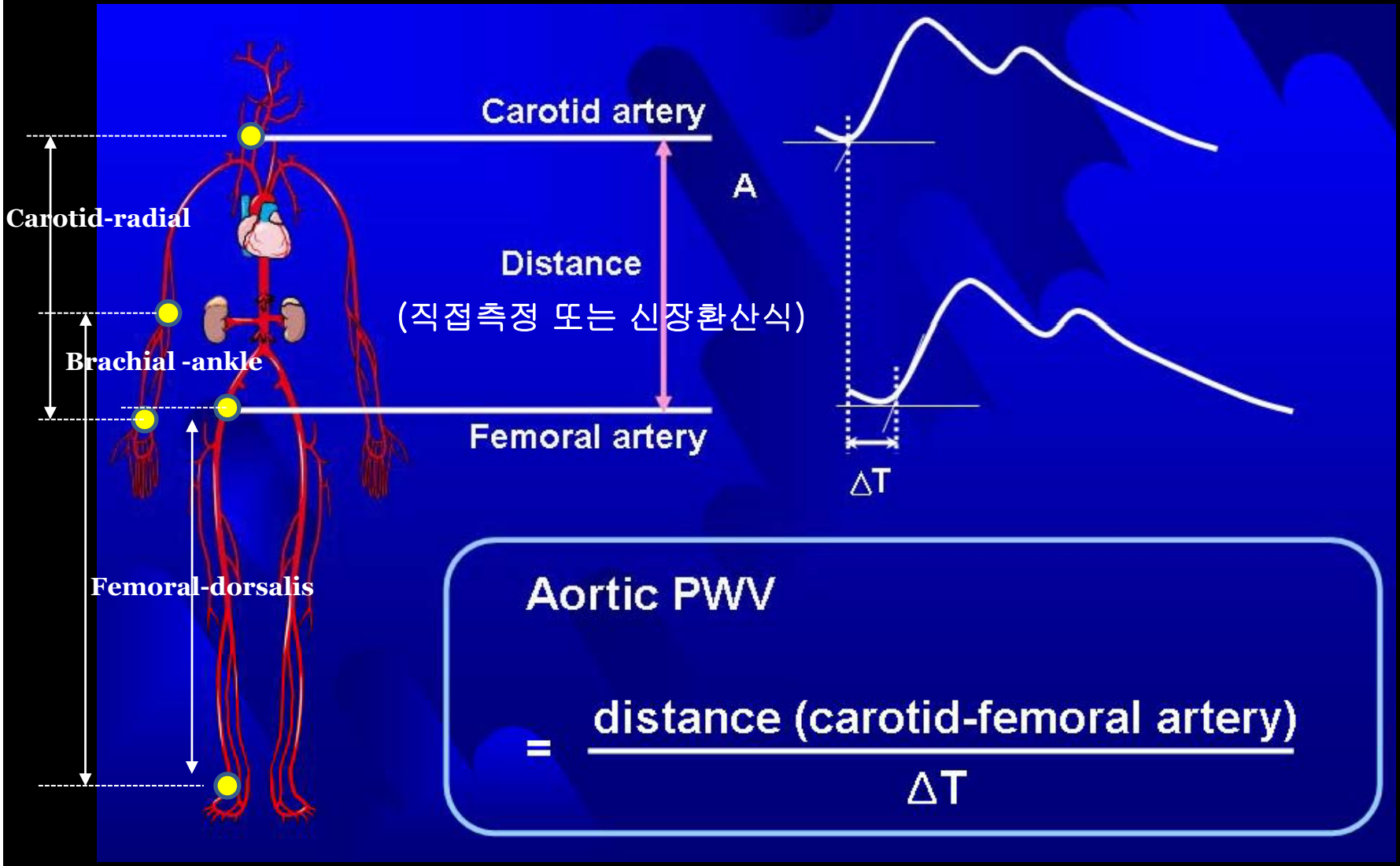
Pulse wave velocity



PWV = Distance (D) / Time delay (DT) m/sec

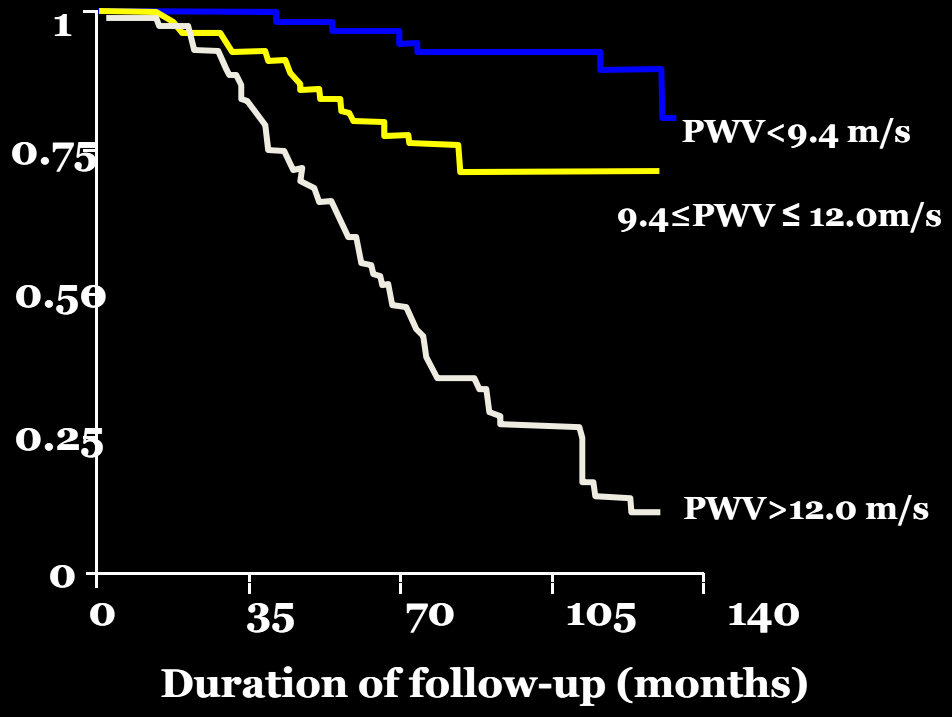
Usually measured over 10 heartbeats.

