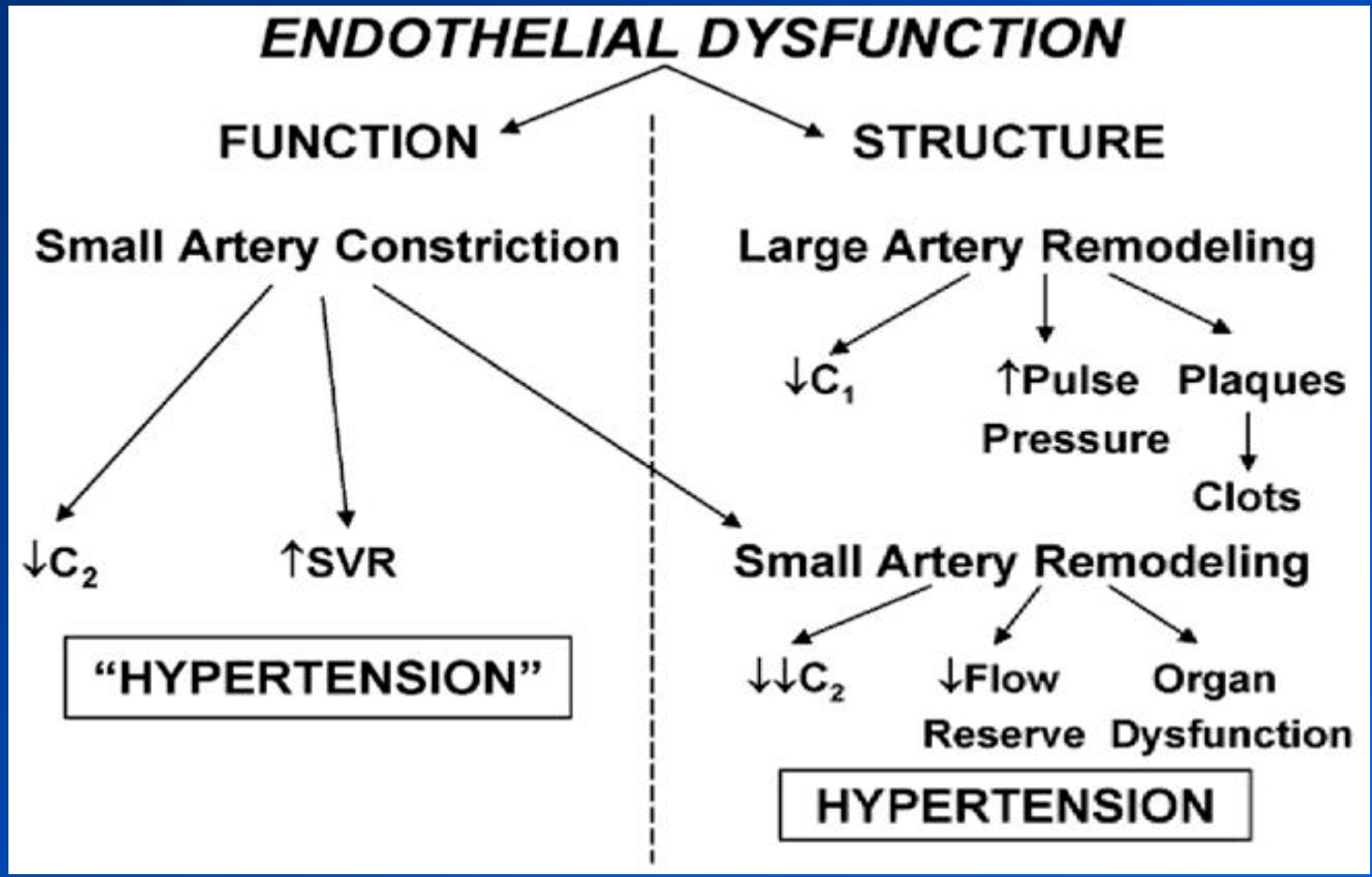
Assessment of Endothelial function: why, when, how

계명의대
김기식

Mechanism

- The endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear stress The effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry (there are no voltage-gated calcium channels in endothelial cells). Calcium activates an enzyme, endothelial nitric oxide synthase (eNOS), and the subsequent generation of NO appears to account for FMD



Role of endothelial dysfunction on small and large arteries. Functional effect is predominantly on small arteries. Structural effects of dysfunction occur later and affect the large and small arteries. Functional abnormalities may raise blood pressure, often within the normotensive range. This so-called hypertension may be complicated by structural changes that further reduce small artery compliance and subsequently large artery compliance and lead to atherothrombotic complications. C1 indicates large artery compliance or elasticity; C2, small artery compliance or elasticity; SVR, systemic vascular resistance

	Methodology Available/ Convenient	Methodology Standardized	Sensitivity/ Specificity for Disease	Identifies Severity of Disease	Tracks With Treatment of Disease
Endothelial dysfunction	+	+	++	++	+
Blood pressure	+++	++	+	++	+++
Arterial stiffness	++	+	++	++	+
Albuminuria	++	++	++	++	++
Ankle–brachial index	+++	+++	+	++	?
Serum collagen marker	+	+	?	?	?

Clinical Applicability of Potential Surrogate Functional Markers for Cardiovascular Disease

Circulation, Volume 109(25).

- Flow Mediated Dilation(FMD):
  - NO derived vasodilation
  - endothelium-derived prostanoids,
    - blocked by indomethacin
    - more than one endothelial mediator is capable of acting as the signal between endothelium and smooth muscle.
    - It is unknown whether other mediators, such as the putative endothelium-derived hyperpolarizing factor, can cause FMD if both NO and prostanoids are deficient.

Very acute changes:

– mediated by the increase in intracellular calcium

Over slightly longer time periods (minutes), shear-stress-induced phosphorylation of eNOS via a serine/threonine protein kinase, Akt/PKB, increases eNOS activity, even at low calcium concentrations, and this may be important to allow continued output of NO. In addition, other posttranslational modifications of the enzyme (myristilation or palmitoylation) or interaction with caveolin can affect intracellular localization of the enzyme and thereby alter its function.

Over longer time periods (many minutes or hours), eNOS gene transcription is activated, and this can result in continued increases in NO generation if shear stress is maintained at high levels.

Subject preparation 1

Factors affect FMD

- temperature,
- food,
- drugs
- sympathetic stimuli,.

Subject preparation 2

- fast for at least 8 to 12 h before the study,
- quiet, temperature-controlled room.
- All vasoactive medications should be withheld for at least four half-lives,
- subjects should not exercise,
- should not ingest substances: caffeine, high-fat foods, vitamin C or use tobacco for at least 4-6 h before the study.
- menstrual cycle, as it too may affect FMD

Equipment

- Ultrasound systems must be equipped with
  - vascular software for two-dimensional (2D) imaging,
  - color and spectral Doppler,
  - an internal electrocardiogram (ECG) monitor and a high-frequency vascular transducer.
  - A linear array transducer with a minimum frequency of 7 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images with sufficient resolution for subsequent analysis. Image resolution is enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers.
  - Timing of each image frame with respect to the cardiac cycle is determined with simultaneous ECG recording on the ultrasound system video monitor

Image acquisition

- The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery.
- The brachial artery is imaged above the antecubital fossa in the longitudinal plane.
- A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging. Currently,

Image acqusition

Cross-sectional imaging of the brachial artery cannot determine maximum diameter or area of the lumen because of inadequate image definition of the lateral walls. Also,

– skew artifacts from cross-sectional imaging limit accurate diameter determination.

M mode and A mode (wall tracking) can be used to continuously measure the diameter, yet these techniques may be more subject to error owing to tracking drift.

veins and fascial planes are noted to help maintain the same image of the artery throughout the study.

A stereotactic probe-holding device can be helpful.

