Endothelial Function and Cardiovascular Prognosis

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3rd Dubrovnik Cardiology Highlights

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Milwaukee, Wisconsin

Green Bay Packers
World Football Champions, 2011

Milwaukee Brewers Baseball

Milwaukee Art Museum
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

1980: Furchgott identified the critical role of endothelium in vasodilation

- 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

- Shear Stress from normal blood flow
- Acetylcholine
- Bradykinin
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
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

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Risk for CAD</th>
<th>Endothelial Dysfunction</th>
<th>Loss of NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>HTN</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Family History of CAD</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Tobacco Use</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Male Gender</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Menopause</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Mental Stress</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Epicard. Anat: Dilator</th>
<th>normal</th>
<th>↑ Chol</th>
<th>Non-Obst.</th>
<th>Obst. CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ach</strong></td>
<td>↑↑</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td><strong>Cold Pressor</strong></td>
<td>↑↑</td>
<td>↑↑</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td><strong>FMD</strong></td>
<td>↑↑</td>
<td>↑↑</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
</tbody>
</table>

- N=11
- N=9
- N=9
- N=9

- 38 patients

- from Zeiher, Drexler et al., Circulation, 1991
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
Reversal of Atherosclerosis in Hypercholesterolemia

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:

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
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

- 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.

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

Hypothesis

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

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

### Protocol

FMD ➔ Exercise ➔ FMD
Effect of Weight Lifting on Flow Mediated Dilation in Conditioned Weight Trainers

![Graph showing the change in FMD (% Change in Diameter) before and after exercise.]
<table>
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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

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**Bar Graph:**

- **X-axis:** SED, CWL, Runner, CT
- **Y-axis:** Absolute Change in Brachial Artery FMD (%)

**Legend:**

- SED: Black bar with an asterisk (*)
- CWL: Gray bar with a hash symbol (#)
- Runner: Gray bar with a hash symbol (#), followed by an accent symbol (†)
- CT: Gray bar with an accent symbol (†)

---

**Text:**

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

Benefit also seen with other stresses: hyperglycemia; lipid bolus

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

Traditional Treatment Strategies
- Cholesterol
- Diabetes
- Tobacco
- Obesity
- FHx
- HTN

Novel Treatment Strategies
- Antioxidants
- Gene Therapy
- Cell Therapy

“atherostasis”

NO^2 O_2

NOS

Cofactors

L-arginine

NO substrates

Replacement NO??
Implications for Detection of CAD Disease Severity

Disease Progression

Pre-Lesion
Early non-obst. Lesion
Mature calcified Plaque
Plaque rupture

Reversible
Permanent

ACS,
EBCT
Cath
NI image,
Stress test
Endothelial function
Endothelial Dysfunction Leads to Atherosclerosis (Shear and Atherosclerosis)
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
Follow-up Regained Menses - FMD

Initial Testing              Follow-up
31 32 33 34 35

Percent Dilation
0
2
4
6
8
10

* P=0.016

Female Tetrad may be a new risk factor for early atherosclerosis

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

<table>
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<th>AHA Diet</th>
<th>Atkins Diet</th>
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<tbody>
<tr>
<td>Fruits</td>
<td>High</td>
<td>Low</td>
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<tr>
<td>Vegetables</td>
<td>High</td>
<td>Low</td>
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<tr>
<td>Carbohydrates</td>
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<td>Low</td>
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<tr>
<td>Fats &amp; Oils</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Salt</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Meat</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>
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

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.

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Subjects (n)</th>
<th>F/U (yrs)</th>
<th>Tx</th>
<th>Result</th>
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<tbody>
<tr>
<td>CHAOS</td>
<td>1996</td>
<td>2002</td>
<td>2</td>
<td>Vit E</td>
<td>+</td>
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<tr>
<td>Azen et al.</td>
<td>1996</td>
<td>146</td>
<td></td>
<td>Vit E</td>
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<tr>
<td>CARET</td>
<td>1996</td>
<td>18314</td>
<td>12</td>
<td>β-carotene</td>
<td>-</td>
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<tr>
<td>MPV</td>
<td>1997</td>
<td>317</td>
<td>0.6</td>
<td>Vit E+C</td>
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<tr>
<td>PART</td>
<td>1997</td>
<td>101</td>
<td>0.6</td>
<td>Probucol</td>
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<tr>
<td>ATBC</td>
<td>1997</td>
<td>1862</td>
<td>3-5</td>
<td>β-carotene</td>
<td>-</td>
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</tbody>
</table>
# Antioxidants and Atherosclerosis

## Primary Prevention: Epidemiological

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

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

Improving the Risk without changing the risk factor

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

Frick et al. JACC 2005

Single measure of FMD vs. sequential
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.
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

- TRENDS (Trial on Endothelial Dysfunction)
- Pts. with CAD but without HTN, CHF, or high Chol.
- 6 month f/u cath
Stem Cells and Endothelial Function