Pulse wave velocity



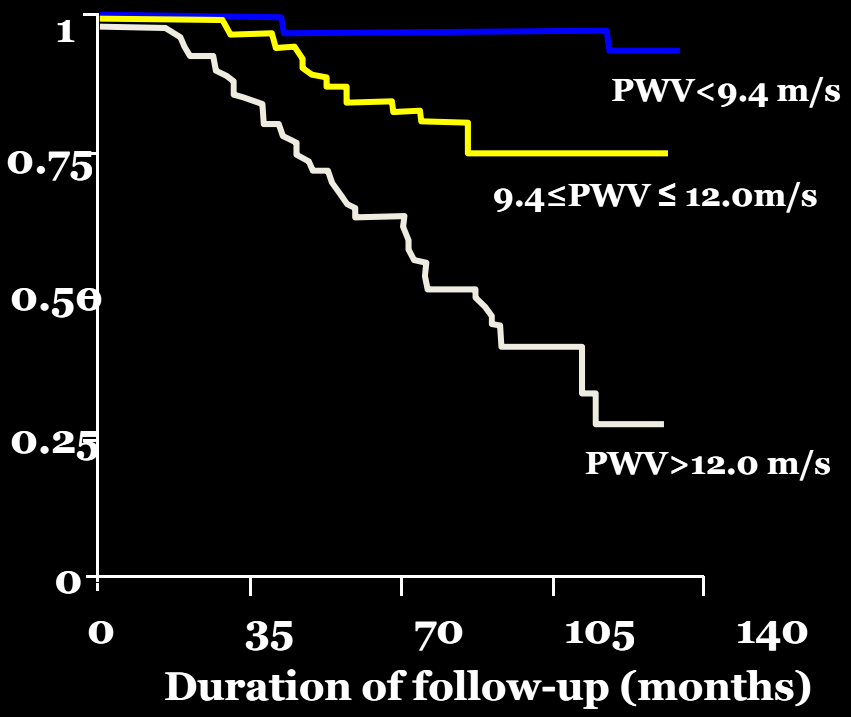
PWV : a determinant of mortality

Probability of overall survival



Probability of event -free survival

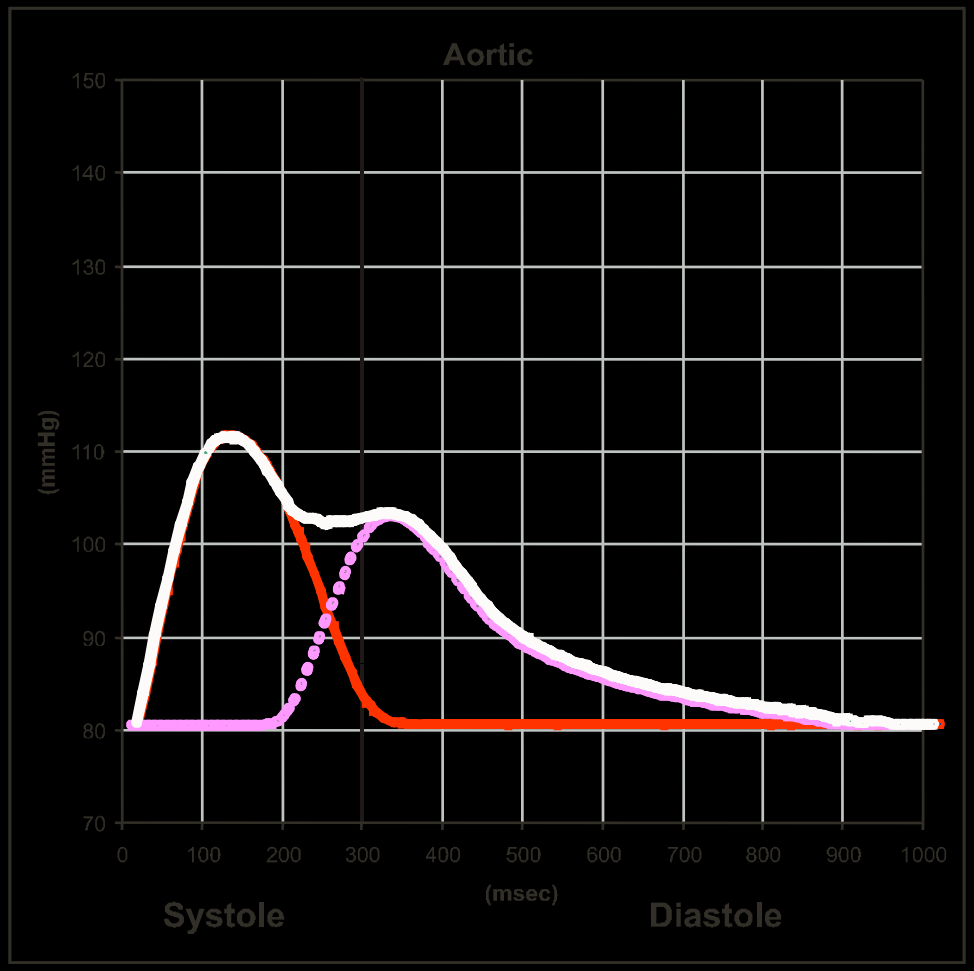
(cardiovascular mortality)

