Learning Objectives
At the end of the session, participants will be able to:
1. Explain a linkage between obesity and endothelial dysfunction.
2. Describe how key vasoactive substances are produced by endothelium and how they regulate blood vessel tone.
3. Explain what causes dysregulation of the bioavailability and/or function of key vasoactive substances produced by endothelium in the presence of overweight/obesity.
4. Explain the principles of methods that are often employed in making a diagnosis of endothelial dysfunction.
5. Explain how endothelial positive adaptive changes occur in response to exercise training.

Obesity-Induced Vascular Dysfunction: Pathophysiology and Treatment with Exercise

APTA Combined Section Meeting, Anaheim, CA, February 18-20, 2016

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Conflicts of Interest
* There is no conflict of interest that needs to be disclosed.

Overweight/Obesity Statistics in the US

<table>
<thead>
<tr>
<th>%</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults, 20+ yrs old</td>
<td></td>
</tr>
<tr>
<td>Overweight or Obese, BMI ≥ 25 kg/m²</td>
<td>68.5</td>
</tr>
<tr>
<td>Obese, BMI ≥ 30 kg/m²</td>
<td>14.9</td>
</tr>
<tr>
<td>**Extremely obese, BMI ≥ 40 kg/m²</td>
<td>6.3</td>
</tr>
<tr>
<td>Children &amp; adolescents, 2-19 yrs old</td>
<td></td>
</tr>
<tr>
<td>Overweight or Obese, BMI for age ≥ 85th percentile</td>
<td>31.8</td>
</tr>
<tr>
<td>Obese, BMI for age ≥ 95th percentile</td>
<td>16.9</td>
</tr>
</tbody>
</table>

Based on survey results in 2009-2010

C. Ogden et al., JAMA 2014, 311:806-814
Overweight & Obesity are Associated with increased Cardiovascular Mortality

B. Cepeda-Valery et al., Nat. Rev. Cardiol. 2011, 8:233–237

Original data from Lancet 2006, 368:666–678

All-cause mortality

Cardiovascular mortality

BMI = Body Mass Index

BMI = Fat mass + Lean BM

BMI lacks discriminatory power to differentiate between body fat and lean mass.

An increase in fat free mass is related to a decrease in mortality.

An increase in fat mass is related to an increase in mortality.

Endothelial Dysfunction Associated with CV Diseases or Risk Factors

Obesity  Physical inactivity  Dyslipidemia  Endothelial Dysfunction  Diabetes  Smoking  CAD  Aging  Hypertension

Heart failure  Stroke  Chronic renal failure

Endothelial Dysfunction

Implications of Endothelial Dysfunction

Consequences of Endothelial Dysfunction

Impaired vascular function

Increased vascular tone

Platelet aggregation

Increased inflammation

Impaired wound healing

Impaired angiogenesis

Reduced nitric oxide availability

Reduced expression of BMP

Stroke

Cognitive decline

Endothelium

vascular muscle blood cells neurons glia

Target Cells
“Vascular dysfunction in the pathogenesis of Alzheimer’s disease — A review of endothelium-mediated mechanisms and ensuing vicious circles”

L. DiMarco et al., Neurobiology of Disease, 2015, 82: 593–606

Vascular Endothelial Function

- It serves as an interface between flow and vascular wall.
- It regulates vascular tone and blood flow by modulating the diameter of vessels.
- It regulates smooth muscle cell proliferation.
- It regulates vessel wall inflammation.
- It regulates coagulation processes.
- It synthesizes and releases vasoactive substances, i.e., NO & endothelin-1.

Key Vasoactive Substances released by Endothelium

- Nitric oxide (NO)
- Endothelin-1 (ET-1)
Role of Nitric Oxide (NO) in Vasodilation

Endothelium

- Nitric oxide (NO)
- Endothelial cell
- Shear stress
- L-arginine (L-Arg)
- Nitric oxide synthase (NOS)
- Nitric oxide (NO)

Smooth muscle

- Endothelium
- Relaxation

Substrates & Stimuli that Affect NO Production through eNOS

- Shear stress
- Acetylcholine, serotonin, thrombin, bradykinin
- NADPH
- O2
- BI4
- L-citrulline
- L-arginine
- eNOS
- GTP
- cGMP
- Relaxation

Endothelin-1 (ET-1) Production & Its Action

VASCULAR WALL

- Endothelin-1 (ET-1)
- Smooth muscle cells
- Vascular smooth muscle cell
- Relaxation
- Constriction
- Endothelial cell
- Smooth muscle

Regulation of Endothelin-1

- Endothelial cell
- Smooth muscle cell
- Vessel lumen
- Constriction
- Relaxation
- MAPK
- P16
- MAPK
- P16
- ANP
- SNP
- NO
- Prostaglandin
- Vascular smooth muscle cell
**Insulin Signaling Pathway in Endothelium**

**Endothelial Insulin Resistance**

- What will happen to the balance between NO and ET-1 in the presence of endothelial insulin resistance?

  **ET-1 >> NO**

  Vasoconstriction + many other effects

How does insulin resistance occur in the presence of obesity?

**Visceral Fat Cells Produce Inflammatory Cytokines**

**Expanded Fat Cells Produce Inflammatory Cytokines**
Imbalance between NO & ET-1 in Endothelium by Adipokines

- Insulin resistance interferes IRS-1/PI3K pathway.
- No interference occurs on MAPK pathway.
- ET-1 production pathway is enhanced.

Insulin Resistance Leads to Vascular Dysfunction

- Insulin resistance interferes IRS-1/PI3K pathway.
- No interference occurs on MAPK pathway.
- ET-1 production pathway is enhanced.

Reactive Oxygen Species (ROS)

- ROS have an unpaired electron in the most outer orbit.
- ROS are very reactive molecules.
- These molecules are produced as byproducts of metabolism of O₂ in normal cells.
- If ROS were produced excessively, they can interact with structural elements of tissues, damaging their functions.
- This situation is called "oxidative stress".

Structure and Function of NADPH Oxidase (NOX)

- NADPH oxidase is a multicomponent enzyme.
- Three cytosolic accessory proteins (Rac, p47<sup>phox</sup>, p67<sup>phox</sup>, & p40<sup>phox</sup>).
- Two membrane-catalytic subunits (Nox, p22<sup>phox</sup>).
- There are multiple Nox isoforms.
- A superscript "phox" means "phagocyte oxidase" as it was found initially from phagocytes.
- Upon being stimulated, the cytosolic components associate with the membrane catalytic subunits.
- It produces superoxide (O₂⁻) by adding a single electron from NADPH to O₂.
Overweight/Obesity-Induced Vascular Dysfunction

Endothelial cells

Smooth muscle cells

Healthy artery

Endothelial dysfunction

Relaxation

Constriction

Endothelial dysfunction refers to reduced endothelial reactivity to vasoactive substances.