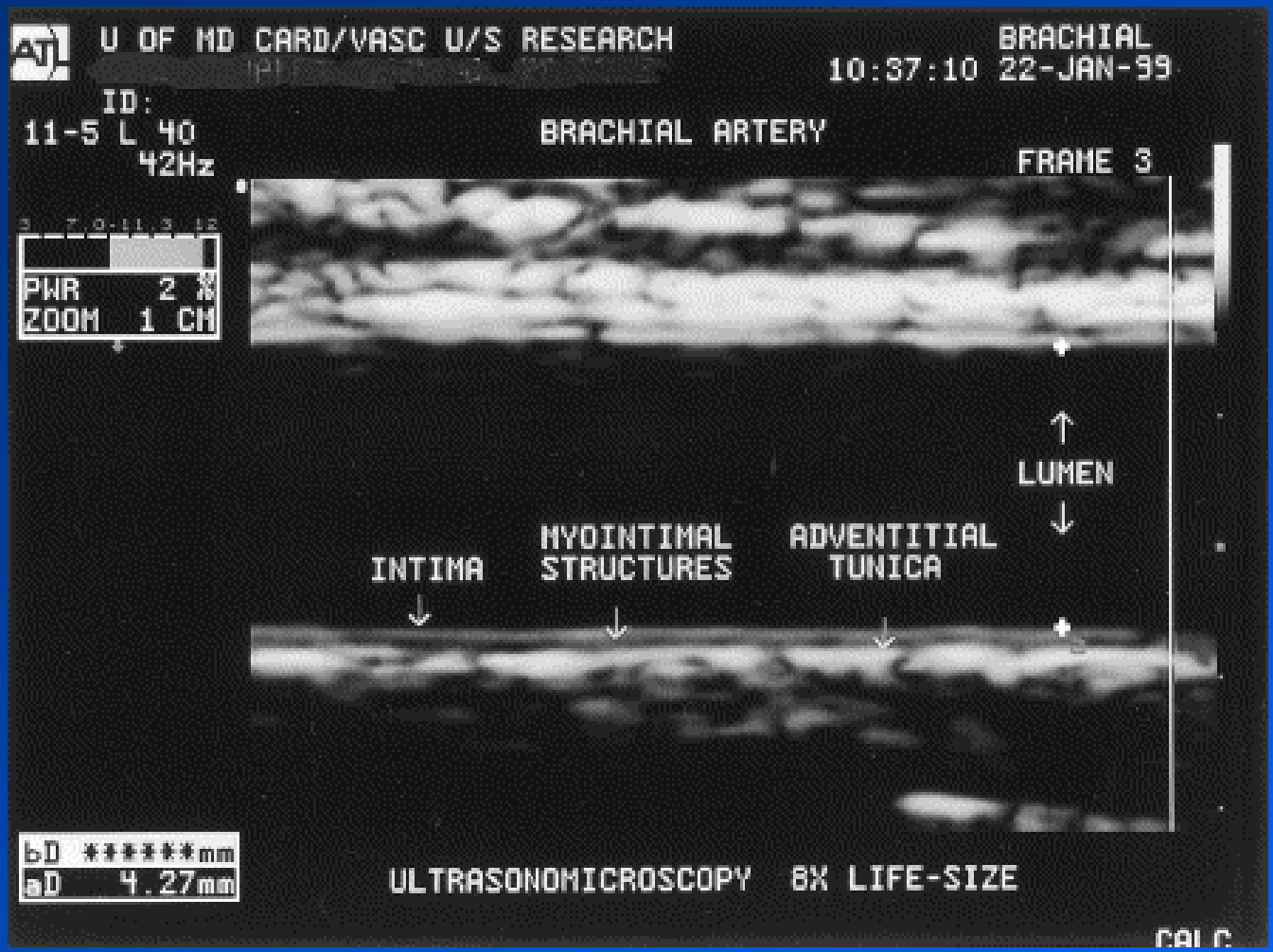
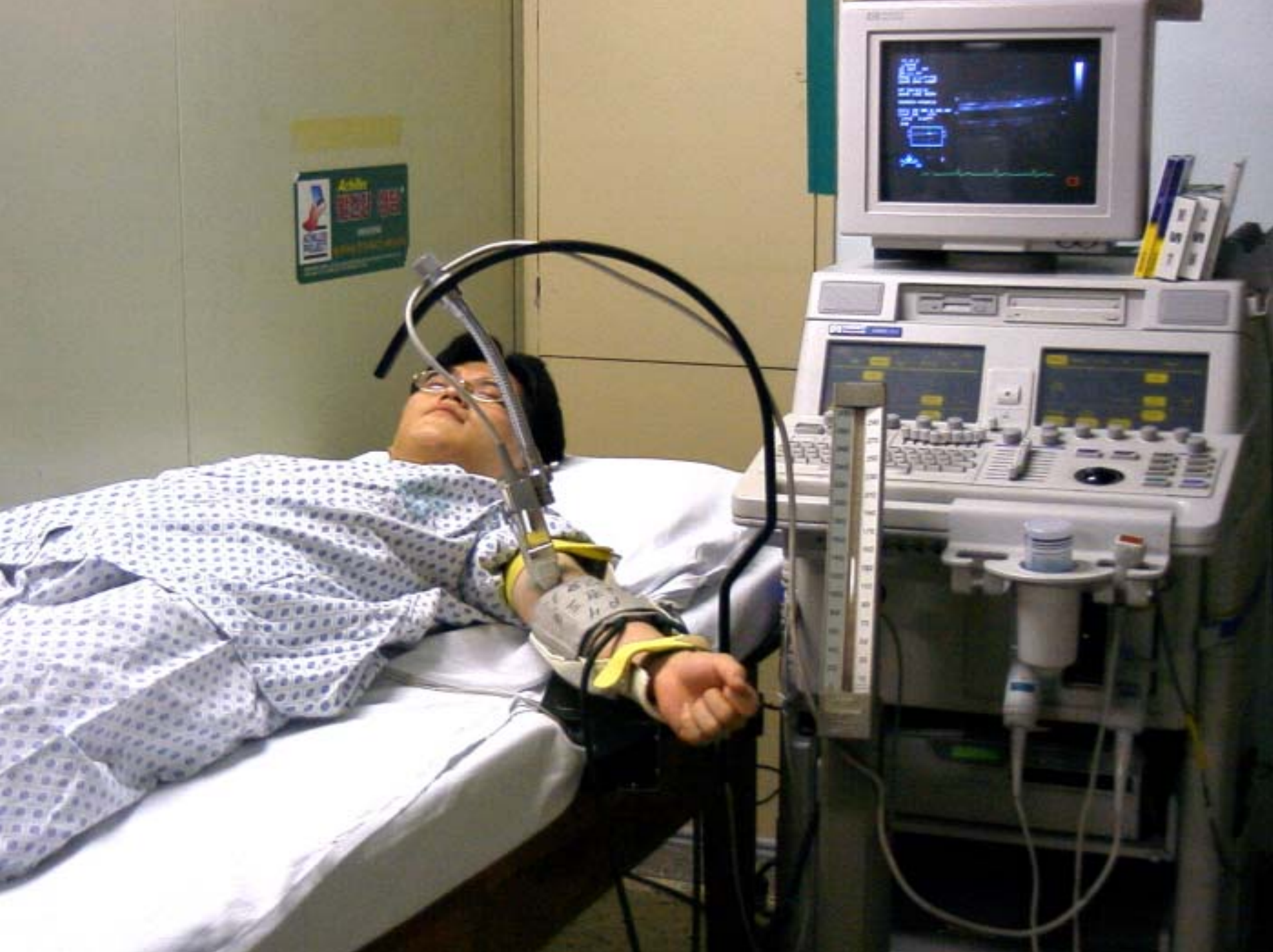


Figure. Ultrasound image of the brachial artery (longitudinally) at 8× magnification, 11-MHz transducer frequency annotated for anatomic landmarks.

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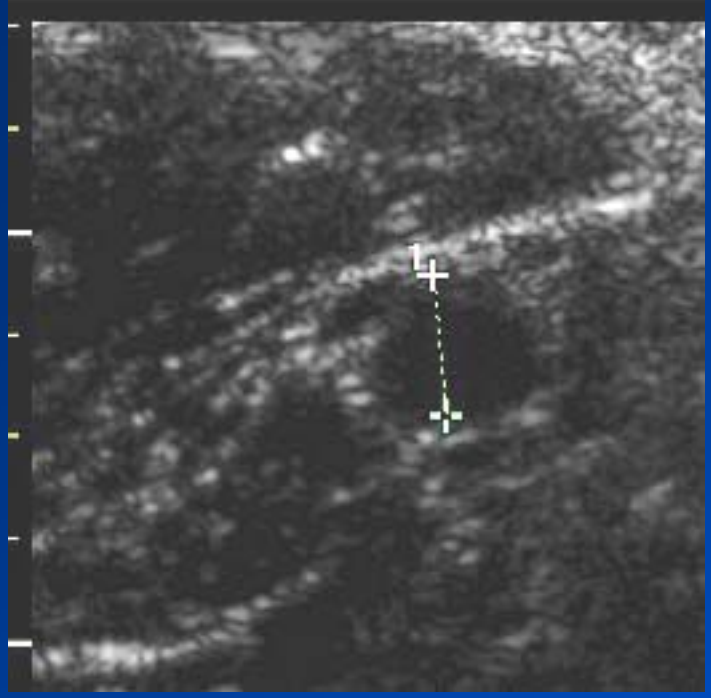
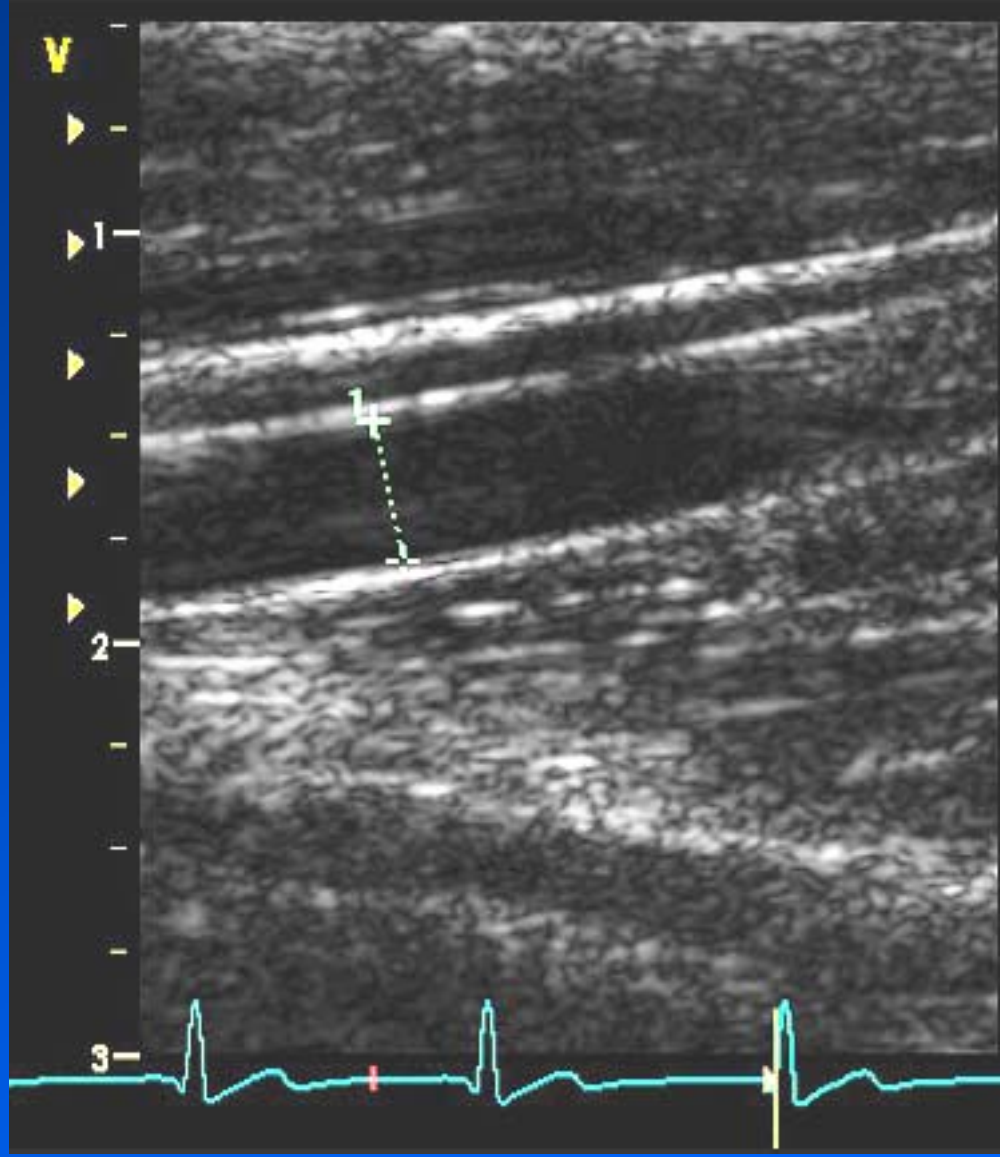
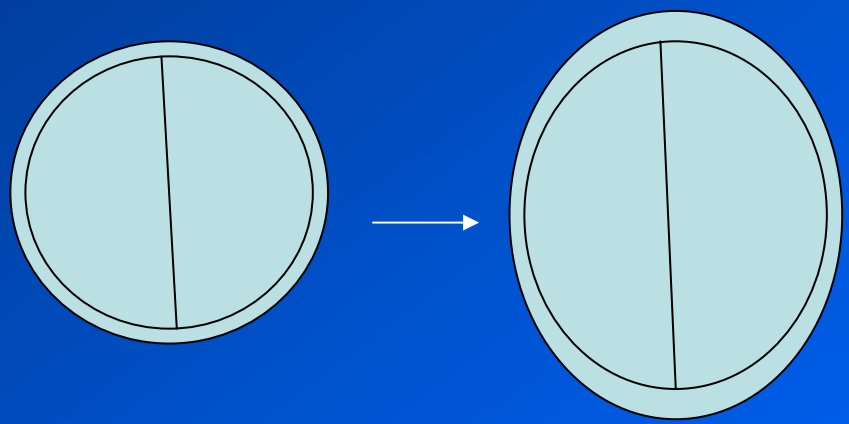