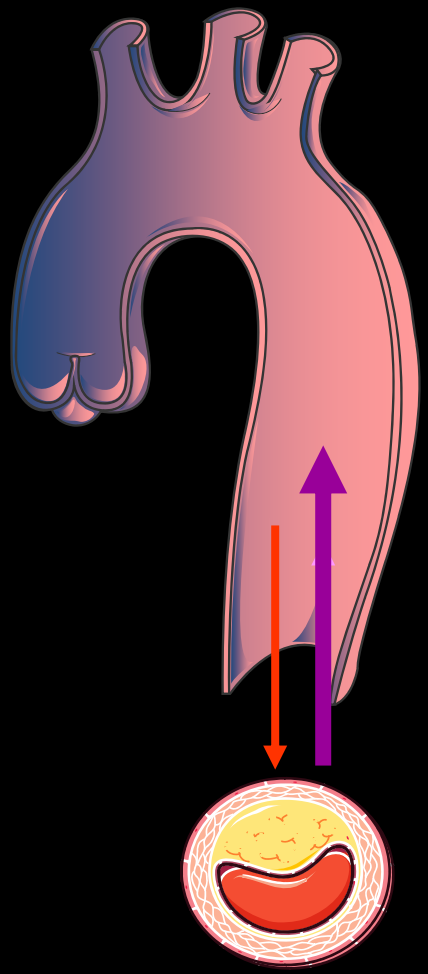


Blacher J, Circulation 1999; 99: 2434-2439

Centrial Aortic Pressure

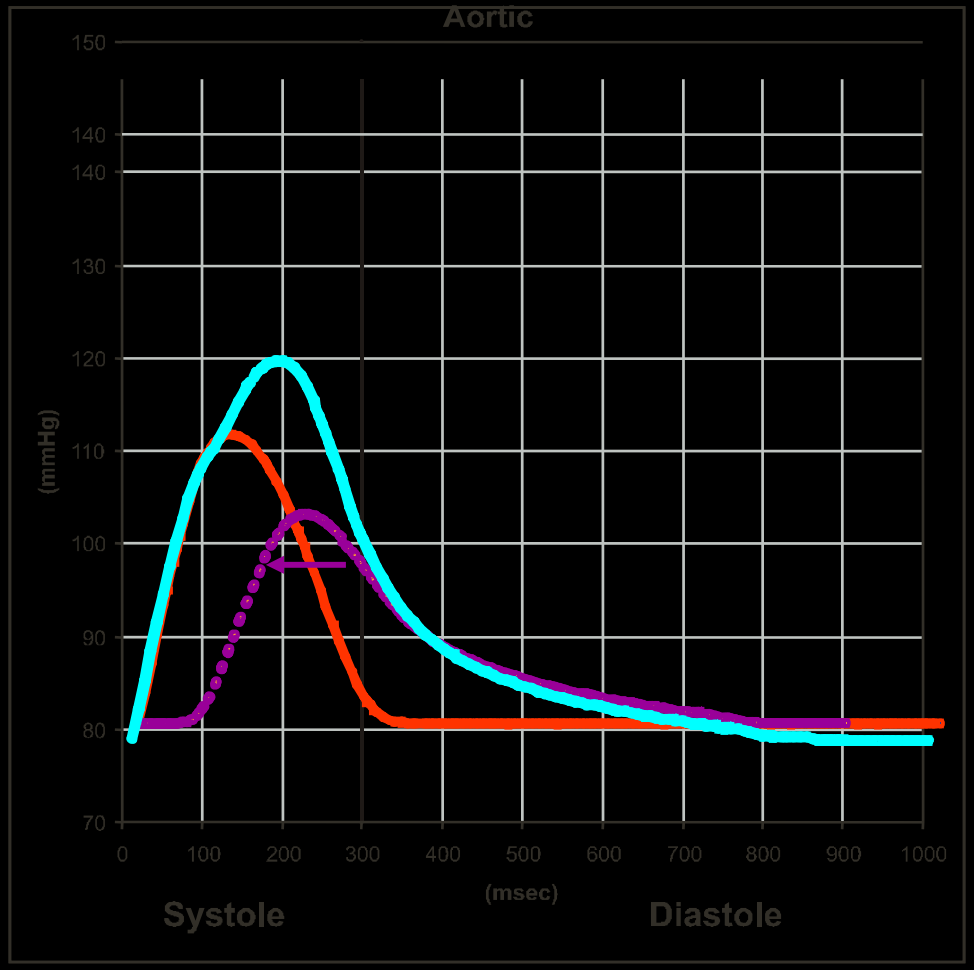
Young Compliant Artery

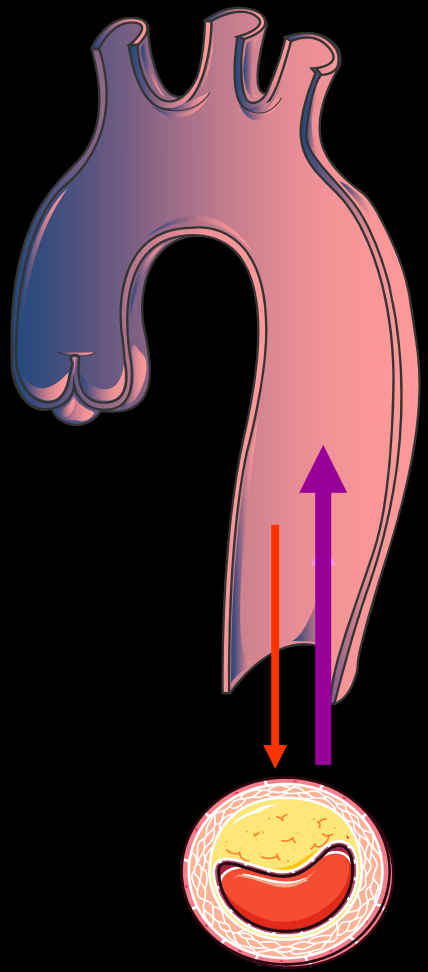