Decreased vasodilation in response to acetylcholine or insulin.

Linkage Between Overweight/Obesity & Endothelial Dysfunction

Overweight/Obesity-Induced Endothelial Dysfunction Leads to Atherosclerosis

Obesity-induced Endothelial Dysfunction Leads to Atherosclerosis

Overweight/Obesity

Dyslipidemia

Insulin resistance

Insulin resistance

Hypoglycemia

Oxidative Stress

Expression of ICAM-1, VCAM-1, MCP-1

Atherosclerosis

Cerebral arterial disease

PAD

CAD

Early Detection of Endothelial Dysfunction

• It is required to prevent the deterioration of endothelial function.
**Assessment of Endothelial Vascular Function**

- **Flow-Mediated Dilation (FMD)**
- **Euglycemic Hyperinsulinemic Clamp technique**
- **Venous Occlusion Plethysmography**

**Flow-Mediated Dilation (FMD)**

**Assessment of Vascular Function & Treatment**
Flow-Mediated Dilation (FMD)

- Ultrasound system
  - Vascular software for 2D imaging
  - Color & spectral Doppler
  - Internal ECG monitor
  - High frequency vascular linear transducer

Experimental Set-Up for the Assessment of FMD

Ultrasound Image of Brachial Artery

Baseline measurement

1 min after deflation of the cuff

Analysis of Data To Determine FMD

- Diameter at baseline: 4.5 mm
- Diameter after ischemic occlusion: 5.0 mm
- Change ($\Delta$) = 5.0 − 4.5 = 0.5 mm

- FMD (%) = ($\Delta$ / baseline diameter) x 100
  = (0.5/4.5) = 11%
Flow-Mediated Dilatation Depends on NO

- **L-NMMA:**
  - Inhibitor of eNOS
  - Inhibition of NO production

![Graph showing radial flow and diameter before and after L-NMMA administration](image)

R. Joannides, Circulation., 1995, 91:1314-1319

Relationship of FMD% with CV Risk Factors

- **FMD%**
- **Risk Factor Score**
- Cholesterol, smoking, mean blood pressure, family history, age, gender.

![Graph showing relationship between FMD% and risk factor score](image)

![Graph showing high correlation between coronary FMD and brachial FMD](image)

B. Takase et al., Am. J. Cardiol., 1998, 82:1535-1539

Evidence of the Presence of Endothelial Dysfunction in Individuals with Overweight/Obesity

![Graph showing evidence of endothelial dysfunction](image)

B. Takase et al., Am. J. Cardiol., 1998, 82:1535-1539
Effect of Obesity on Vascular Health

**Characteristics of Subjects:**
- Sedentary and obese individuals
- No smoking
- No established CV disease
- No hyperlipidemia
- No use of birth control drugs
- No Tx with hormone replacement
- Total cholesterol <200 mg/dl
- Fasting glucose <126 mg/dl
- No family history of premature CV disease

**Blood Metabolic Profiles in Healthy Sedentary Individuals with Obesity**

<table>
<thead>
<tr>
<th>Metabolic Trait</th>
<th>Recommended Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>&lt;200</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>&lt;100</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>&gt;40 for men &amp; women</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Insulin, uU/dl</td>
<td>&lt;11</td>
</tr>
<tr>
<td>FFA, mEq/L</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL particle size, angstrom</td>
<td>267±12, normal large phenotype</td>
</tr>
</tbody>
</table>

**Abdominal Obesity on Vascular Health**

- WHR≥0.85 indicates abdominal obesity.
- WHR<0.85 indicates peripheral obesity.

**Relationship between FMD & Waist/Hip Ratio**

Decrease waistline is important:
- <35” (88 cm) for women
- <40” (102 cm) for men
Inverse relationship between FMD & Body Mass

<table>
<thead>
<tr>
<th>BM, kg</th>
<th>Before diet</th>
<th>After diet</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>98±11</td>
<td>124±7</td>
<td>161±17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BM, kg/m²</td>
<td>38±4</td>
<td>45±5</td>
<td>55±7</td>
</tr>
<tr>
<td>Flow-mediated diameter increase, mm</td>
<td>0.36±0.106</td>
<td>0.32±0.17</td>
<td>0.27±0.18</td>
</tr>
<tr>
<td>Reactive hyperemia, % increase</td>
<td>694±352</td>
<td>520±298</td>
<td>518±398</td>
</tr>
<tr>
<td>Nitroglycerin-mediated dilation, %</td>
<td>12.6±5.3</td>
<td>11.5±7.3</td>
<td>11.1±5.8</td>
</tr>
</tbody>
</table>

- Endothelium-dependent dilation is abnormal.
- Endothelium-independent dilation is normal.

The results of these studies suggest that

- Weight loss likely improves FMD.

Does Weight Loss Improve Endothelium-Dependent Vasodilation in Individuals w/ Obesity?

<table>
<thead>
<tr>
<th>Lean &amp; normotensive N=15</th>
<th>Obese &amp; hypertensive N=11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>64.1</td>
</tr>
<tr>
<td>BMI</td>
<td>24.4</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>114.6</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>68.8</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.8</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>16.1</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.99</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>3.48</td>
</tr>
<tr>
<td>Plasma leptin, ng/l</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*P<0.05 vs. before diet; +P<0.05 vs. control

S. Sasaki et al., Am. J. Hypertension, 2002, 15:302-309

Endothelium-Dependent Vasodilation after Wt. Loss in Individuals w/ Obesity

- No change in endothelium-independent vasodilation

S. Sasaki et al., Am. J. Hypertension, 2002, 15:302-309

Basal Blood Flow (ml/min, femoral)

Acetylcholine (µg/min)
Combination of Diet & Exercise on FMD

- Obese subjects (n=24, 49.3 yrs, BMI 36.7) with insulin resistance
- Calorie restriction with 500 kcal less than usual/day
- Supervised exercise 3x/wk + home exercise, total of at least 150 min/wk
- 6-month intervention

They improved significantly:
- HDL level
- HbA1c
- Insulin sensitivity
- Weight (from 106.1 to 98.7 kg)
- BMI (from 36.7 to 34 kg/m²)
- WHR

They improved significantly:

Improvement in FMD after 6-month Lifestyle Change

- The whole group (WG) showed improvement in FMD.
- Each subgroup showed significant improvement.
  - Normal glucose tolerance group (N)
  - Impaired glucose tolerance group (IGT)
  - The group with diabetes (DM)

Positive Relationship between Improvement in FMD & Wt. Loss

Is Weight Loss Required for Improvement in FMD with Exercise Training?
**Adolescents with Obesity**

- Nineteen adolescents with obesity (14.4 yrs) volunteered for a study.
- They underwent a circuit training for 8 weeks.