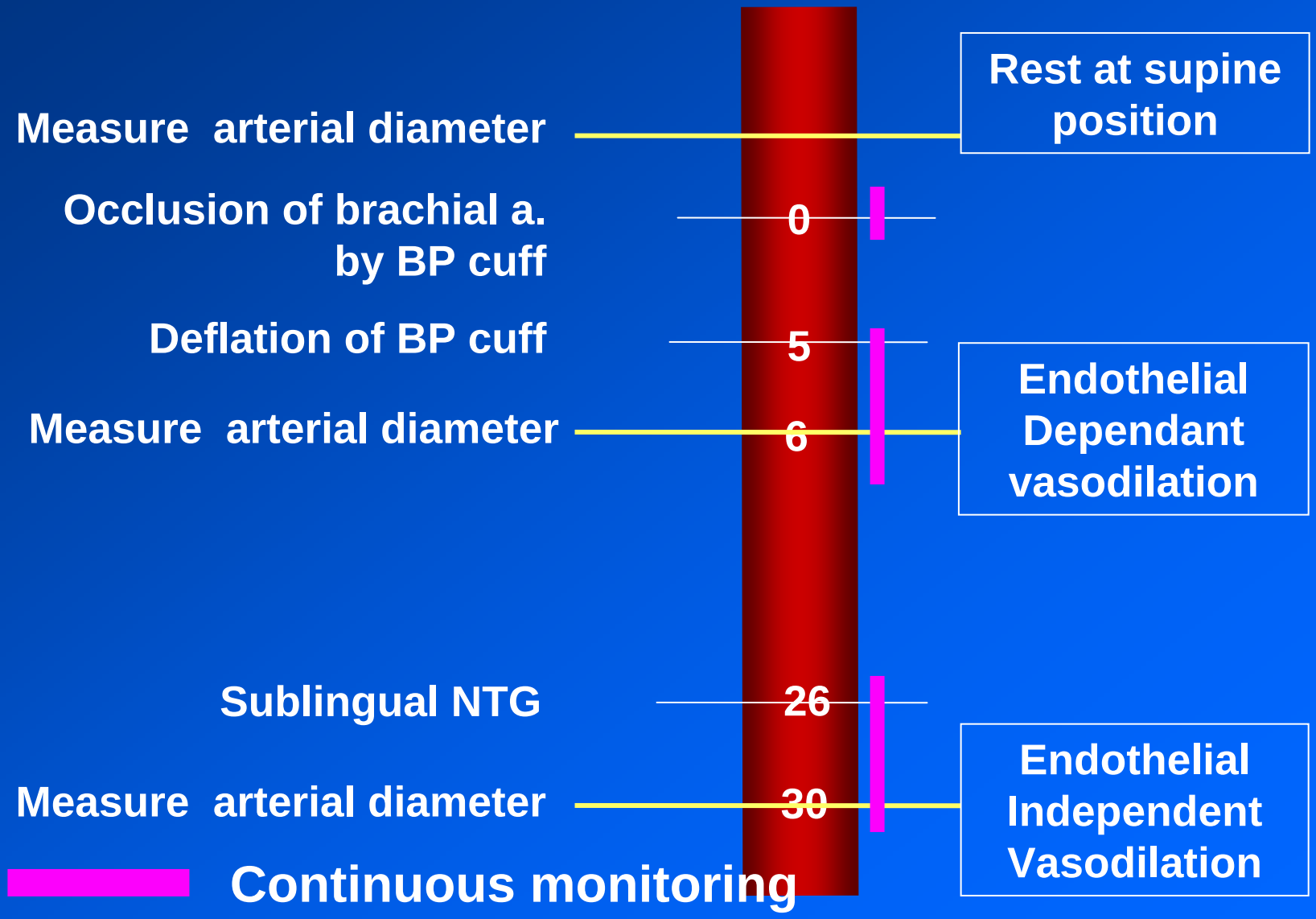


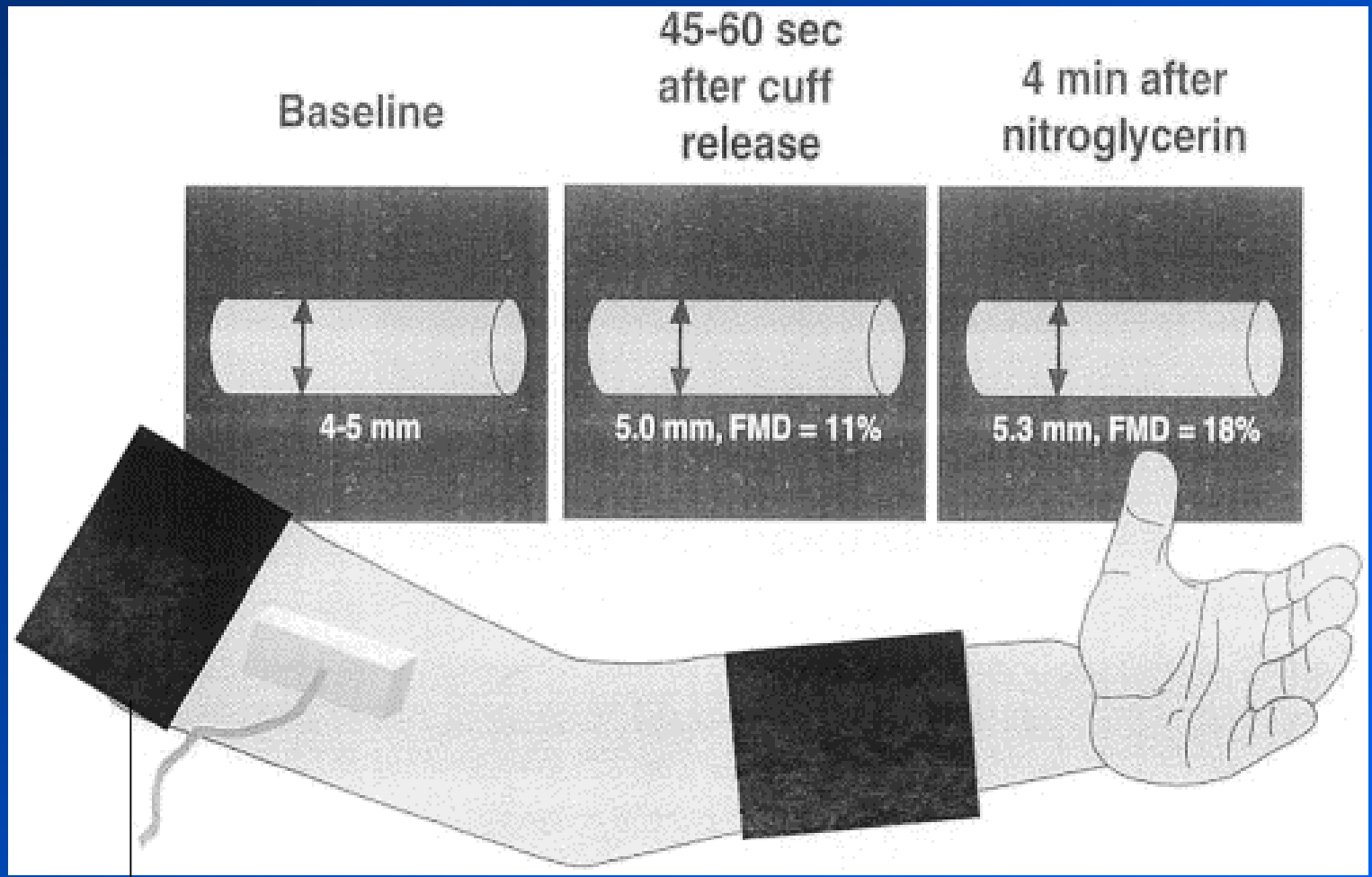
Skew artifact

Endothelium-dependent FMD

1. Blood pressure cuff is first placed either above the antecubital fossa or on the forearm.
2. A baseline rest image is acquired,
3. blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume.
4. arterial occlusion is created by cuff inflation to suprasystolic pressure. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a standardized length of time.
5. The longitudinal image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation.
6. A midartery pulsed Doppler signal is obtained upon immediate cuff release and no later than 15 s after cuff deflation to assess hyperemic velocity.

Protocol for detection of endothelial function





cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm

1. Greater flow stimulus resulting from recruitment of more resistance vessels or possibly to direct effects of ischemia on the brachial artery.

2. However, upper-arm occlusion is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue.

Duration of Ischemia

1. The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 s to 5 min. The change in diameter is similar after 5 and 10 min of occlusion; therefore, the more easily tolerated 5-min occlusion is typically used.
2. FMD may be studied in the radial, axillary and superficial femoral arteries. Notable caveats are that arteries smaller than 2.5 mm in diameter are difficult to measure, and vasodilation is generally less difficult to perceive in vessels larger than 5.0 mm in diameter.