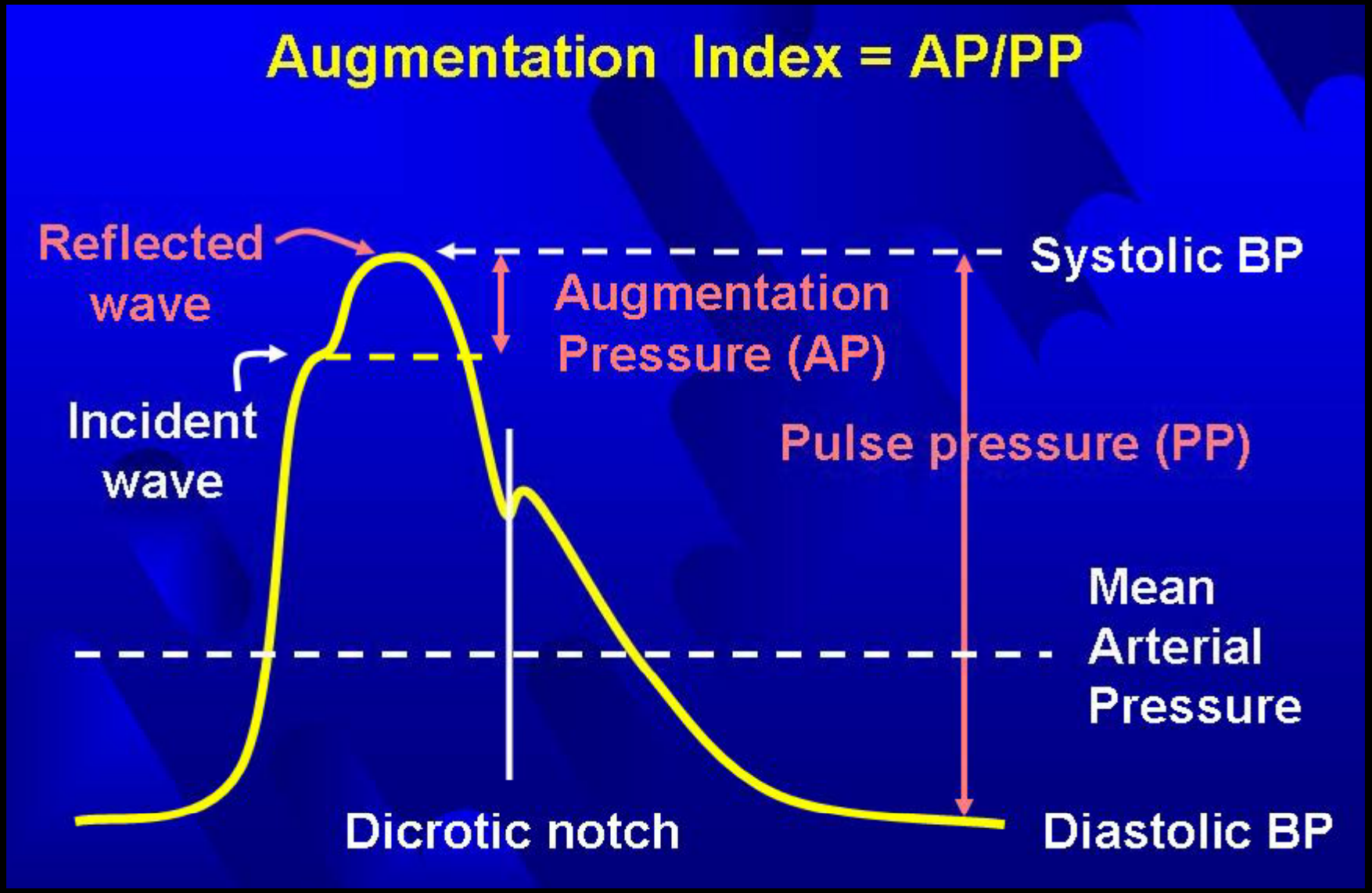
Centrial Aortic Pressure

Elderly Stiff Artery

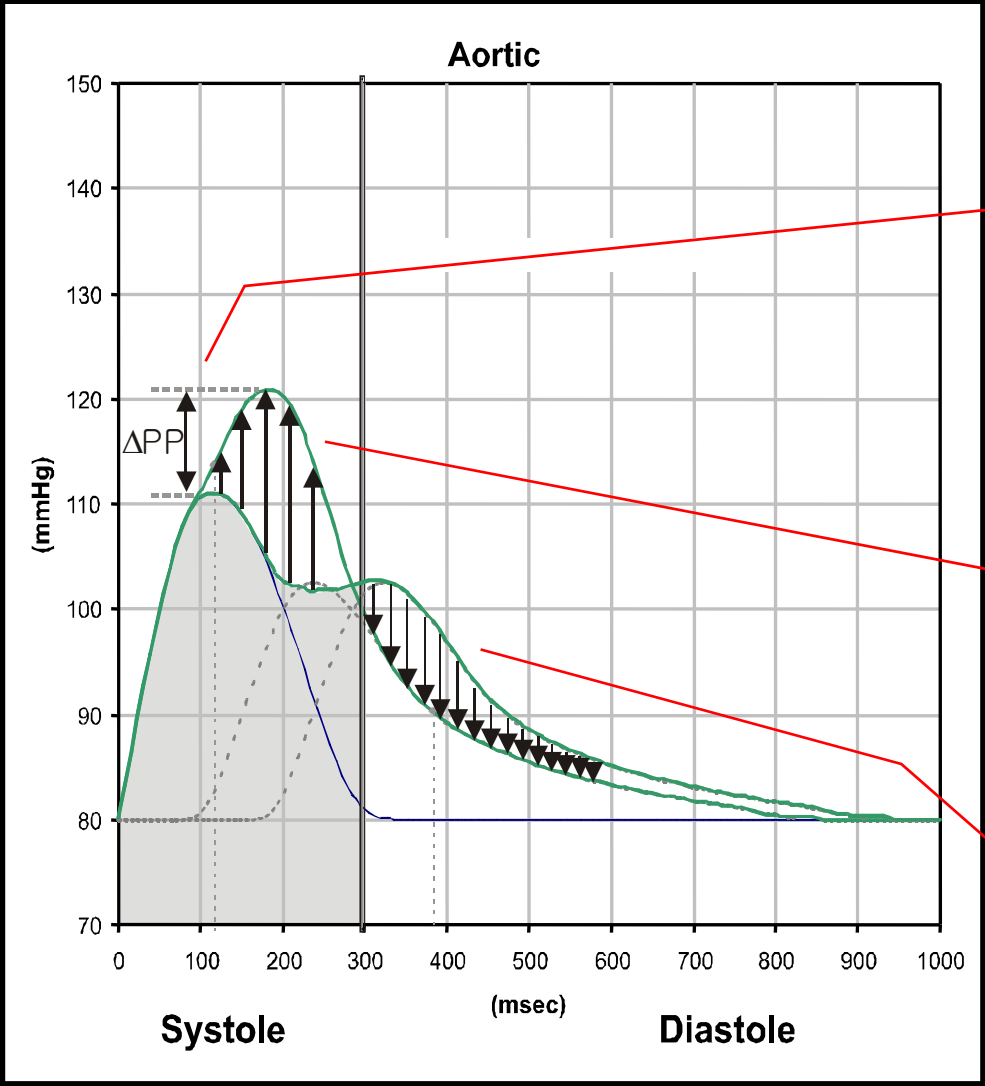




Augmentation Index



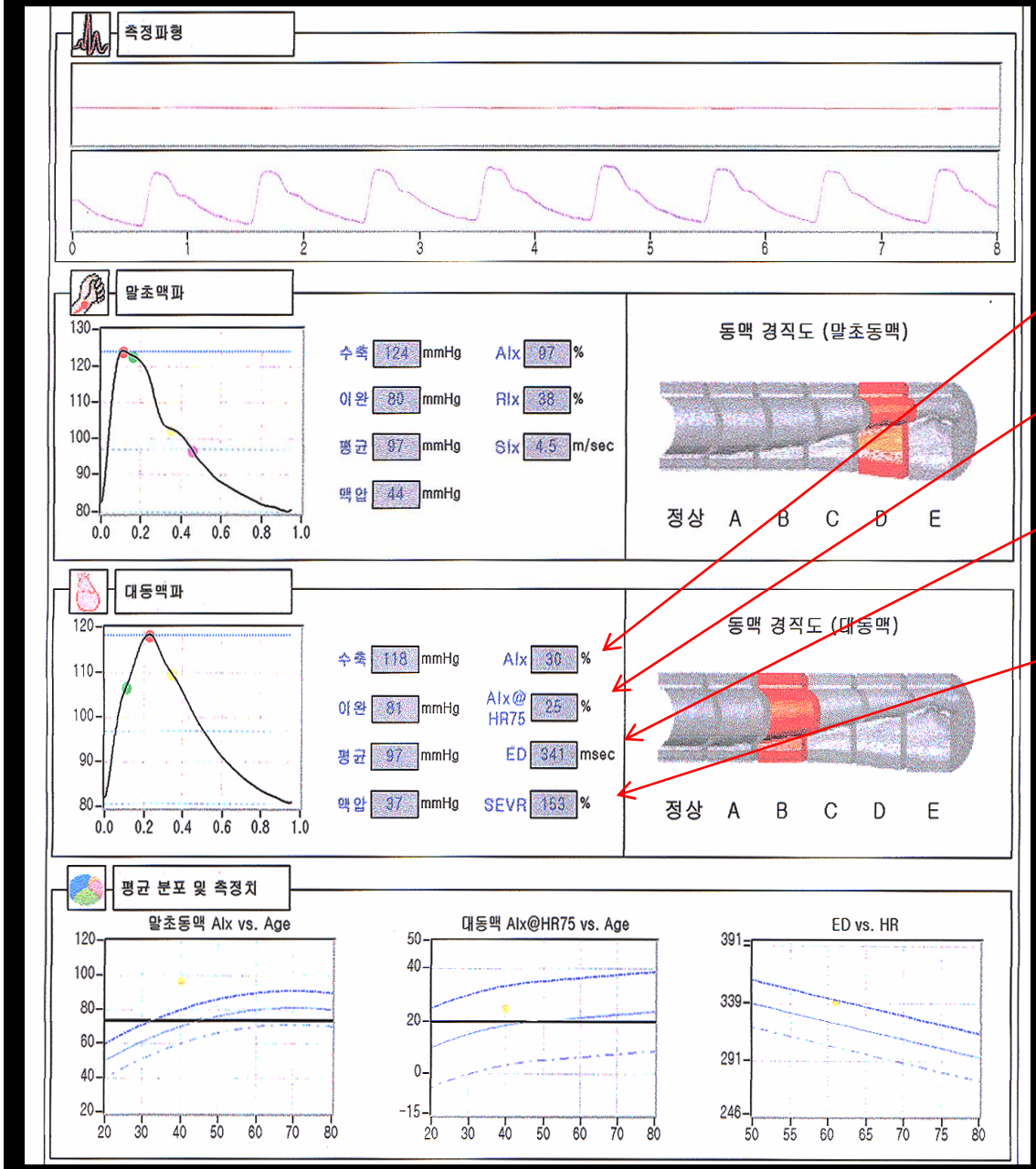
The impact of the early wave reflection



1. Increase in the central pulse pressure that drives cerebral blood flow ->**increases stroke risk and renal failure**

2. Increase in left ventricular load (LV load) accelerates increase in LV mass - **increases risk of LV hypertrophy and HF**