<table>
<thead>
<tr>
<th>Lean control, n=20</th>
<th>Subjects w/ obesity, n=19</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>BM, kg</td>
<td>57.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.2</td>
</tr>
<tr>
<td>Waist girth, cm</td>
<td>71.7</td>
</tr>
<tr>
<td>Body fat%</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>41.2</td>
</tr>
<tr>
<td>Trunk fat mass, kg</td>
<td>19</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td>18.3</td>
</tr>
<tr>
<td>Abdominal fat mass, kg</td>
<td>8.6</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td>8.0</td>
</tr>
<tr>
<td>Muscle strength</td>
<td>274.2</td>
</tr>
<tr>
<td>Sum of 5 max</td>
<td>274.2</td>
</tr>
<tr>
<td>contractions</td>
<td>274.2</td>
</tr>
</tbody>
</table>

**Exercise Training**

- Randomized crossover study design:
  - 8-wk exercise training or 8-wk non-training period
- Exercise training: 1-hr session of circuit training
  - Cycle ergometer: 65-85% of HRmax
  - Resistance: 55% to 70% of pre-training maximum strength
- Significant HR response after training

**Change in FMD% After Training In Adolescents with Obesity**

- Normalized endothelium-dependent vasodilation after training.
- This results tell us:
  - Wt. loss is not obligatory for improvement in FMD with training.
  - Shear stress-mediated up-regulation of the expression of eNOS might be a mechanism.

**Change in FMD After Training In Children with Obesity**

- 14 children with obesity (8.9 yrs, BMI of 29.9, waist girth 86 cm)
- They underwent circuit training for 8 weeks.
- There was no change in BM, BMI, & waist girth after training.
- Significant improvement in FMD% occurred after training.
Further Study Is needed

- Weight loss and Exercise Training cause an additive effect on improvement in FMD.

Obesity-Associated Endothelial Insulin Resistance

- How can you assess endothelial insulin resistance?

Assessment of Endothelial Insulin Resistance

- To assess endothelial insulin resistance, investigators have employed euglycemic hyperinsulinemic clamp technique.

- We need to understand the principle of this technique

Euglycemic Hyperinsulinemic Clamp (EHC)

- Glucose concentration is maintained at a basal level (euglycemic condition).
  - Maintaining at 90 mg/dl by varying glucose infusion rate

- Insulin level is raised to a much higher level than a basal level (hyperinsulinemic condition).
  - Raising to 100 uU/ml from 10 uU/ml in basal

- This technique allows to assess insulin sensitivity of a whole body.
**Euglycemic Hyperinsulinemic Clamp (EHC)**

- Leg blood flow & glucose uptake can be assessed during the EHC.
- Leg blood flow using thermodilution technique
- Leg glucose uptake (LGU) per Fick principle
  - LGU = arteriovenous difference in glucose x leg blood flow
- Leg blood flow and LGU reflect endothelial insulin sensitivity.

**Insulin Causes Vasodilation Leading to Increased Blood Flow**

- Six healthy men (32 yrs old, 68 kg) & six healthy obese men (37 yrs old, BMI>27 kg/m^2)
- The Subjects underwent euglycemic hyperinsulinemic clamp with various insulin infusion rates to determine a insulin-dose curve.
- Leg blood flow was determined by thermodilution technique.
- Leg blood flow increased in a sigmoidal fashion.
- Basal & maximum leg blood flow were not different between the two groups.
- Half-maximum (ED50) dose of insulin differed.
  - The lean group: 256 pmol/l
  - The obese group: 957 pmol/l ($P<0.01$)
- Insulin resistance was present at endothelial level in the group with obesity.

**Leg Glucose Uptake Is Impaired with Obesity**

- LGU = ([Arterial glu.] - [Venous glu.]) x leg BF

---

**Typical pattern of changes in plasma glucose, insulin, and glucose infusion rate during the EHC.**

- Glucose Infusion Rate
  - High for individuals with high insulin sensitivity
  - Low for individuals with insulin resistance

---


Linkage between Leg Blood Flow & Endothelial Insulin Resistance

Insulin Infusion \(\rightarrow\) Vasodilation \(\rightarrow\) Increased Leg Blood Flow

What mediates insulin-induced vasodilation?

Insulin Infusion

Test of NO Involvement in Leg Blood Flow Under Hyperinsulinemic Condition

• Coinfusion of insulin and eNOS inhibitor, L-NMMA, abolished insulin-induced vasodilation.

• The graph shows no change or a slight decrease in LBF by using L-NMMA.

A. Baron et al., J. Diabetes & Complications, 2003, 16:92-102

How can we confirm the Role of NO that Lads to Insulin-induced Vasodilation?

• A strategy that is commonly used is to inhibit eNOS activity with an inhibitor.

• \(\text{N}^\circ\)-monomethyl-L-arginine (L-NMMA)

Another Way of Testing of NO-Mediated Vasodilation

• Acetylcholine (Ach) is used.

• a neurotransmitter at neuromuscular junction.

• known as an agonist for the activation of eNOS.
Reduced Endothelium-Dependent Vasodilation with Obesity or T2D


Additive Effect of Obesity & HTN on the Suppression of Endothelium-Dependent Vasodilation


What Causes Impaired Glucose Tolerance with Obesity?