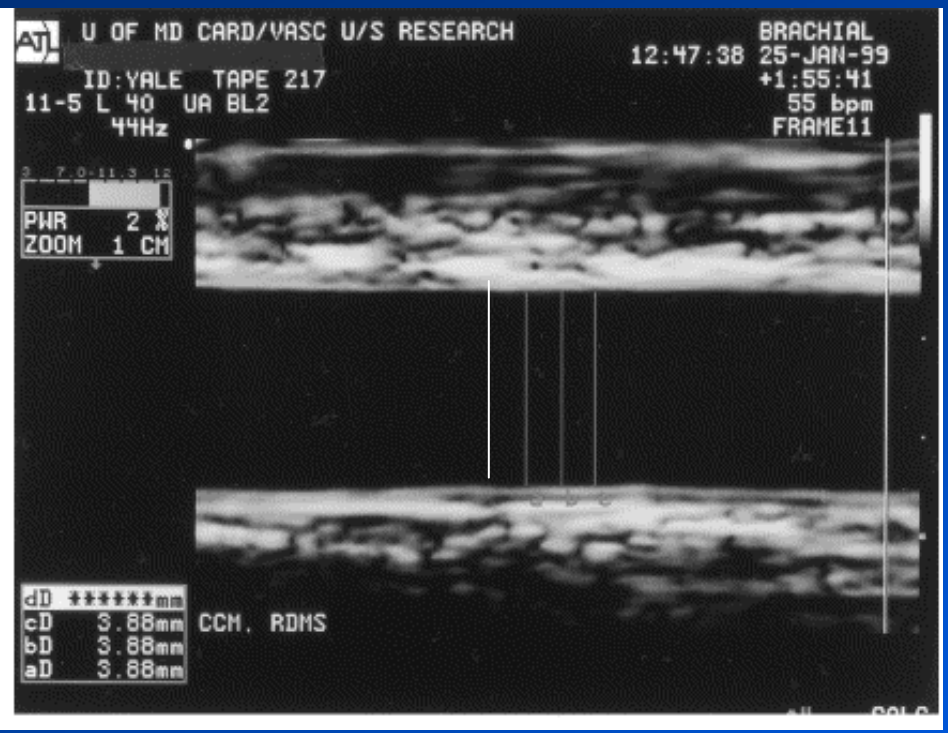
Endothelium-independent vasodilation with nitroglycerin

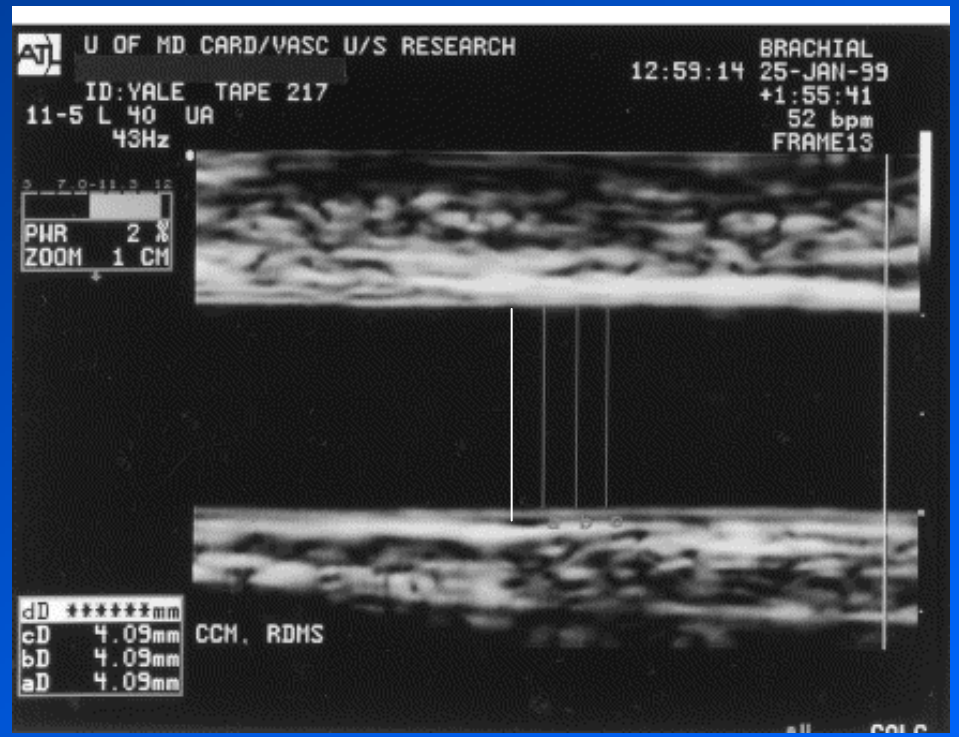
- At least 10 min of rest is needed after reactive hyperemia (i.e., FMD) before another image is acquired to reflect the reestablished baseline conditions. In most studies to date, an exogenous NO donor, such as a single high dose (0.4 mg) of nitroglycerin (NTG) spray or sublingual tablet has been given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation reflecting vascular smooth muscle function. Peak vasodilation occurs 3 to 4 min after NTG administration; images should be continuously recorded during this time, and NTG should not be administered to individuals with clinically significant bradycardia or hypotension. Determining the vasodilator responses to increasing doses of NTG, rather than a single dose, may further elucidate changes in smooth muscle function or arterial compliance that might be playing a role in any observed changes in FMD.

Anatomic landmarks

The diameter of the brachial artery

- longitudinal images in which the lumen-intima interface is visualized on the near (anterior) and far (posterior) walls).
- Once the image for analysis is chosen, the boundaries for diameter measurements (the lumen-intima or the media-adventitia interfaces) are identified manually with electronic calipers or automatically using edge-detection software.
- The variability of the diameter measurement is greatest when it is determined from a point-to-point measurement of a single frame, and least when there is an average derived from multiple diameter measurements determined along a segment of the vessel



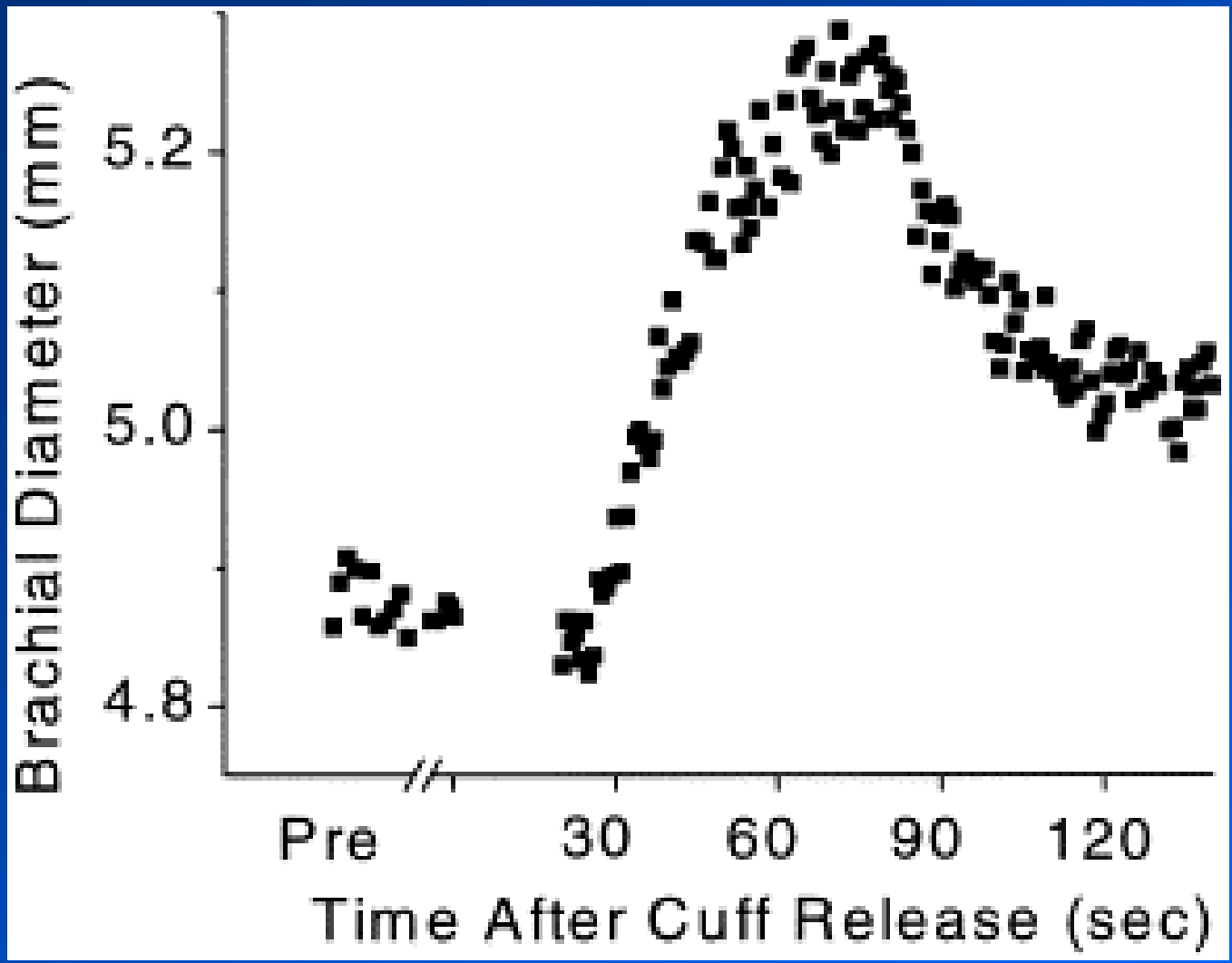


Before

After ischemia

Timing of FMD

- maximal increase in diameter occurs approximately 60 s after release of the occlusive cuff, or 45 to 60 s after peak reactive hyperemic blood flow.
- The increase in diameter at this time is prevented by the NOS inhibitor NG-monomethyl--arginine, indicating that it is an endothelium-dependent process mediated by NO.
- Other measures of vasodilator response include time to maximum response, duration of the vasodilator response and the area under the dilation curve.



Time course of brachial artery flow-mediated vasodilation (FMD) in a healthy individual