3. Decreased coronary artery perfusion pressure in diastole -> **increases risk of coronary events**



AIx

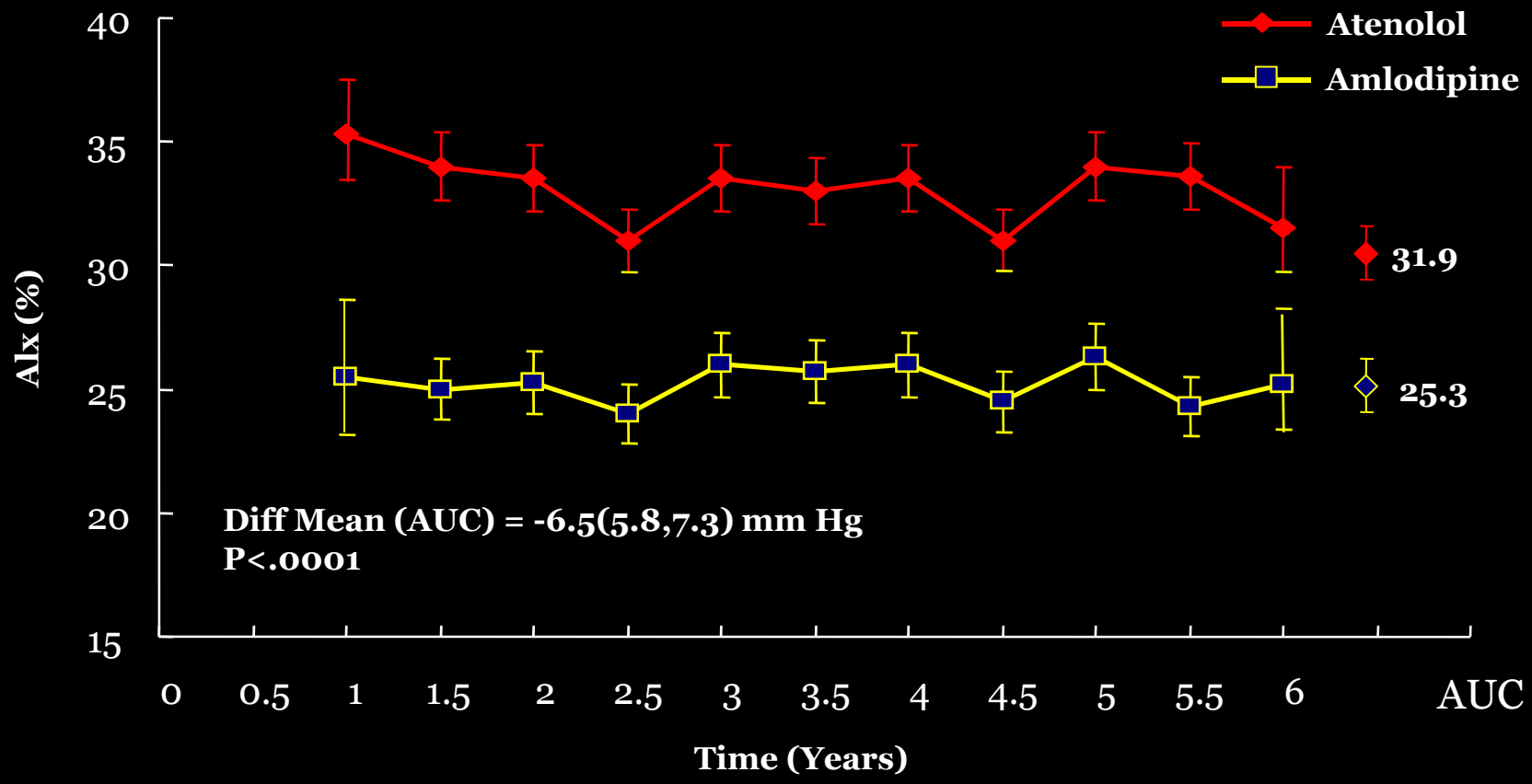
AIx at HR 75

ED (ejection duration)

SEVR (subendocardial viability ratio ;diastolic area/systolic area)