T. Olver et al., Appl Physiol Nutr Metab 2012, 37:176-183

Femoral Arterial Blood Flow during OGGT

T. Ruan et al., Appl Physiol Nutr Metab 2012, 37:176-183

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lean (n = 8)</th>
<th>Obese (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23.3±2.4</td>
<td>25.5±4.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75±0.1</td>
<td>1.79±0.1</td>
</tr>
<tr>
<td>Total mass (kg)</td>
<td>80.6±2.0</td>
<td>86.7±1.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>13.6±1.5</td>
<td>17.2±1.0</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>48.4±2.5</td>
<td>69.5±2.9</td>
</tr>
<tr>
<td>Percent fat (%)</td>
<td>22.3±2.8</td>
<td>37.2±6.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3±1.0</td>
<td>29.8±4.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>69±5</td>
<td>91±10</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.8±0.03</td>
<td>0.87±0.09</td>
</tr>
<tr>
<td>Fasting capillary blood glucose (mmol/L)</td>
<td>4.5±0.4</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>2-h postprandial blood glucose (mmol/L)</td>
<td>5.5±0.4</td>
<td>7.2±1.3</td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/L)</td>
<td>4.9±1.7</td>
<td>11.3±10.2</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1±0.4</td>
<td>2.6±2.3</td>
</tr>
</tbody>
</table>

Femoral Arterial Blood Flow during OGGT

T. Ruan et al., Appl Physiol Nutr Metab 2012, 37:176-183
What Causes Endothelial Insulin Resistance?

Glucose, FFA, & Insulin level in obese individuals

High Level of Plasma FFA Impairs Endothelial-Dependent Vasodilation

Does Chronic Elevated Plasma FFA Level Cause Endothelial Insulin Resistance?

Glucose area under curve: ND
FFA area under curve: obese (P<0.02) > non-obese
Insulin area under curve: obese (P<0.01) > non-obese

• Normal healthy lean individuals were studied.
  • Normal blood pressure
  • Normal glucose tolerance
FFA Inhibits Endothelium-Derived NO Production

- Endothelial nitric oxide synthase (eNOS) from cultured bovine pulmonary artery endothelial cells were studied.
- The purpose was to determine if some of 18-C-FFA with different level of saturation inhibit the Activity of eNOS.
- Oleic acid (18:1cis), linoleic acid (18:2cis), elaidic acid (18:1trans), & stearic acid (18:0)
- Oleic acid inhibited eNOS activity in a dose-dependent manner.

Composition of FFAs in Individuals with Obesity

<table>
<thead>
<tr>
<th></th>
<th>Lean normotensive, n=8</th>
<th>Obese HTN, n=9</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m2</td>
<td>23.8</td>
<td>28.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Arterial pressure, mmHg</td>
<td>84</td>
<td>81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>0.72</td>
<td>1.80</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Stearic acid, umol/l</td>
<td>30</td>
<td>47</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oleic acid, umol/l</td>
<td>113</td>
<td>172</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Linoleic acid, umol/l</td>
<td>57</td>
<td>78</td>
<td>NS</td>
</tr>
<tr>
<td>Total NEFA, umol/l</td>
<td>313</td>
<td>626</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Oxidative Stress is contributing factor to Endothelial Dysfunction with Obesity

Endothelial insulin resistance  →  Endothelial inflammation  →  Hyperglycemia  →  Dyslipidemia  →  Endothelial oxidative stress  →  Vascular dysfunction

Effect of Vitamin C infusion on Endothelium-Dependent Vasodilation

- Subjects: White Caucasian
- Group A: Lean, BMI<25
- Group B: Overweight, 25<BMI<29
- Group C: obese, BMI≥30
- Achbasal: Achbasal
- Infused into brachial artery
- Forearm blood flow was assessed using venous occlusive plethysmography.
Will Exercise Training Improve Endothelial-Dependent Vasodilation in Individuals with CV Risk Factors?

**Subject Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Overweight</th>
<th>Obesity w/ Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject #, (male/female)</td>
<td>2/3</td>
<td>4/5</td>
<td>7/7</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>32</td>
<td>37</td>
<td>43, p&lt;0.05 vs Lean</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>62</td>
<td>78, p&lt;0.05 vs Lean</td>
<td>89, p&lt;0.05 vs Lean</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>23</td>
<td>29, p&lt;0.05 vs Lean</td>
<td>31, p&lt;0.05 vs Lean</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>5.1</td>
<td>5.4</td>
<td>8.7, p&lt;0.05 vs Lean &amp; Overwt.</td>
</tr>
<tr>
<td>FFPI, pmol/l</td>
<td>15</td>
<td>42, p&lt;0.05 vs Lean</td>
<td>76, p&lt;0.05 vs Lean &amp; Overwt.</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.0</td>
<td>5.3</td>
<td>8.1, p&lt;0.05 vs Lean</td>
</tr>
</tbody>
</table>


**Training Program**

- Only individuals with overweight or obesity with diabetes participated in exercise training program.
- All subjects exercised using a stationary cycle ergometer except one who used treadmill.
- Total length of training program was 8 weeks:
  - 3x/wk at 60% VO2max for 20 min progressively increased to 4x/wk at 70% VO2max for 45 min
  - Subject also squeezed a spring-loaded handgrip during leg exercise:
    - 30x/min every 5 min with a non-dominant arm
  - All exercise sessions were supervised.


**Insulin Sensitivity & Endothelium-Dependent Vasodilation**

- Insulin sensitivity was assessed using euglycemic hyperinsulinemic clamp technique.
- Endothelium-dependent vasodilation was assessed by injecting acetylcholine into brachial artery through a catheter at various dosages.
- Forearm blood flow was assessed using venous occlusion plethysmography.

**Improvement in Aerobic Capacity & Insulin Sensitivity**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Overweight</th>
<th>Obesity w/ diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2 peak before training</td>
<td>29</td>
<td>33</td>
<td>42</td>
</tr>
<tr>
<td>VO2 peak after training</td>
<td>37, 27.5%, P&lt;0.05</td>
<td>42, 27.2%, P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Insulin-mediated glucose disposal before training

|                  | 7.9  | 6.5        | 3.8                 |
| Glucose disposal after training | 7.2, 11%, P<0.05 vs before | 4.2, 11%, P<0.05 vs before |

**Impaired Endothelium-Dependent Vasodilation with overweight or obesity w/ T2D**

Acetylcholine-induced Forearm blood flow in people with overweight and/or obesity w/ diabetes was smaller compared with that in lean control group.

