Hormones and Atherosclerosis - Insights From Studies of Nonhuman Primates

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Macaques Experience Similar Menstrual Cycle Hormone Profiles to Women

Ovarian Follicle Populations Decline with Age in Macaques

- Appt et al., 2009
- Nichols et al., 2004 (Human Reproduction)
Antimullerian Hormone is a Marker of Follicle Numbers in Macaques

- Naturally and oophorectomized postmenopausal macaques
- Elevated FSH and LH
- Lower estradiol, progesterone & AMH

Postmenopausal Hormone Patterns are Similar between Macaques and Women

- Perimenopausal monkeys
  - Defined by irregular menstrual cycles
  - Significant elevations in peak FSH concentrations
  - Lower AMH

Atherosclerosis

Sources of Coronary Artery Blocks
- Adventitia
- Media
- Internal Elastic Lamina
- Intimal Area (Plaque Area)

Measurement of Intimal (Plaque) Cross-sectional Areas

Macaques Have Similar Coronary Artery Anatomy to Women
Coronary Artery Atherosclerosis following Exposure to a Western Diet

Appt et al., unpublished

Iliac Artery Biopsy Technique Allows Baseline or Interim Measures of Atherosclerosis

The iliac artery biopsy approach has proven to have powerful experimental advantages.

Surgical Menopause Increases Coronary Artery Atherosclerosis in Nonhuman Primates

Adams et al., 1985

Surgical Menopause, Hormone Treatment and Atherosclerosis in Macaques

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Timing of Initiation of HT Affects Coronary Atherosclerosis Outcomes in Oophorectomized Monkeys

<table>
<thead>
<tr>
<th>Timing</th>
<th>Baseline</th>
<th>Placebo</th>
<th>CEE</th>
<th>CEE+MPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>0.20</td>
<td>0.30</td>
<td>0.40</td>
<td>0.30</td>
</tr>
<tr>
<td>Late</td>
<td>0.20</td>
<td>0.30</td>
<td>0.40</td>
<td>0.30</td>
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</tbody>
</table>

Early Intervention: p < .05
Late Intervention: p = NS

CEE Prevents Initiation / Progression of Small Atherosclerotic Plaques in Female Monkeys

<table>
<thead>
<tr>
<th>Tertile</th>
<th>Baseline PQ size (mm²)</th>
<th>Plaque size at Outcome (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>&lt;0.12</td>
<td>0.0001</td>
</tr>
<tr>
<td>Middle</td>
<td>0.12-0.60</td>
<td>p = 0.33</td>
</tr>
<tr>
<td>High</td>
<td>&gt;0.60</td>
<td>p = 0.71</td>
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CEE Prevents Initiation / Progression of Small Atherosclerotic Plaques in Female Monkeys

E2 Reduced Arterial Pro-Inflammatory Gene Expression in Early, but Not Late Menopausal (Oophorectomized) Monkeys

<table>
<thead>
<tr>
<th>Gene</th>
<th>Relative mRNA Abundance (AU)</th>
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<tbody>
<tr>
<td>MCP-1</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>TNFα</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Macrophage (CD68)</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Premenopausal Influences on Atherosclerosis Progression
Premenopausal Oral Contraceptives Reduced Postmenopausal Atherosclerosis
(Subordinate Psychosocially Stressed Monkeys)

Why Would Premenopausal Monkeys Benefit from Hormone Treatment?

Ovarian Follicle Varies Significantly between Monkeys of the same Age

Could this be an indication of variation in ovarian aging and subsequent risk for atherosclerosis, irrespective of biologic age?

Plasma AMH Prior to Consumption of a Western Diet Predicted Iliac Artery Atherosclerosis Extent 2 years Later

Wake Forest Baptist Medical Center
Premenopausal Monkeys with Low AMH Prior to Western Diet Develop Larger Iliac Artery Plaques

- 2.5 years: AMH LO (0.15), AMH HI (0.3)
- 4.5 years: AMH LO (0.2), AMH HI (0.35)

$p = 0.04, 0.05$

Monkeys with Low AMH Prior to OVX and 2 years Exposure to Western Diet Develop Larger Coronary Artery Plaques

- AMH LO: Mean IA (SQRT mm²) 0.5
- AMH HI: Mean IA (SQRT mm²) 0.6

$p = 0.04, n = 19$

Premenopausal Monkeys with Low AMH Prior to Western Diet Develop Larger Coronary Artery Plaques 4 years later

- Low AMH: Plaque Area (SQRT mm²) 0.65
- High AMH: Plaque Area (SQRT mm²) 0.4

$p = 0.06$

Premenopausal Monkeys with Low AMH Prior to 4 years of Western Diet Have Greater Arterial Stiffness and Diastolic Blood Pressure

- AMH (ng/ml) vs. PWV (m/s)
- Predictors of PWV: SBP $R^2 = 0.37$, DBP $R^2 = 0.43$, $p < 0.004$
- Whole body fat $R^2 = 0.44$, $p = 0.002$

$r = -0.39, p = 0.03$
**AMH is Correlated with Markers of Inflammation**

- In a separate study of premenopausal monkeys consuming a western diet (n=49)
  - Significant inverse relationship between baseline serum AMH and plasma monocyte chemoattractant protein-1 (MCP-1) after 30 months of diet ($r = -0.34$, $p=0.02$)
  - AMH and iliac artery gene expression of inflammatory markers were correlated ($p's <= 0.05$)
    - IL-6 $r = -0.34$
    - IL-2 $r = -0.37$
    - CD 68 $r = -0.36$

**AMH Protein and AMHR2 mRNA are Present in NHP Arteries**

- Immunohistochemistry
  - Staining is seen consistently in the smooth muscle cell layer
  - Arterial smooth muscle cells are integral in arterial responses to stressors such as changes in blood pressure, inflammation and oxidative stress.
  - Using human TGFβ / BMP signaling pathway PCR array, AMHR2 mRNA expression was identified in NHP arteries and ovary

**Summary**

- In Postmenopausal monkeys, estrogen treatment prevents atherosclerosis progression when lesions are small and uncomplicated
- Premenopausal monkeys with low ovarian reserve (AMH) prior to challenge with Western diet appear to be at greater risk for atherosclerosis, arterial stiffness, increased diastolic blood pressure and arterial inflammation
- AMH protein and AMHR2 mRNA are present in Macaque arteries
Conclusions

- Together these data suggest a potential window of opportunity during pre- and perimenopause to decrease postmenopausal atherosclerosis progression
- Raise the question of whether AMH is a marker of vascular aging and susceptibility to CHD

Additional Slides

Ovarian Follicle Number Prior to Western Diet is Inversely Related to Iliac Artery Atherosclerosis 2 years Later

\[ r = -0.41 \]
\[ p = 0.10 \]
PWV is Associated with Atherosclerosis

PWV (m/s)

Plaque Area (SQRT mm²)

$P_WV = 0.44$

$p = 0.01$

$n = 33$

AMHR2 Relative mRNA Expression in Tissue Collected from Premenopausal Macaques

AMHR2 Relative mRNA Expression in Tissue Collected from Pre- and Postmenopausal Macaques

$P_{AMHR2} = 0.13$