45 patients with CAD; 15 controls


7 patients with PVD; autologous BM stem cells

Higashi et al. Circ 2004
Studies in LDL receptor-null mice (LDL R-/-)

ROS and Vasomotor Function

Disease Severity

ROS Generation

Vasodilator Mechanisms

Atherogenic Potential

ROS

H$_2$O$_2$

ONOO$^-$

superoxide

NO

EET

H$_2$O$_2$
Endothelial Nitric Oxide Synthase

L-Arginine → cNOS
BH₄
FAD
FMN
Ca²⁺
caveolin

→ NO⁻
→ citrulline

HSP 90
caimodulin
Ca²⁺
Endothelial Function and Prognosis in CHF

Kaplan-Meier plot of survival over time

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

>K median FMD
< median FMD

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.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio for in-stent restenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD &lt;7%</td>
<td>4.5 (2.4-12.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4.0 (1.3-7.9)</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>0.4 (0.3-0.5)</td>
</tr>
<tr>
<td>Stent diameter &lt;3 mm</td>
<td>2.0 (1.2-4.3)</td>
</tr>
<tr>
<td>IIb-IIIa inhibitors</td>
<td>3.1 (0.8-7.9)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>2.8 (0.7-7.8)</td>
</tr>
<tr>
<td>NMD</td>
<td>2.4 (0.8-6.3)</td>
</tr>
</tbody>
</table>

Patti et al. Circ. 2005
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)

A. Patients with no CAD

B. Patients with CAD

Miura et al. Circulation 2001
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
- PGI₂
- Ach

- Lumen
- Endothelium
- Vascular Smooth Muscle

- NO
- EDHF
- NOS
- NADPH oxidase
- O₂⁻
- \( \text{Ca}^{++} \)
- \( \text{K}^{+} \)
- \( \text{Ca}^{++} \)
- \( \text{K}^{+} \)
- \( \text{O}_2^- \)
- \( \text{GTP} \)
- \( \text{AA} \)
- \( \text{PLC} \)
- \( \text{CYP450} \)

- \( \text{I-Arg} \)
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

<table>
<thead>
<tr>
<th></th>
<th>nNOS (NOSI)</th>
<th>iNOS (NOSII)</th>
<th>eNOS (NOSIII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium dependent</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Calmodulin dependent</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Constitutively active</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Caveolin sensitive</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>NO Production</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Neurons</td>
<td>+++</td>
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</table>
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
NO PGI2 schema

Lumen

Endothelium

Vascular Smooth Muscle

Shear
BK
Ach

Ca++

I-Arg

NOS

NO

K+

Ca++

dilation

GTP

X

K+

cGMP

GC

GTP

?
Endothelial Nitric Oxide Synthase

L-Arginine → BH4 → cNOS → FAD/FMN → NO⁻ → citrulline

Buffered by calmodulin and caveolin with Ca²⁺ and HSP 90
### Nitric Oxide Synthase Isoforms

<table>
<thead>
<tr>
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<th>iNOS (NOSII)</th>
<th>eNOS (NOSIII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium dependent</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Calmodulin dependent</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Constitutively active</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Caveolin sensitive</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>NO Production</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Neurons</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Possible Etiologies of EDHF

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

**Endothelium**

- Shear
- BK
- Ach
- AA
- PLC
- COX
- CYP450
- I-Arg
- NOS
- NO
- EDHF
  - (EET)

**Lumen**

- K⁺
- Ca²⁺

**Vascular Smooth Muscle**

- ATP
- AC
- cAMP
- cGMP
- GC
- PGI₂
- EDHF
- NO
- K⁺
- Ca²⁺
- GTP
- dilatation

**Contraction**

- K⁺
- AC
- cAMP
- cGMP
- GC
- GTP
- ATP
Shear | BK | PGI₂ | Ach
---|---|---|---
PLC | CYP450 | EET | EDHF
K⁺ | Ca++ | NO | NOS
I-Arg | NADPH oxidase | O₂ | SOD
H₂O₂ | Hypercholesterolemia | Diabetes | Smoking

Lumen | Endothelium | Vascular Smooth Muscle
---|---|---
K⁺Ca | Ca++ | K⁺Ca
EDHF | GTP | O₂
Is NO-mediated Vasodilation Really Important?

Atherosclerosis

- Vasospasm
- syndrome X
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

Risk Factor
- Diabetes Mellitus
- Hypercholesterolemia
- Hypertension
- Tobacco

NOS
HSP 90
BH

\( \text{O}_2 \text{^(-)} \)

\( \text{ADMA} \)

\( \text{NO}^* \)

\( \text{ONOO}^- \) (peroxynitrite)
Development of Atherosclerosis

- Injury
  - glucose
  - oxLDL
  - mechanical (HTN)

- Inflammatory Response
  - lymphocytes
  - platelets
  - monocytes

- Migration and transformation

- Fully developed plaque
  - foam cells
  - fibroblast migration

- subintimal thickening

- Development of Atherosclerosis
  - intima
  - media
  - adventitia

- Foam cells
  - Fibroblast migration

- Subintimal thickening