**Effect of Exercise Training on Endothelium-Dependent Vasodilation Induced by Oral Glucose Ingestion**

- Eleven overweight or obese sedentary individuals (53 yrs old, 95.5 kg) with T2D were studied
- Exercise training protocol: 60-min aerobic exercise/day at 60-75% HRR (considered vigorous intensity exercise) for 7 consecutive days
- 20-min walk on a treadmill, 20-min stationary cycling, and 20-min walk on a treadmill
- HR was monitored using Polar heart rate monitor

**Characteristics**

- BMI, kg/m²: 33.7 with range of 25-43
- Fat %: 34.5
- HbA1c, %: 6.63
- Time since diagnosis, yr: 5
- Medications: Biguanides (n=8), sulfonylurea (n=5), combination (n=3), statins (n=7), & other (n=10)

**Before Training**

<table>
<thead>
<tr>
<th></th>
<th>7.5</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBF (ml/100 g/min)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ACh (µg/min)</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td></td>
<td>7.5</td>
<td>15</td>
<td>30</td>
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<td>FBF (ml/100 g/min)</td>
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<tr>
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<td>30</td>
</tr>
<tr>
<td>FBF (ml/100 g/min)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ACh (µg/min)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
Study Method

- Additional snacks were provided during each day of exercise to compensate for the energy cost of exercise. Avoiding energy imbalance.
- Femoral blood flow (FFB) during OGTT was assessed using a Doppler ultrasound.
- Glucose ingestion with OGTT served as a stimulus for endogenous insulin release.
  - All subjects drank glucose solution (75 g).

Dependent variables:
- Glucose, insulin, & c-peptide responses to OGTT & a whole body insulin sensitivity.
- Femoral blood flow (FFB) during OGTT.
- Postprandial glucose response to a breakfast meal.

Changes in Glucose, Insulin, C-peptide, & Insulin Sensitivity during OGTT

- Blood glucose response was monitored using continuous glucose monitoring system before and after ingesting a standardized breakfast meal (462 kcal: 60% from CHO, 24% from fat, & 16% from protein) for 2 hrs.
- Data were averaged for 3 days before training and the last 3 days of training.

The significance of the results:
- Minimizing blood glucose excursion after meal is important in controlling overall HbA1c level.
Overweight/Obesity & Endothelin-1 (ET-1)
• ET-1 is a powerful vasoconstrictor.
• Does it affect vascular health negatively in individuals with overweight/obesity?

Assessment of Endothelial Function using Venous Occlusion Plethysmography

Basic Principles of Venous Occlusion Plethysmography


Principles of Venous Occlusion Plethysmography
• The cuff on the upper arm inflates to 40 mmHg to prevent venous blood outflow.
• Arterial blood flow into the forearm is not interfered.
• The forearm volume increases linearly in proportion to the volume of arterial blood inflow.
• The cuff on the wrist inflates above systolic blood pressure to cut off blood flow into the hand.

A strain gauge (mercury-in-elastic gauge) is attached to a monitor.
Infusion of Vasoactive Substances

• To assess vascular function, one can infuse vasoactive substance through a catheter inserted into brachial artery.

• Infusion of Ach
  • to determine if endothelium responds normally with vasodilation.

• Infusion of an inhibitor of ET-1 receptor
  • to assess the influence of ET-1 on vascular tone.

Commonly Tested Substances

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Effect on vessel</th>
<th>Effect on blood flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>Vasodilation</td>
<td>Increased blood flow</td>
</tr>
<tr>
<td>BQ-123</td>
<td>ET-1 receptor A (ET,) antagonist Vasodilation (Bk.; Pharmacol. 2001 Oct; 136(3): 488-496)</td>
<td>Increased blood flow</td>
</tr>
<tr>
<td>BQ-788</td>
<td>ET-1 receptor B (ET,) antagonist Vasodilation (not consistent, Goc., 2004, 158:1186-1193)</td>
<td>Increased blood flow</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Vasoconstriction</td>
<td>Reduced blood flow</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>Vasoconstriction</td>
<td>Reduced blood flow</td>
</tr>
<tr>
<td>NG-monomethyl-L-arginine (L-NMMA)</td>
<td>Vasodilation</td>
<td>Increased blood flow</td>
</tr>
</tbody>
</table>

The Slope of a Recorded Graph Reflects Blood Flow

Strategy to Dissect out the Influence of ET-1 on Vascular Response

<table>
<thead>
<tr>
<th>Status of endogenous ET-1 level</th>
<th>Vessel response</th>
<th>ET-1 receptors</th>
<th>ET-1 infusion</th>
<th>Infusion of ET-1 blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>High endogenous ET-1 level</td>
<td>Vasocostricion</td>
<td>Reduced expression</td>
<td>No or small effect w/ vasoconstriction</td>
<td>Big effect w/ vasodilation</td>
</tr>
<tr>
<td>Normal endogenous ET-1 level</td>
<td>No vasoconstricion</td>
<td>Normal expression</td>
<td>Big effect w/ vasoconstriction</td>
<td>No or small effect w/ vasodilation</td>
</tr>
</tbody>
</table>

* One can infer the status of endogenous ET-1 level based on response to either ET-1 infusion or the infusion of ET-1 blockade.
Increased Vasoconstrictor Tone in Individuals with Overweight/Obesity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Weight</th>
<th>Overweight</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>34</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td>Sex: male/female</td>
<td>16/18</td>
<td>10/12</td>
<td>17/8</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>56±4</td>
<td>56±4</td>
<td>57±4</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>67±5</td>
<td>74±9</td>
<td>86±13</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±6</td>
<td>27±6</td>
<td>32±8</td>
</tr>
<tr>
<td>BMI</td>
<td>28±5</td>
<td>32±8</td>
<td>34±8</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>94±1</td>
<td>91±1</td>
<td>105±2</td>
</tr>
<tr>
<td>Waist: Hip ratio</td>
<td>0.7±0.2</td>
<td>0.7±0.2</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73±1</td>
<td>79±1</td>
<td>79±1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>5.5±0.1</td>
<td>5.3±0.1</td>
<td>5.3±0.1</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>3.3±0.1</td>
<td>3.4±0.1</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>1.1±0.3</td>
<td>1.4±0.3</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Rinnic acid, mg/dl</td>
<td>57.0±3.3</td>
<td>54.7±7.1</td>
<td>71.1±8.4</td>
</tr>
<tr>
<td>SEMA-5 model assessment</td>
<td>1.2±0.1</td>
<td>1.8±0.2</td>
<td>2.4±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. normal weight adults; †P < overweight adults.


Forearm Blood Flow in Response to ET-1 Infusion

- Significantly less decrease (P<0.05) in the Overweight & Obese Groups (5%) than in the control group (15%).
- This suggests that increased constrictor tone with high ET-1 level in individuals with overweight/obesity.