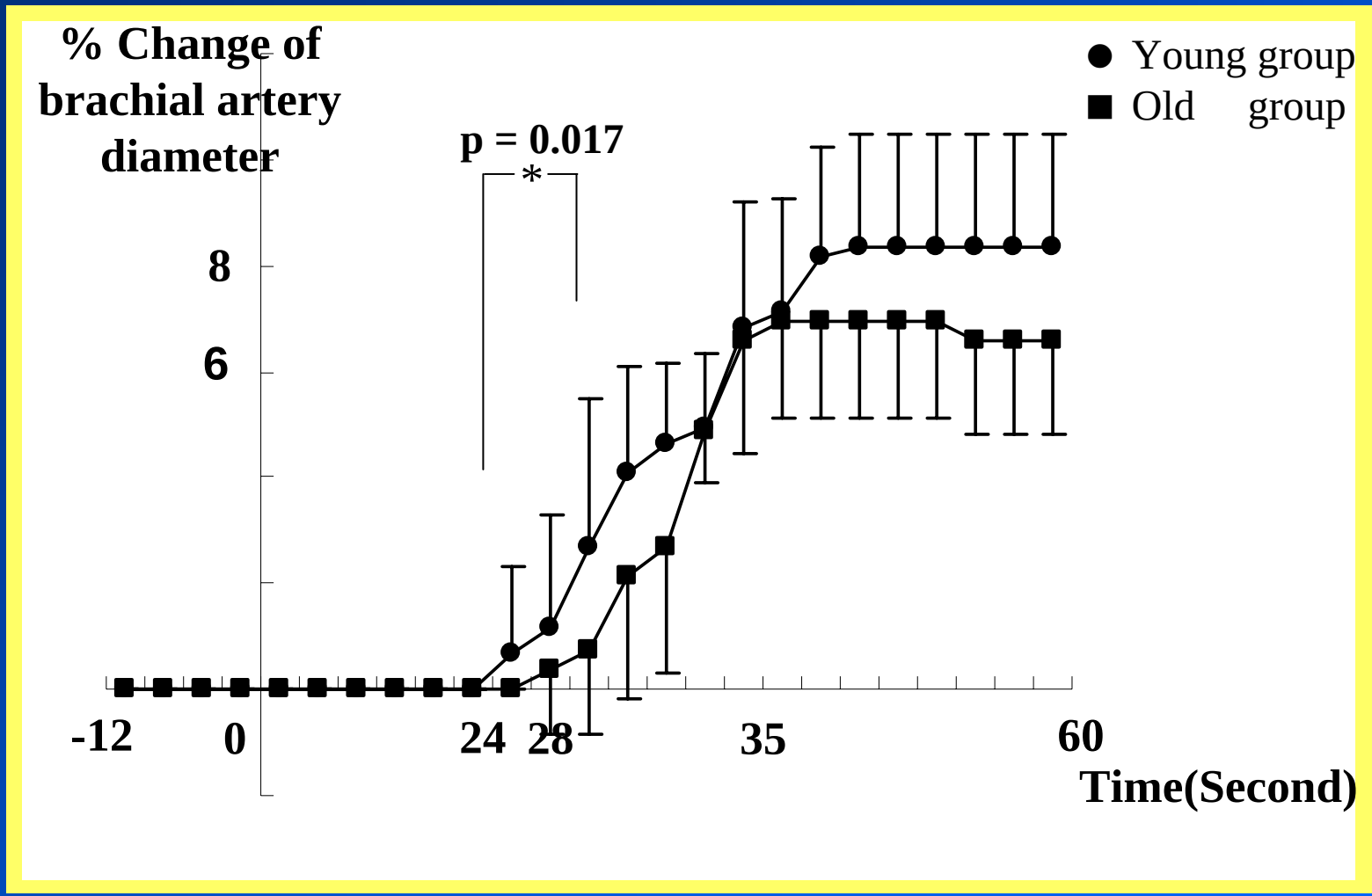


Fig. 2. Continuous monitoring of endothelial dependent vasodilation. The initiating time of vasoactivity is more faster in young group and young group has higher tendency in peak vasoactivity.

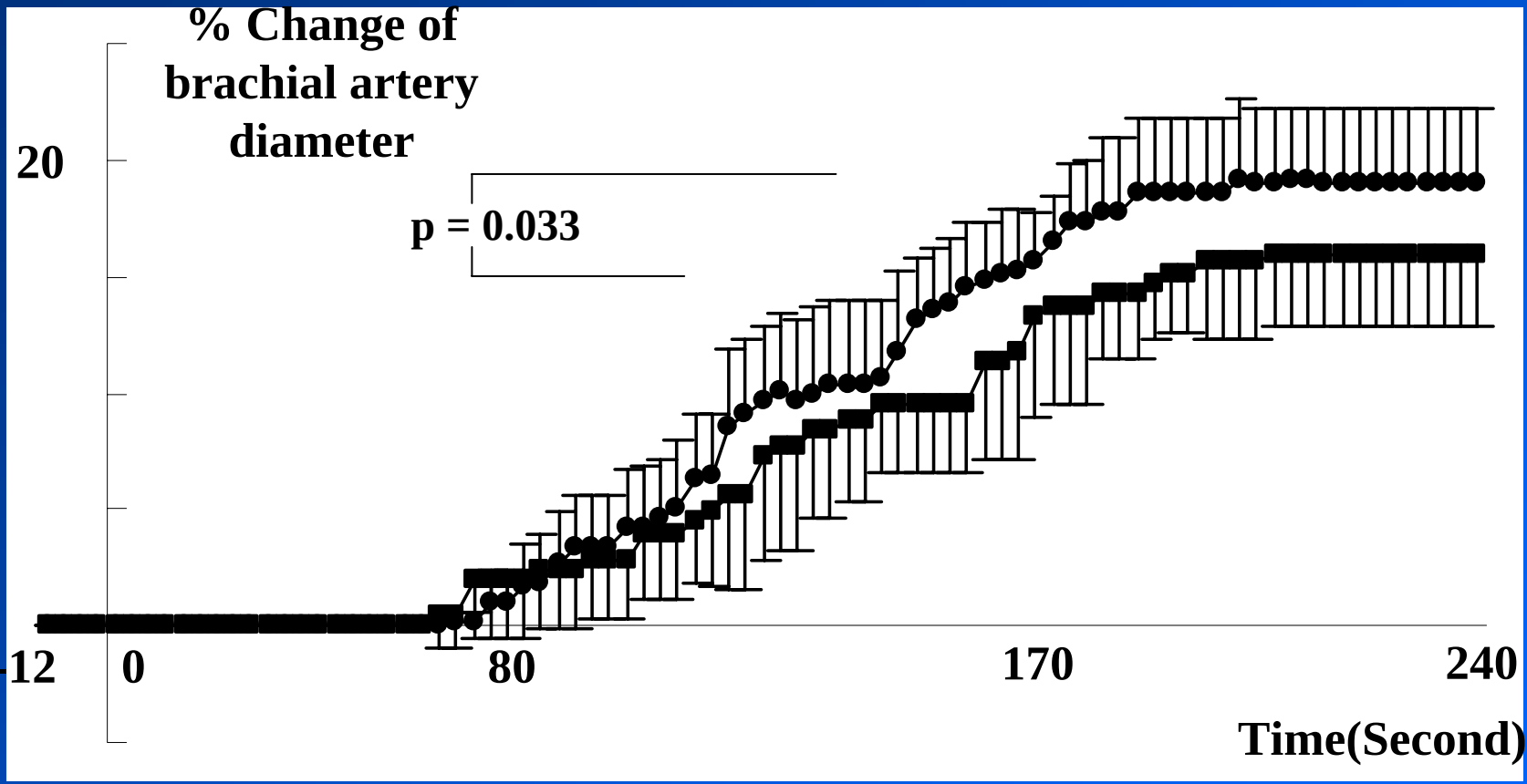


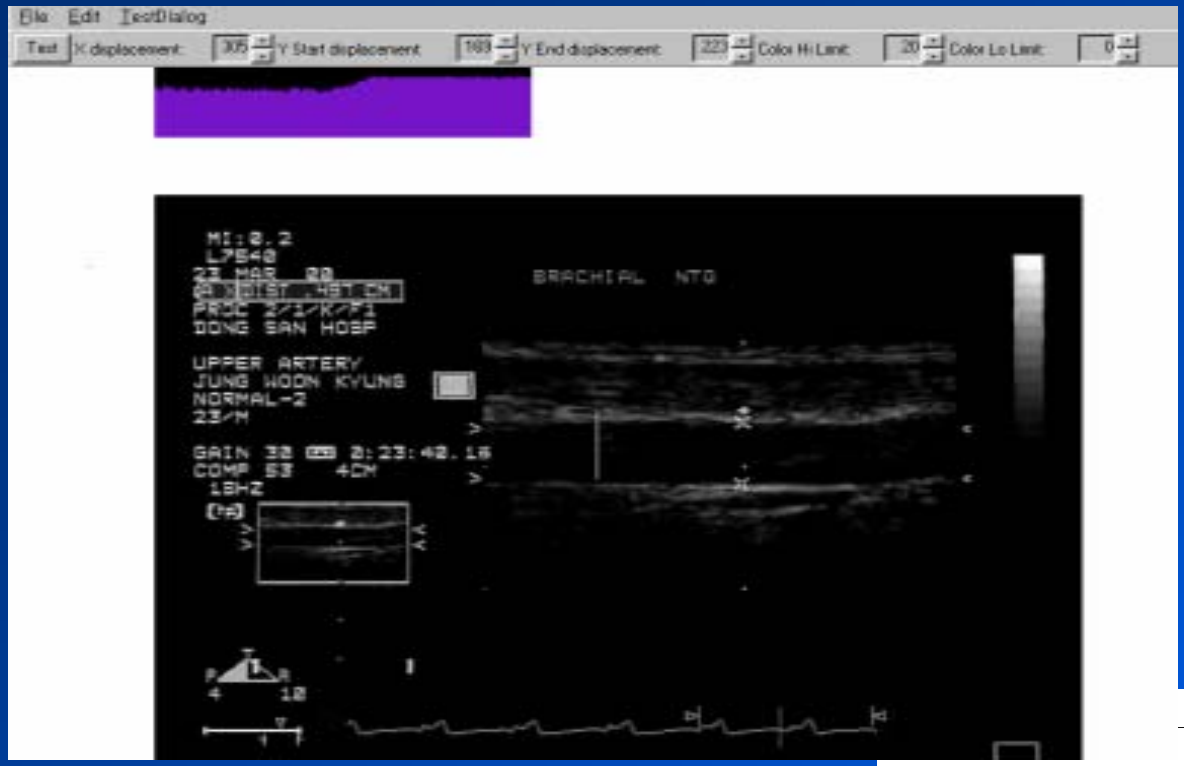
Fig. 4. Continuous monitoring of endothelial independent vasodilation. Initiating time and peak activity time are nearby, but peak vasoactivity is more higher in old group.

Timing of the measurement during the cardiac cycle

- The onset of the R-wave : end diastole,
- the peak of the T-wave : end systole.

- Peak systolic diameter is larger than end systolic diameter, because the vessel expands during systole to accommodate the increase in pressure and volume generated by left ventricular contraction.

- The magnitude of systolic expansion is affected by the vessel compliance, and it may be reduced by factors such as aging and hypertension (possibly by reduced bioavailability of NO). Thus, functional characteristics of the brachial artery may obfuscate the measurement of FMD if diameter is measured during end systole; however, this concern has not been tested in a rigorous trial.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BRACHIAL NTG


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Characterizing FMD

Baseline diameter

- First, a larger baseline diameter yields a smaller measure of percent change. Reporting absolute change in diameter will minimize this problem.
- Second, smaller arteries appear to dilate relatively more than do larger arteries.
- Percent change might be the easiest method to use if baseline diameter remains stable over time. However, the best policy may be to measure and report baseline diameter, absolute change and percent change in diameter.