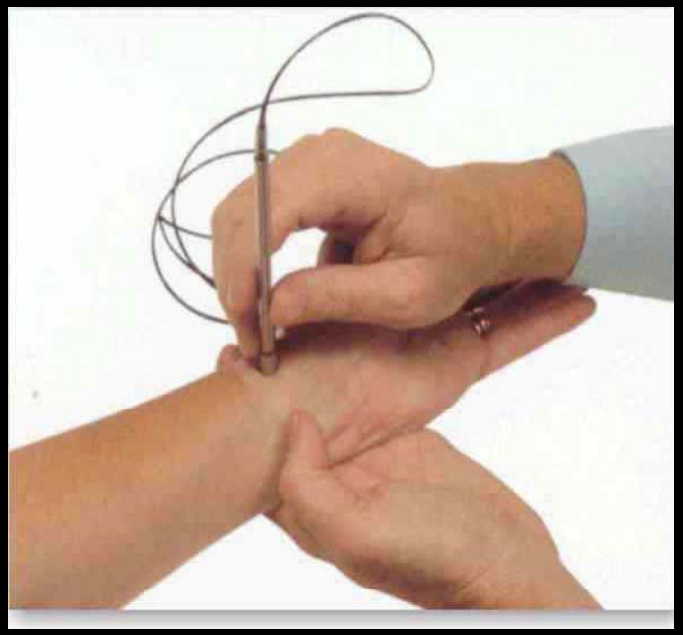
CAFE Results

Augmentation Index (%) by Treatment Arm

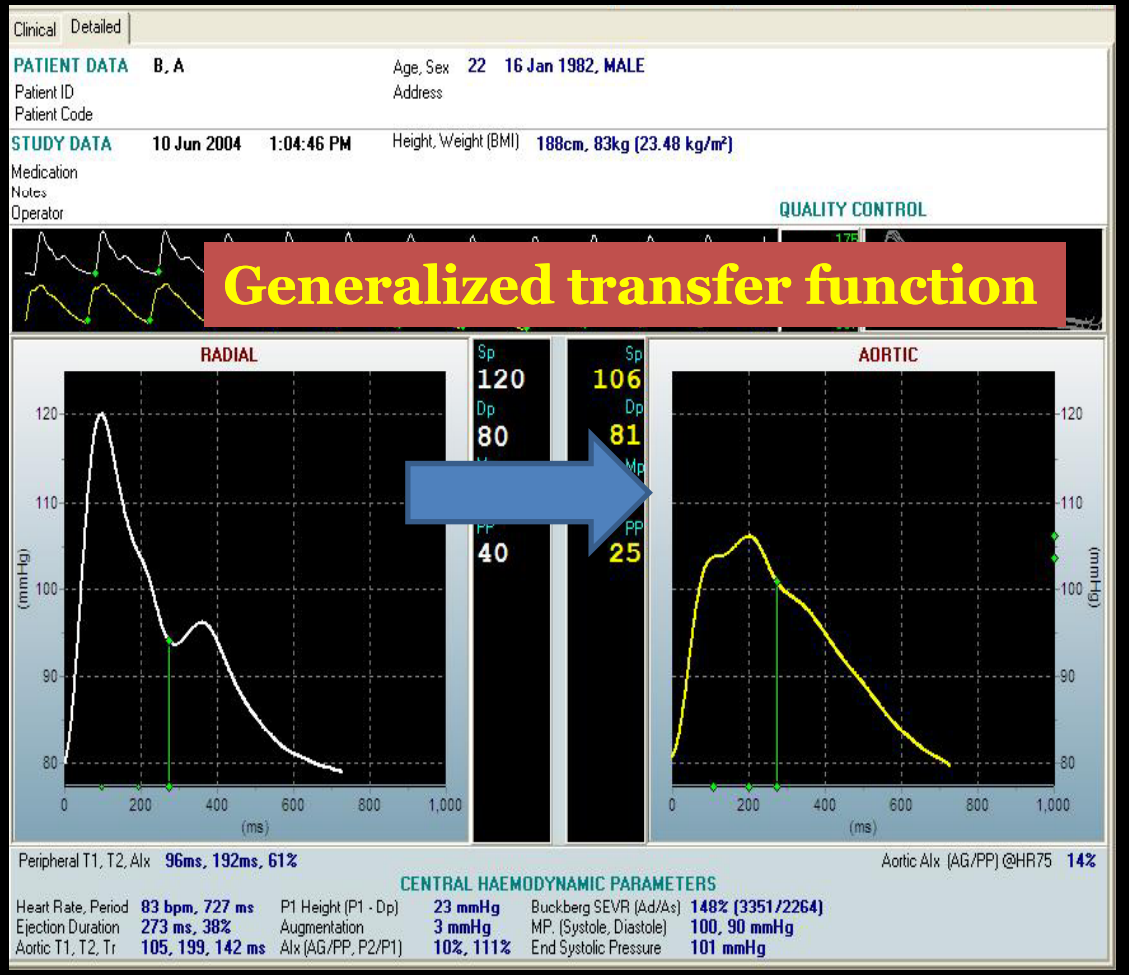


Circulation. 2006;113:1213-25.

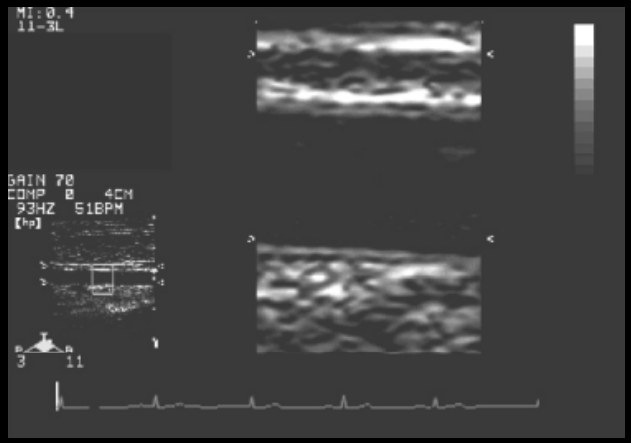
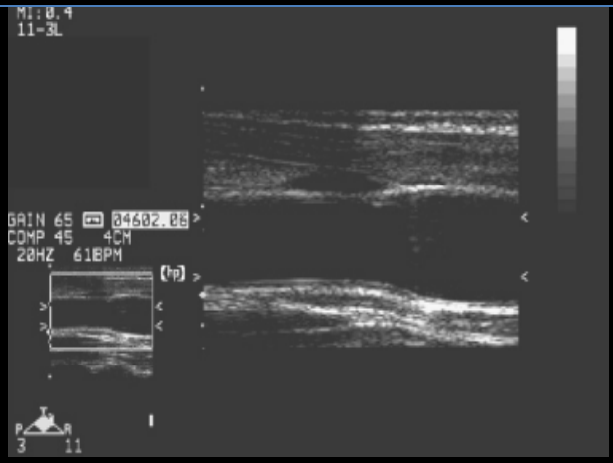
Sphygmocor



Pulse Wave Velocity
& Augmentation Index
Uses Arterial tonometer (radial)



Cardiovascular MRI

	Brachial a	Carotid a
MRI		
USG		

Journal of Cardiovascular Magnetic Resonance (2006) ***8, 381–387***

Cardiovascular MRI

Endothelial dependent response	Change of vessel size after FMD -Absolute change of area (Post-Pre) -Proportional change of area (Post-pre/pre *100)
Endothelium independent response	Change of vessel size after nitrate (SL or spary)
Vascular distensibility	$(A_{max}\text{-}A_{min})/A_{min}*(P_{max}\text{-}P_{min})$
Pulse wave velocity	Arrival time of the pulse wave at each level was defined as the time point when the mean velocity reached half of its maximum. Curve fitting of velocity data (Software Version 7, OriginLab Corporation, Northampton, MA)

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Cardiovascular MRI