Endothelium-Dependent Vasodilation Was Restored After Blocking ET-1 receptors

- Co-infusion of Ach and GH-123 caused 30% improvement in the overweight/obese group.
- The co-infusion produced no much effect in the control group.
- There was no difference in Ach-induced increase in FBF between the two groups with co-infusion.
- A decrease in Ach-induced vasodilation was caused predominantly by ET-1 vasoconstrictor tone.
- Tx strategy for individuals w/obesity
  - NO bioavailability
  - ET-1
Higher level of ET-1 Expected with Overweight/obesity

- These results (e.g., Weil et al., Am J Physiol Heart Circ Physiol., 2011, 301:H689-H695) suggest that there will be greater production in ET-1 by endothelium or by other tissues in individuals with overweight/obesity or insulin resistance.

- Then one can expect a level of plasma ET-1 in individuals with overweight/obesity or insulin resistance higher than that in lean individuals.

Is metabolic derangement associated with elevated ET-1?

- Individuals w/ or w/o in the presence of T2D were studied in comparison of control group.
- What causes high ET-1 level in individuals w/ diabetic condition or insulin resistance?
- Elevated insulin level in the presence of insulin resistance may cause an increase in ET-1 production.

How does insulin modulates ET-1?

- Eight women (41 yrs old) with obesity (BMI = 36 kg/m²) were studied.
- The subjects were on 10-wk diet with calorie restriction.
- High insulin level during euglycemic hyperinsulinemic clamp caused an increase in ET-1 before and after weight loss (the upper panel).
- A decrease in basal ET-1 level was positively correlated (r = 0.32, P<0.01) with a decrease in basal insulin level after body weight loss with calorie-restricted diet set up (lower panel).
- A decrease in ET-1 in basal after wt. loss was inversely correlated (r = -0.55, P=0.05) with an increase in insulin sensitivity.
- Keeping low level of insulin prevents ET-1 production.

Insulin modulates plasma ET-1 in Humans

- Basal (left column) Hyperinsulinemic clamp (right column)
- Endothelial dysfunction monitored with NO release
- ET-1 release monitored with ET-1 ELISA
- Insulin stimulation increased NO release
- Insulin stimulation increased ET-1 release

Overweight/Obesity

Insulin receptor

Endothelial cells

X. Petersen et al., Am J Physiol Endocrinol Metab 2009, 297:E568-E577
Imbalance in Vascular Endothelin-1 System With Overweight/Obesity & Diabetes

Healthy Condition

- Endothelin-1 (ET-1) Causes Superoxide Production by Human Coronary Artery
  - ET-1 caused a significant increase in superoxide production.
  - Superoxide production was dependent on ET_A.
  - An inhibitor of ET_A blocked ET-1-induced production of superoxide.
  - ET_A may play a minimal role in superoxide production induced by ET-1.
  - An inhibitor of ET_A did NOT cause a further reduction of superoxide production.

Overweight/Obesity & Diabetes Condition

- Visual Stain of Superoxide in Artery tissues
  - Red stains indicate superoxide.

Does Endothelin-1 (ET-1) Promote ROS Production?

- Internal mammary arteries (IMA; n=73) from patients who were undergoing CABG were collected at the time of surgery.
- IMAs were incubated for 45 min with or without ET-1 (0.1 nM) in appropriate buffer (Krebs-HEPES buffer).
- Some of IMAs were also incubated for 20 min with:
  - BQ123 (inhibitor of ET_A)
  - BQ123 + BQ788 (inhibitor of ET_B)
**ET-1 promotes the breakdown of vascular NO**

- Normal coupling:
  - eNOS
  - BH4
  - NO

- Un coupling:
  - eNOS
  - BH4
  - ET-1
  - O2
  - NO
  - ONOO-

  Vasodilation
  - Antithrombotic activity
  - Anti proliferative activity

  Endothelial dysfunction

**Negative Impact of High Level of ET-1 on Blood vessel**

- It increased basal constrictor tone.
- It opposes dilatory action of NO.
- It increases oxidative stress.
- It decreases NO bioavailability through oxidative stress

**Is ET-1 constrictor vascular tone modifiable?**

- Can exercise training decrease its tone?

**Subject Characteristics**

<table>
<thead>
<tr>
<th>Metric</th>
<th>Young control, n=13</th>
<th>Older, n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>26 (range 21-34 yrs)</td>
<td>62 (range 52-70 yrs)</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>74.3</td>
<td>84.2, <em>P&lt;0.05</em></td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>23.5</td>
<td>26.8, <em>P&lt;0.05</em></td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82.0</td>
<td>95.7, <em>P&lt;0.05</em></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>132</td>
<td>138, <em>P=0.05</em></td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>70</td>
<td>78</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.3</td>
<td>5.1, <em>P&lt;0.05</em></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.6</td>
<td>3.5, <em>P&lt;0.05</em></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.8</td>
<td>5.2, <em>P=0.05</em></td>
</tr>
<tr>
<td>Trig, pmol/L</td>
<td>30.0</td>
<td>33.6, <em>P=0.05</em></td>
</tr>
<tr>
<td>C-reactive protein, mg/dl</td>
<td>0.7</td>
<td>3.1, <em>P=0.05</em></td>
</tr>
<tr>
<td>oxLDL, U/L</td>
<td>44.3</td>
<td>58.2, <em>P&lt;0.05</em></td>
</tr>
</tbody>
</table>

Exercise Training Program

- Home-based aerobic exercise training program
- Frequency: 5-7x/week
- Intensity: 65-75% HRmax for 40-50 min/session (Moderate intensity)
- Training period: 3 months
- Most subjects walked and some incorporated jogging into their exercise session within prescribed HR range.
- Compliance was checked every 2 weeks by downloading data from a heart rate monitor (Polar Electro) and from exercise logs.

Response to the Infusion of Exogenous ET-1

- Exogenous ET-1 was infused (5 pmol/min) for 20 min.
- FBF was measured during the last 3 min.
- Old individuals: 7% reduction in resting FBF
- Young controls: 19% reduction in resting FBF

Less decrease w/ ET-1 injection

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>-0.38</td>
<td>0.05</td>
</tr>
<tr>
<td>%BF</td>
<td>-0.47</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.45</td>
<td>0.05</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.43</td>
<td>0.05</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.53</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Comparison of Responses Before and After Training

Response to ET-1 receptor Blockades

- An antagonist of ET-1 receptor A (BQ-123) was infused (200 pmol/min) through brachial artery for 60 min of the experiment period.
- BQ-123 & BQ-788, an antagonist of ET-1 receptor B, was infused together for an additional 60 min.
- Forearm blood flow was measured every 10 min.