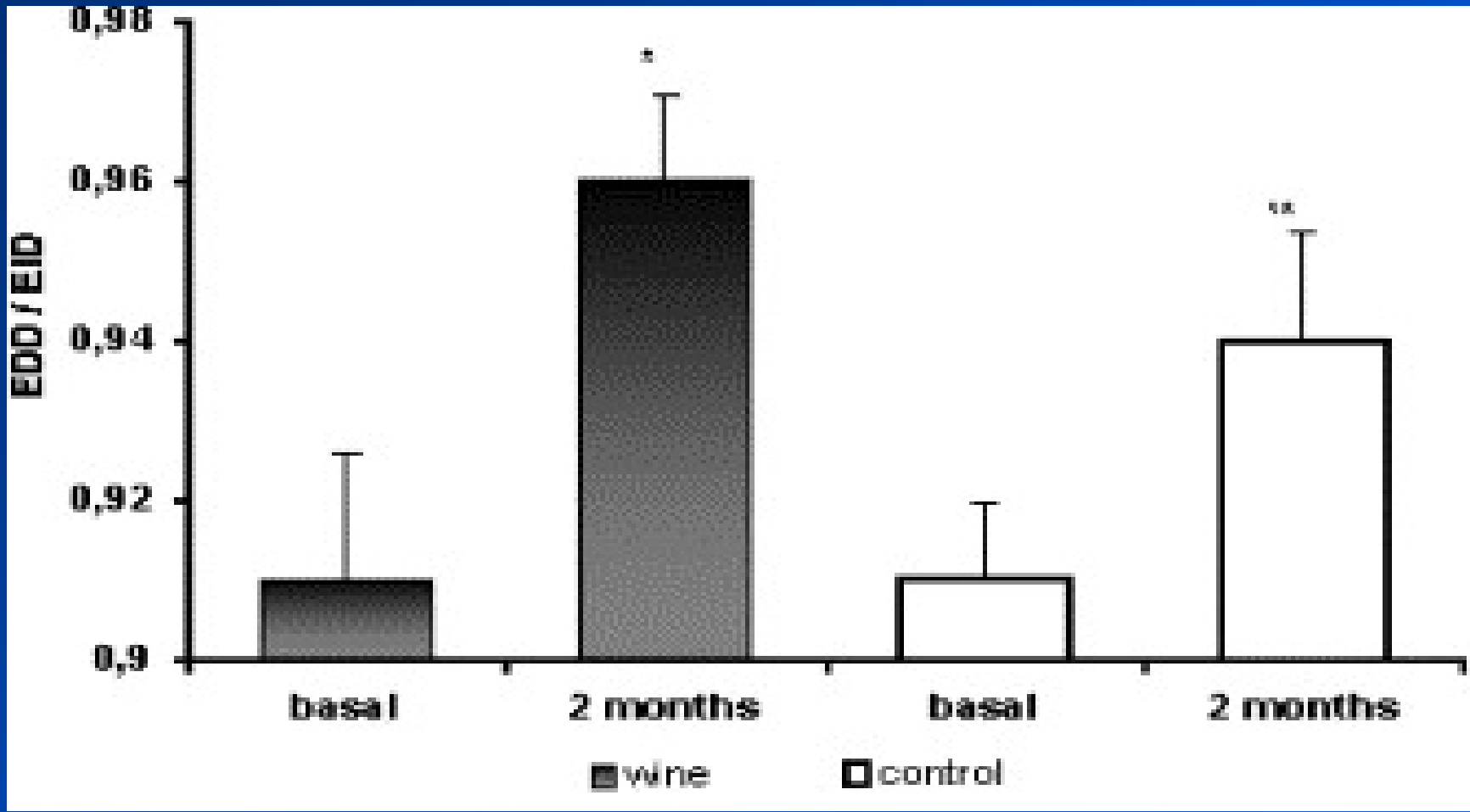


Fig. 1. Flow mediated dilation at baseline and after 2 months in groups Wine and Control. EDD/EID=endothelial dependent dilatation/endothelial independent dilatation ratio (normal reference value=0.95±0 03). *p<0.005 basal vs. 2 months in Wine; **p<0.01 basal vs. 2 months in Control. Differences between Control vs. Wine at baseline and at 2 months were not statistically significant

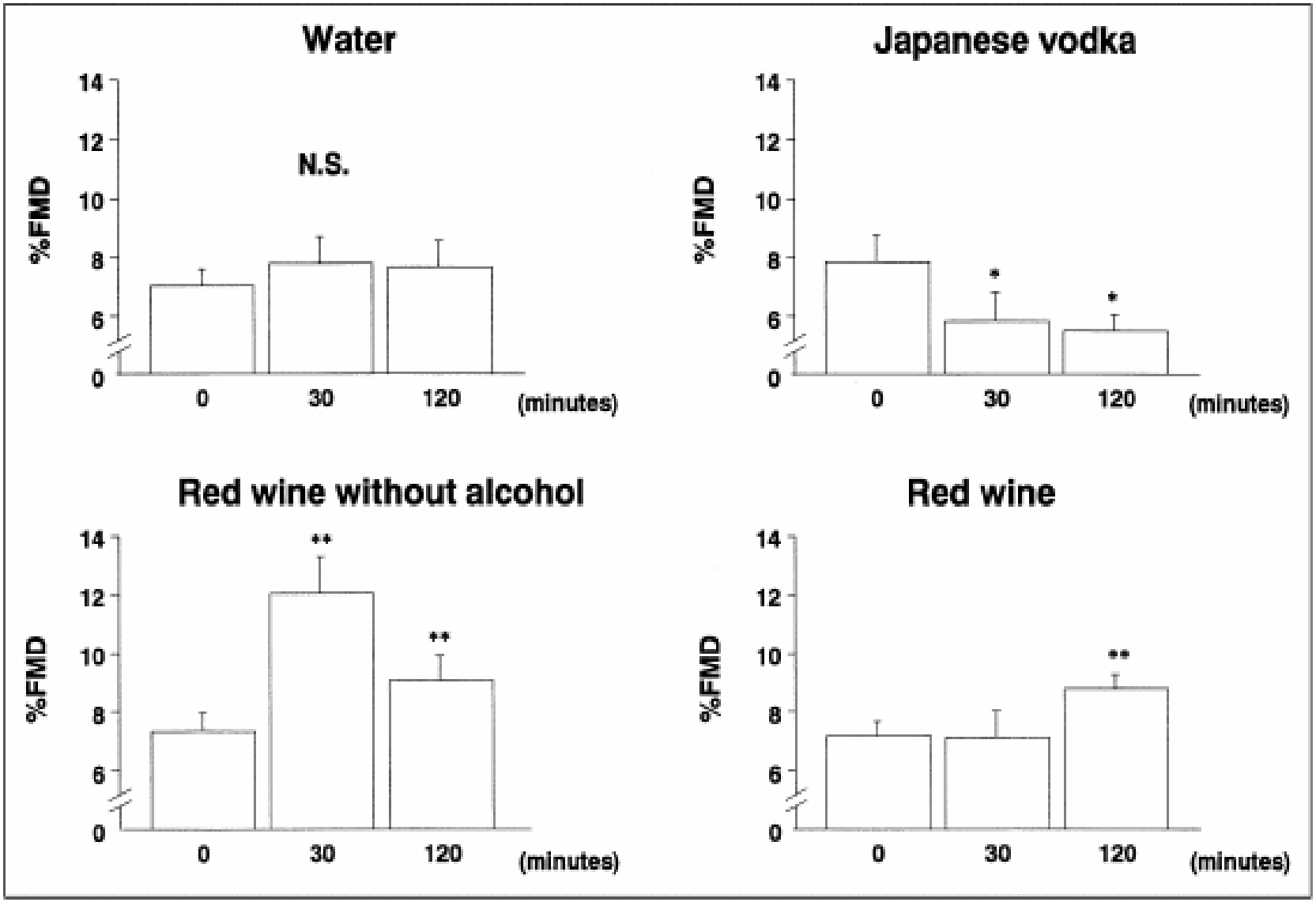
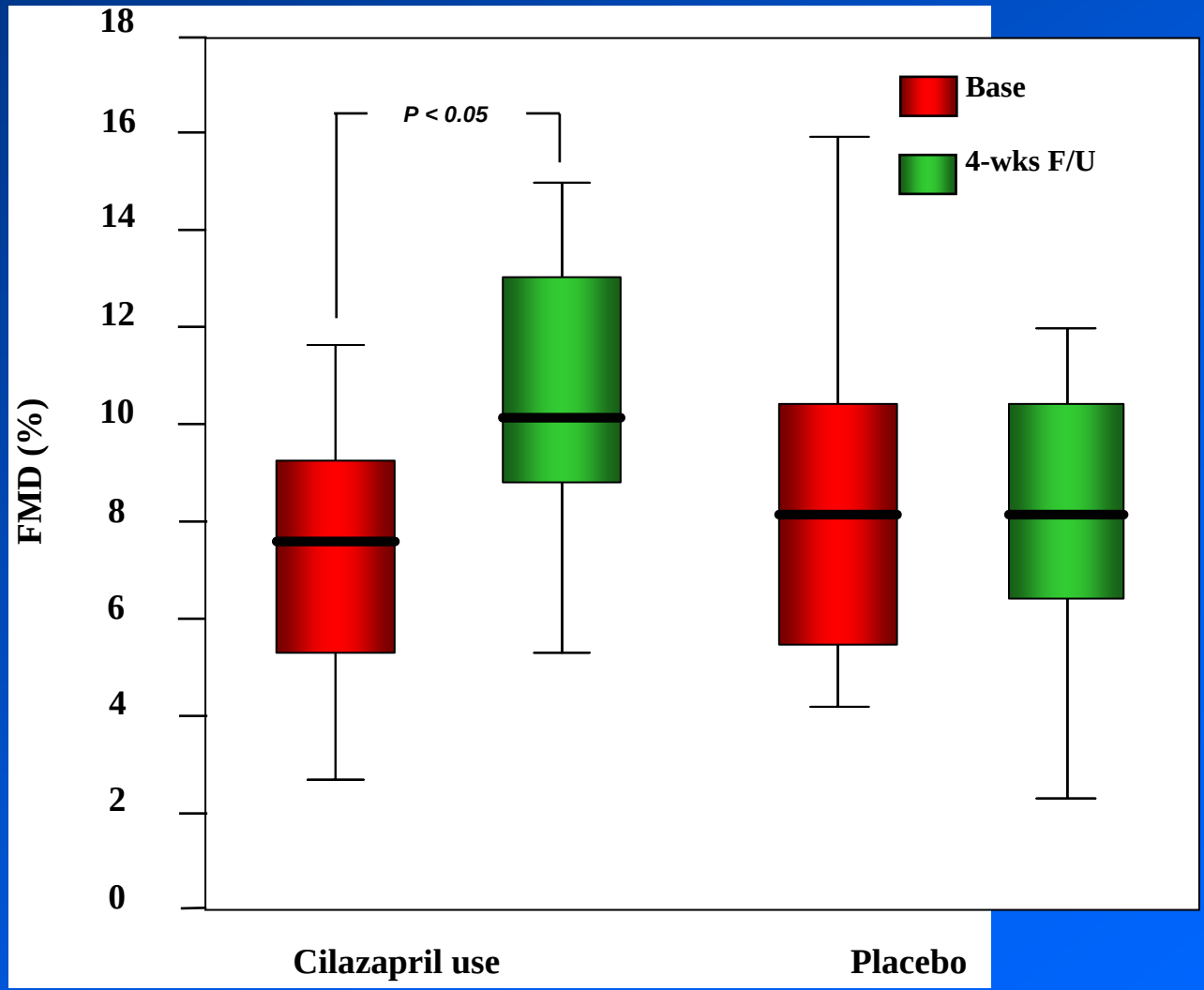
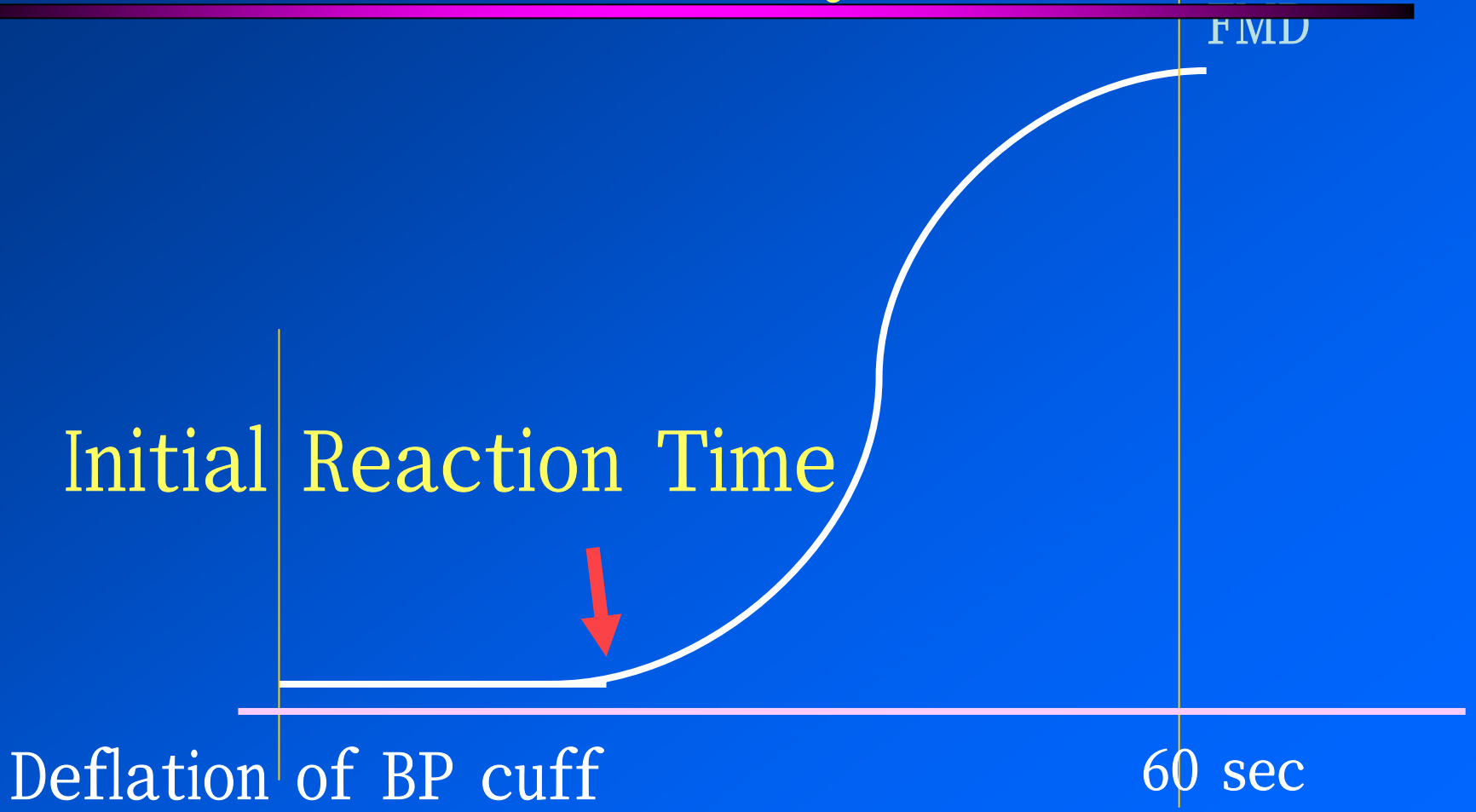