Comparison of brachial and carotid artery measures. Difference between CMR and ultrasound represents the mean (SD) of the differences in the vascular measures obtained by CMR and ultrasound in each individual. Results are presented as Mean (SD)

	CMR	Ultrasound	Difference between CMR & ultrasound	Correl^{n}	P for correl^{n}
Resting diastolic brachial area in mm^2	12.6 (4.3)	13.3 (4.4)	–0.7 (2.2)	0.87	<0.0001
Resting systolic brachial area in mm^2	13.7 (5.0)	14.1 (4.6)	0.4 (2.1)	0.90	<0.0001
Post-cuff diastolic brachial area in mm^2	13.7 (4.5)	14.5 (4.9)	–0.8 (2.6)	0.85	<0.0001
Post-GTN diastolic brachial area in mm^2	19.3 (4.5)	18.3 (5.4)	1.0 (2.7)	0.96	<0.0001
Carotid diastolic area in mm^2	35.5 (9.6)	37.9 (6.7)	–2.4 (7.7)	0.60	0.02
Carotid systolic area in mm^2	42.8 (10.6)	44.6 (8.2)	–1.8 (7.8)	0.72	0.01

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Conclusion

1. *Vascular function represents a final pathway integrating the net effects endothelial cells and is a logical physiologic marker of vascular health and prognosis.*
2. *However, before the curtain rises to welcome endothelial function testing onto clinical stage, further study is needed for the **reproducibility** , **standardization** and **clinical implications**.*