In response to BQ-123
- Older men improved 20% in FBF.
- No significant change in the young control group.
- No significant change in FBF in both groups

Conclusion
- A significant increase in the blood flow in older subjects suggests that aging w/ overweight causes ET-1 vasoconstrictor tone on blood vessel.

Response to ET-1 Blockades

- After 3 months of aerobic training, forearm blood flow increased 20% in both groups.

Conclusion
- A significant increase in the blood flow in older subjects suggests that aging w/ overweight causes ET-1 vasoconstrictor tone on blood vessel.
Overweight/Obesity & other CV Risk Factors

- Oxidative stress
- Hyperinsulinemia
- Endothelial insulin resistance
- Decreased NO bioavailability
- Impaired endothelial-dependent vasodilation
- ET-1 production
- Increased ET-1 constrictor tone on blood vessel
- Exercise training
- No effect on endothelial-independent vasodilation
- Exercise training
- Decreased muscle Glucose uptake
- Improved or normalization of vascular function

How Does Exercise Training Work?

- We are asking about biological mechanisms underlying training-induced improvement in endothelial function.

Exercise Training has been shown effective: strong & abundant evidence

How does it work? Looking for biological mechanisms:

- Mechanism-driven PT practice
- Hypothesis-driven PT practice
- Mechanism-driven patient care

• Dev. & maturation of knowledge in the area of Rehabilitation Science
• Convincing your patient about Tx
• Becoming a top notch clinician

How does exercise training work for improvement in endothelium-dependent vasodilation after training?

Exercise

- Increased Laminar Blood Flow
- Increased Shear Stress

• Creation of intracellular signals
• Activation of proteins involved in signaling process
• Activation of eNOS activity
• Increased eNOS protein expression through gene activation

Hemodynamic Forces Acting on Endothelium

- Shear stress is the tangential frictional force acting the vessel wall due to blood flow.
- Normal stress is due to hydrostatic pressure acting on the vessel wall perpendicularly.
- Tensile stress is force acting on the vessel wall in the circumferential direction due to stretch of the vessel wall.

Role of Shear Stress for Improvement of FMD

Change in FMD% with or without shear Stress

Subjects performed a 30-min handgrip exercise with a cuff on one arm and without a cuff on the other arm.

FMD was evaluated at the completion of the handgrip exercise.

Shear Rate on Change in FMD

Direct Relationship Between a change in FMD Shear Stress AUC

Shear stress is not often measured during FMD test.

Shear stress = viscosity x (flow velocity/diameter)

"Shear rate area-under-the-curve (AUC)" can be viewed as a main stimulus for the peak diameter of blood vessel.

Shear rate = flow velocity/diameter
Intensity of Exercise on Flow Velocity & Shear Rate AUC

- If one assumes that "shear rate area-under-the-curve (AUC)" can be viewed as a main stimulus for the peak diameter of blood vessel, one needs to employ exercise intensity, which maximizes "shear rate area-under-the-curve (AUC)."

- This means that an combination of high shear rate and slow decay rate of reactive hyperemia will produce greater "shear rate area-under-the-curve (AUC)."

- Under high intensity of exercise (vs. low intensity exercise), one can expect:
  - Higher flow velocity
  - Higher flow rate

FMD%: better adaptation to training

Do we have results that support the concept?

Time Course of Endothelial Adaptation to a Single Bout of Exercise

- 28 pts with MetS randomized into:
  - Control (n=9)
  - Continuous moderate exercise (n=8)
  - Aerobic interval training (n=11)

- Walking/running uphill on a treadmill
- 3x/wk for 16 wks
- Continuous moderate exercise
- 47 min/session on a treadmill at 70% HRmax
- Aerobic interval training (AIT)
- 10 min warm up at 70% HRmax
- 4 min run at 90-95% HRmax
- 3 min walk/run at 70% HRmax
- 5 min cool down
- Total 40 min/session

- FMD was assessed immediately after a bout of AIT or continuous moderate exercise before and after training.

High Intensity Interval Training vs. Continuous Moderate Training

- 32 pts with MetS randomized into:
  - Control
  - Continuous moderate exercise
  - Aerobic interval training

- Walking/running uphill on a treadmill
- 3x/wk for 16 wks
- Continuous moderate exercise
- 47 min/session on a treadmill at 70% HRmax
- Aerobic interval training (High intensity interval training)
  - 10 min warm up at 70% HRmax
  - 4 min run at 90% HRmax
  - 3 min walk/run at 70% HRmax
  - 5 min cool down
  - Total 40 min/session

- FMD was assessed before & after training.
Can reduced physical activity cause the deterioration of Endothelial Function?

- Reduction in physical activity
- Longer Sitting Hours
- Decreased blood flow
- Decreased shear stress
- Decreased FMD

Reduced Daily Steps on FMD%

<table>
<thead>
<tr>
<th>Days</th>
<th>Steps</th>
<th>Average METs</th>
<th>Ketals &gt;3 METs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>12550 ± 345</td>
<td>1.68 ± 0.17</td>
<td>1214 ± 151</td>
</tr>
<tr>
<td>RA1</td>
<td>3666 ± 381*</td>
<td>1.60 ± 0.15*</td>
<td>453 ± 82*</td>
</tr>
<tr>
<td>RA2</td>
<td>3603 ± 190*</td>
<td>1.65 ± 0.19*</td>
<td>464 ± 135*</td>
</tr>
<tr>
<td>RA3</td>
<td>4067 ± 197*</td>
<td>1.55 ± 0.18*</td>
<td>568 ± 105*</td>
</tr>
<tr>
<td>RA4</td>
<td>3641 ± 360*</td>
<td>1.55 ± 0.12*</td>
<td>220 ± 43*</td>
</tr>
<tr>
<td>RA5</td>
<td>3734 ± 275*</td>
<td>1.61 ± 0.15*</td>
<td>446 ± 99*</td>
</tr>
</tbody>
</table>

- Eleven recreationally active subjects (25 yrs) were instructed to reduce steps <5,000 steps/day for 5 days.
- FMD was assessed on brachial and popliteal arteries at baseline, 1,3,5 days of reduced activity.
- FMD on brachial artery was not changed from the baseline.
- FMD on popliteal artery on the day 5 was reduced (P<0.05)

Effect of Long-Hour Sitting on FMD

- Individuals (n = 12) who did not meet physical activity guideline of Center for Disease Control & Prevention (CDC) were studied.
- Sitting quietly for 3 hrs without an interruption (SIT).
- Sitting with interruptions at the middle of sitting hour (ACT).
- Walking on a treadmill at 2 mph for 5 min.
- FMD was assessed using superficial femoral artery at the beginning and at every hour of sitting time.
- Automatic BP cuff was placed on R thigh about 7 cm above the knee to induce hyperemia after occlusion.