FIGURE 1. Percent FMD changes after 4 kinds of beverage. Data are expressed as mean ± SEM. *p <0.05; **p <0.01 versus before intake. N.S. = not significant.

Short-term Effects of cilazapril on Vascular Response in Heart Failure



Compare initial reaction time of Vasoactivity between young age diabetes and healthy volunteers



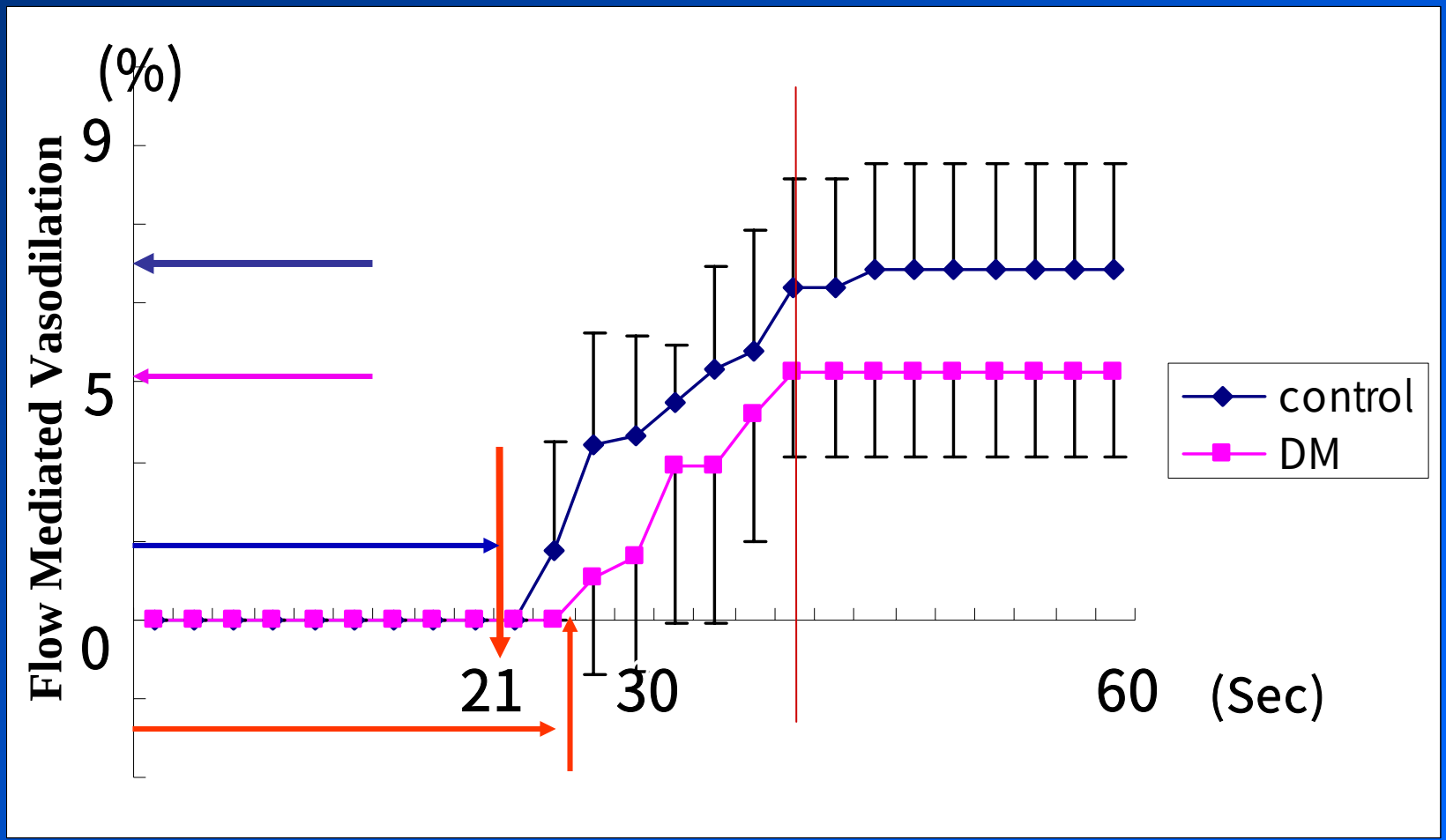
The results of FMD and EID

		Normal (n=12)	DM (n=12)
FMD	L. d (cm)	0.376 ± 0.068	0.366 ± 0.057
	VA (%)	8.9 ± 2.7	6.3 ± 2.1 *
	Initial-rx(sec)	21.3 ± 3.3	28.4 ± 5.3 **
EID	VA (%)	19.7 ± 3.8	18.5 ± 5.5
	Initial-rx(sec)	83.4 ± 12.9	86.7 ± 15.7

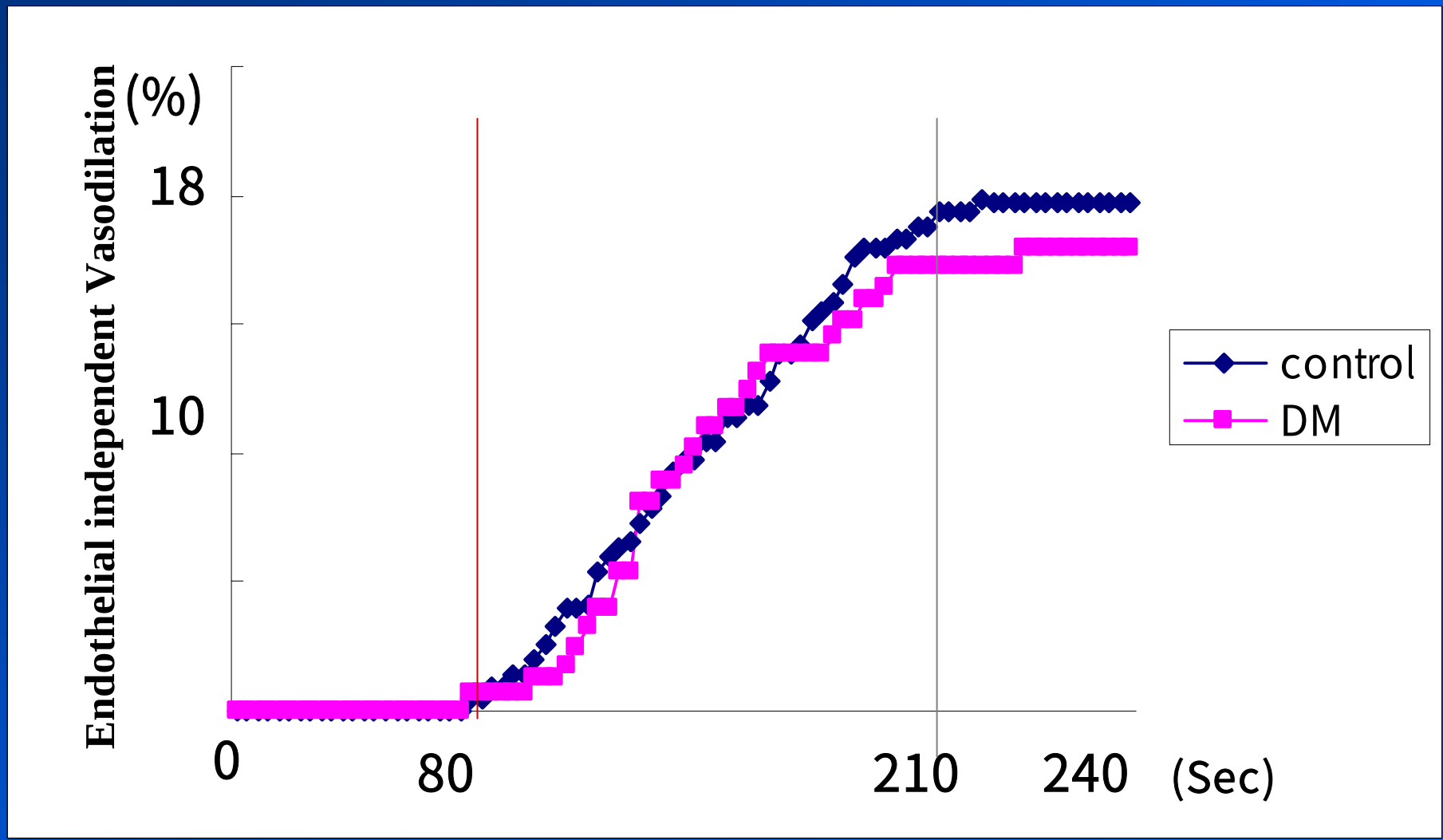
L.d:lumen diameter, VA:vasoactivity, Initial-rx: initial reaction time