Sitting Causes a Decrease in FMD in LE blood Vessel

- Sitting without interruption (SIT)
- Sitting with interruptions (ACT)
Testing the Role of Shear Rate in Sitting-Induced Reduction in FMD

- Healthy young men (n = 10; 26 yrs old, BMI = 26.8 kg/m²) were studied.
- Each sat without leg movement for 3 hrs.
- One foot remained in open air while the other foot was submerged up to the ankle in a foot spa with H₂O temperature maintained at 42°C.
- Popliteal artery FMD in both legs was assessed in supine position before and after sitting (solid arrow).
- Popliteal artery blood flow and shear rate in both legs were assessed in supine as well as in sitting position (dotted arrow).

Changes in Shear Rate in Popliteal Artery w/ or w/o Leg Heating During Sitting

- Time: p<0.001
- Leg: p<0.003
- Interaction: p<0.001
- *P<0.05 between legs

An Increase in Shear Rate Prevents Reduction in FMD During Sitting

- Time: p<0.011
- Leg: p<0.002
- Interaction: p<0.003
- *P<0.05 between legs
Does Resistance Training Improves FMD?

Total of 40 subjects (males and females; >20 yrs old; BMI>30 kg/m²)

- Strength training (n=13)
  - 15 min of static training exercises
  - 6 series of leg press (5 RM; 10% of HRmax)
  - 5 series of abdominal & back exercises (3 RM; 30 sec break between series)
  - 2 warm-ups before monitored
  - 1 session/wk at home
  - 12 wks training

- Moderate-intensity training (n=12)
  - 60-70% HRmax
  - 47 min/session
  - 2 sessions/wk monitored
  - 1 session/wk at home
  - 12 wks training

- High-intensity aerobic interval training (n=14)
  - 35 min warm-up at 50-60% HRmax
  - 6-9 min intervals at 85-90% HRmax
  - Deep seated knee while walking/jogging on 45° and 60° slopes
  - 5 sets cool down
  - 40 min/session
  - 3 sessions/wk monitored
  - 1 session/wk at home
  - 12 wks training

Improvement in VO2 max & peak O2 pulse after strength training

- Improvement in VO2 max
  - 10% in strength training group (P<0.001)
  - 16% in moderate-intensity training group (P<0.001)
  - 33% in high-intensity training group (P<0.001)
  - Greatest improvement

- Peak O2 pulse (an index of stroke volume)
  - Improved all groups
  - Greatest improvement in high-intensity training group

Effect of Combined Aerobic & Resistance Exercise Training in FMD

- N = 16 pts w/ T2D
- Circuit exercise program for 6 weeks
- 7 resistance exercises + 8 cycling stations + walking on treadmill
- Resistance exercise:
  - Dead weight leg press
  - B & L hip extension
  - Pectoral & shoulder extensions
  - Seated abdominal flexion
  - Dead leg flexion
- Intensity and duration were increased progressively.
- Exercise on cycle or treadmill at 70-85% peak HR
- Resistance training at 55-65% maximum voluntary contraction
- FMD improved significantly after training.
- Endothelium-independent vasodilation did not change with training.
- Glyceryl trinitrate (GTN) was used for the test.
Remodeling of Endothelium Induced by Exercise Training

- Increased protein expression of eNOS and other isoforms of NOS, i.e., iNOS
- Increased protein expression of superoxide dismutase
- Decreased expression of NAD(P)H oxidase
- Decreased expression of nuclear factor kB (NF-kB)

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Expression of NOX, NF-kB & Superoxide Dismutase in Human Endothelial Cells

Expression of NOX, NF-kB & Superoxide Dismutase in Human Endothelial Cells

Exercise Training on Endothelial Dysfunction in Pts w/ CHF

- Pts w/ CHF (n = 11, LVEF = 26%) performed bicycle exercise at 70% peak O2 for 10 min, 6x/day for 4 wks.
- Eleven pts (LVEF = 24%) served as control.
- Vasodilative response of radial artery to Ach (30 ug/min infusion) was assessed at beginning and after 4 wks of exercise training.

Rats ran on treadmill at 30 m/min & 10% grade for 60 min/day, 5 days/wk for 8-12 wks.
- Gastrocnemius feed artery (GFA)
- Gastrocnemius 1st gen. artery (G1A)
- White gastrocnemius 2nd gen. artery (WG2A)
- Red gastrocnemius 2nd gen. artery (RG2A)

Expression of NOX, NF-kB & Superoxide Dismutase in Human Endothelial Cells

Exercise Training on Endothelial Dysfunction in Pts w/ CHF

<table>
<thead>
<tr>
<th>Training</th>
<th>Control</th>
</tr>
</thead>
</table>
| FMD, %   | A in internal diameter was induced by Ach infusion

- NOX, NF-kB, & Superoxide Dismutase Changes
- Young inactive vs Old inactive vs Old active
- VO2max, ml/kg/min: 41 vs 29* vs 42+
- BMI, kg/m2: 26 vs 27 vs 23*+
- PA, MET-hrs/wk: 37 vs 35 vs 78*+
- FMD, %: 7.1 vs 6.9* vs 6.1+

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Remodeling of Endothelium Induced by Exercise Training

- Increased protein expression of eNOS and other isoforms of NOS, i.e., iNOS
- Increased protein expression of superoxide dismutase
- Decreased expression of NAD(P)H oxidase
- Decreased expression of nuclear factor kB (NF-kB)
**Relationship between Improvement in Vasodilative Response and a Change in VO2 max**

- This suggests that O2 delivery was a limiting factor due to limited vasodilation during exercise before exercise training.

**References for key articles**


**Overweight/Obesity**

- Summary
  - Decreased endothelial NO bioavailability
  - Increased production of vasoconstrictors, i.e., endothelin-1
  - Increased oxidative stress
  - Increased inflammatory cytokines

- Endothelial Dysfunction
  - CAD
  - Heart dysfunction
  - Stroke
  - Atherosclerosis

- Summary
  - Reduced body mass & BF%

- Improved quality of life
  - Early death
  - Poor quality of life

**References for key articles**


The End

Thank you for your attention.

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