**:$p<0.0005$ vs control *:$p<0.05$ vs control

Continuous Monitoring of Flow Mediated Vasodilation



Continuous Monitoring of Endothelial Independent Vasodilation



Compare initial reaction time of Vasoactivity between young age diabetes and healthy volunteers

Initial–reaction time(IRT) is very sensitive marker to detect endothelial dysfunction in young diabetic patients.

Coronary artery disease and endothelial function:

Initial reaction time vs conventional FMD

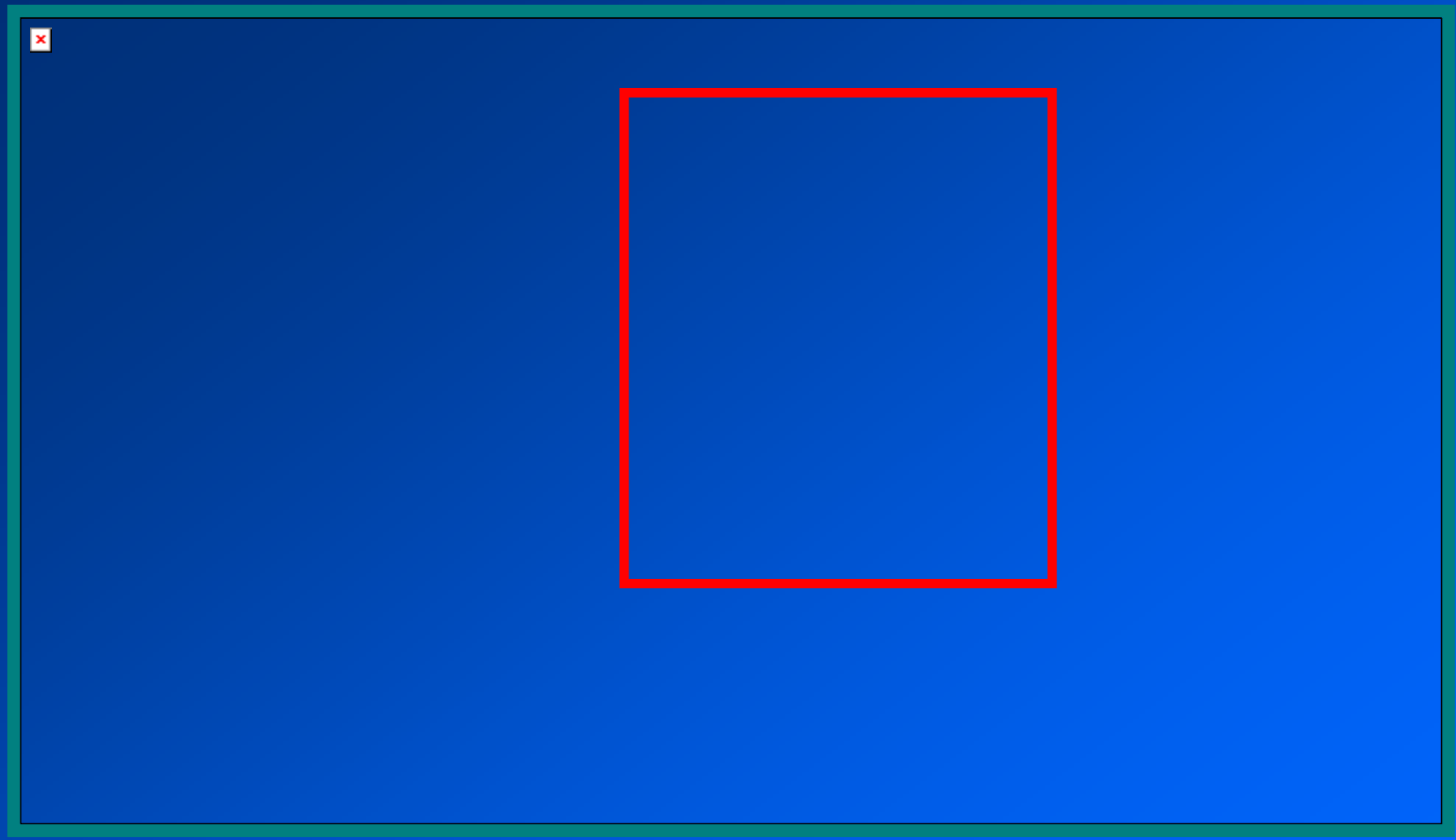


Figure 1. Percent change of brachial artery in response to hyperemic flow.
Vasoactivity: flow-medicated, endothelial dependent vasodilation, CADs: severe stenosis of coronary artery, CADm: minimal stenosis of coronary artery, Ca: age-matched control group, Cy: young healthy control group

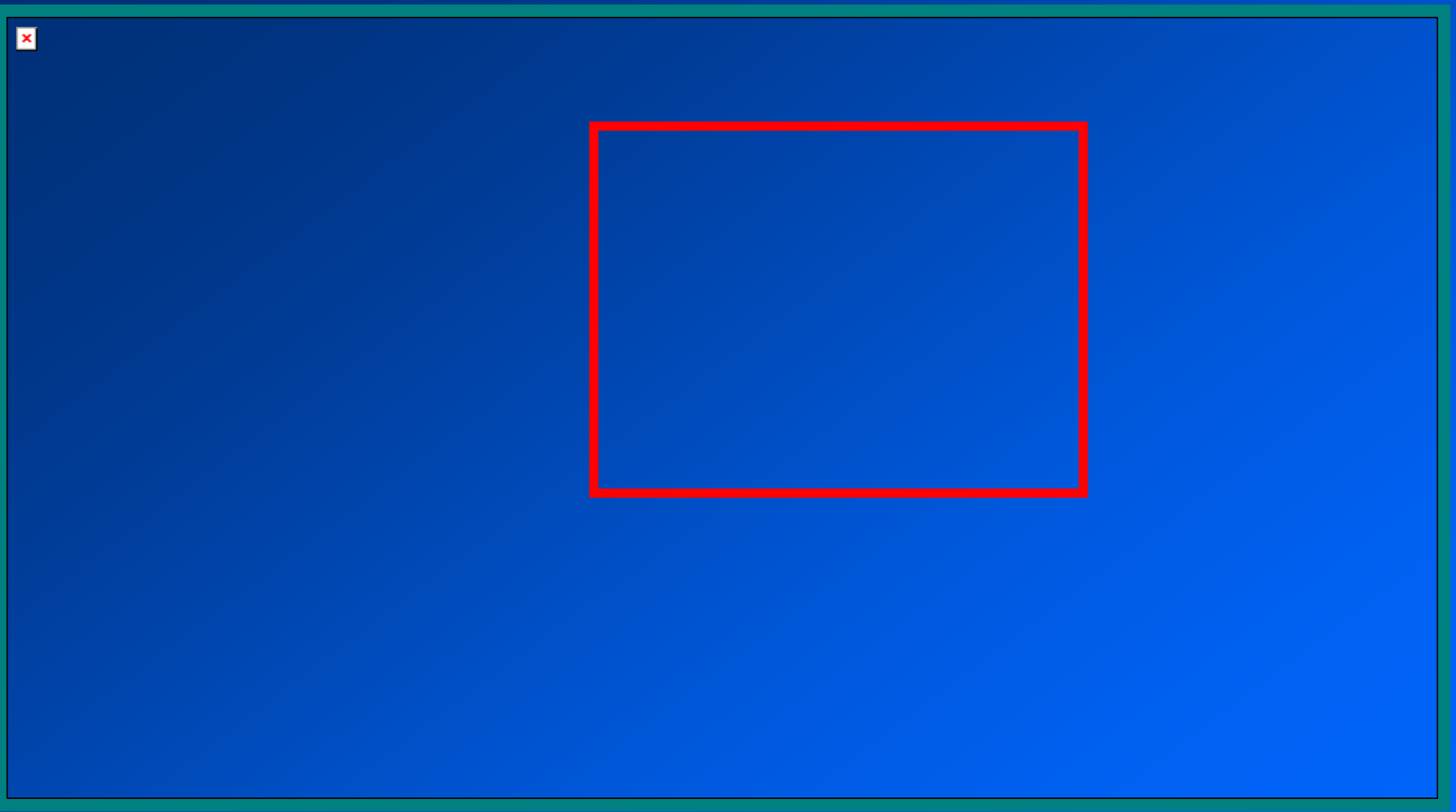


Figure 3. IRT, Lag time to initial change of brachial artery in response to hyperemic flow. IRT: initial reation time, CADs: severe stenosis of coronary artery, CADm: minimal stenosis of coronary artery, Ca: age-matched control group, Cy: young healthy control group

Conclusion

- FMD 측정은 비교적 간단히 할 수 있는 방법이며 심혈관계 질환, 특히 동맥 경화증의 조기 발견에 많은 도움을 주고 여러 가지 약효의 판정에도 도움이 되는 검사 방법이다.
- 새로운 parameter인 IRT는 좀더 연구가 필요한 변수이나 쉬운 측정 방법이 나오면 도움이 될 것으로 보인다.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