Soy, flaxseed and fish oil: effects on bone health throughout the life cycle

Wendy E. Ward, Ph.D.
Department of Nutritional Sciences
Faculty of Medicine
University of Toronto

Osteoporosis: an emerging epidemic!

- 2000-2010 Decade of Bone & Joint, WHO
- Surgeon General Report 2004: 1 in 2 Americans will have poor bone health predisposing to fragility fractures by 2020
- Similar estimates for other developed countries

*Prevention versus Treatment*
Our Population is Aging….

...Seniors are fastest growing segment of the population....

>65 years of age
1981 10% of population
1998 12% of population
2021 18% of population

Diet & Physical Activity

Common Sites of Fragility Fracture

- Hip
- Spine/Vertebra
- Wrist

Destruction of bone microarchitecture:

↓ mineral
↓ matrix proteins
What is Osteoporosis?

A skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture.

Bone strength reflects the integration of two main features:

1. Bone density ➡️ Dual energy x-ray absorptiometry (DEXA or DXA)
2. Bone quality ➡️ Challenging to measure in humans
   History of fragility fracture

Ultimate goal of prevention & treatment strategies:

- reduce risk of fragility fracture
How to assess bone health?

Bone Mineral Density (BMD) by DXA

Changes in BMD measured over time, months or years depending on species studied.

- Mineral
- BMD (g/cm²)
Biochemical Markers of Bone Turnover

Used as surrogate measures of changes in BMD: altered in weeks

Markers of bone formation
  - serum or plasma
  - production of bone matrix proteins by osteoblasts: bone-specific alkaline phosphatase

Markers of bone resorption
  - urine
  - breakdown of bone matrix proteins by osteoclasts: deoxypyridinoline, pyridinoline, N-telopeptide

Changes in biochemical markers of bone turnover provide a crude prediction of changes in BMD.

Quantitative Computed Tomography:
  bone quality
Bone Biomechanics: bone quality & risk of fragility fracture

Three point bending

Compression

Load Displacement Curve

- Stiffness
- Yield Load
- Toughness
- Resilience
- Peak Load
- FRACTURE

Load N vs. Displacement mm
Optimize Bone Health Throughout the Life Cycle

Peak Bone Mass:
- Genetics
- Hormones
- Physical activity
- Nutrition

Fracture Threshold

Growth & Development   Early Adulthood   Late Adulthood
Optimize Bone Health Throughout Life

- Growth & Development
- Early Adulthood
- Late Adulthood

Peak Bone Mass:
- Genetics
- Hormones
- Physical activity
- Nutrition

Fracture Threshold
10+ years?

Soy Isoflavones, Flaxseed, Fish Oil

Optimize Bone Health Throughout Life

- Growth & Development
- Early Adulthood
- Late Adulthood

Menopause & Aging:
- Loss of sex steroids

Fracture Threshold
Optimize Bone Health Throughout Life

- **Food Synergy**: Soy Isoflavones, Ca, Flaxseed, Fish Oil
- **Food-Drug Synergy**: Low dose estrogen

Menopause & Aging: Loss of sex steroids

Fracture Threshold

Mechanisms of Foods/Food Components

- **Isoflavones** bind ER - genomic & non-genomic events
  - ↑ osteoblastic activity
  - ↓ osteoclastic activity
  - modulate cytokines, growth factors

- **Omega-3 fatty acids**
  - anti-inflammatory effects
  - modify cytokines, growth factors

Other mechanisms.........
AGING

- food-synergy
- food-drug synergy
- prevent or attenuate deterioration of bone tissue

AGING: *Food synergy*

Sprague-Dawley rats, 8 week intervention

- Sham
- Ovx
- Soy
- Ca
- Soy+Ca

Combination of soy + Ca preserves spine BMD more than soy or Ca alone.

...but peak load of LV5 is not improved by any interventions.

**AGING: Food synergy**

Sprague-Dawley rats, 8 week intervention
- Sham
- Ovx
- Isoflavones
- Ca
- Isoflavones+Ca

*Breitman et al. Bone 2003;33:597-605*
### Bone mineral density (BMD) of lumbar vertebrae (LV1–LV6)\(^{a,b}\)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>BMD (mg/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>257 ± 13(^a)</td>
</tr>
<tr>
<td>OVX</td>
<td>225 ± 10(^c)</td>
</tr>
<tr>
<td>IE</td>
<td>216 ± 9(^c)</td>
</tr>
<tr>
<td>Ca</td>
<td>224 ± 11(^c)</td>
</tr>
<tr>
<td>IE + Ca</td>
<td>239 ± 12(^c)</td>
</tr>
</tbody>
</table>

Combination of isoflavones + Ca preserves spine BMD more than isoflavones or Ca alone

---

### AGING: Food synergy

Daidzein converts to equol

**C57BL/6 mice, 12 week intervention**

- Sham
- Ovx
- Daidzein (2 doses)
- Ca
- Daidzein+Ca

*Fonseca and Ward. Bone. 2004;35:489-497*
AGING: Food synergy

- Daidzein+Ca or Ca alone preserved BMD and biomechanical bone strength at femur and lumbar vertebrae to a similar extent


Daidzein increased with higher daidzein dose

Equol produced at both daidzein doses

Bars with different letters, p<0.05
Daidzein and equol are both present in long bones

Bars with different letters, p<0.05

AGING: Food synergy

Do commonly used strains of mice in bone health studies all convert daidzein to equol?

- C57BL/6
- C3H
- CD-1
- Swiss Webster

As expected, serum daidzein was elevated with daidzein feeding.

All four strains convert daidzein to equol, with outbred strains having higher conversion.

### AGING: *Food synergy*

CD-1 mice, 12 week intervention

- Sham
- Ovx
- Isoflavone
- Fish oil
- Isoflavone + Fish oil

*Fonseca and Ward. Molecular Nutrition and Food Research 2007;51:824-831*
Metabolism of n-3 and n-6 PUFA

n-3 PUFA

- α-Linolenic Acid (ALA)
- Eicosapentaenoic Acid (EPA)
- Docosahexaenoic Acid (DHA)

Inhibit bone loss

n-6 PUFA

- Linoleic Acid (LA)
- Arachidonic Acid (AA)
- Eicosanoids (PGE$_2$)

Promote bone loss

Inhibit bone loss

Proinflammatory mediators:
IL-6, IL-1, TNF-α, PGE$_2$
Fish oil and fish oil +isoflavones improved BMD at lumbar spine

Combination of fish oil and isoflavones improved peak load of LV4, surrogate marker of fracture risk

Bars with different letters, p<0.05

Fonseca and Ward. Molecular Nutrition and Food Research 2007;51:824-831

AGING: Food-drug synergy
June 2002 – Findings from the Women’s Health Initiative Study

Headlines:

Hormone-replacement therapy is riskier than advertised.

What’s a woman to do?

Women’s Health Initiative Study

E2 + Progestin
5.2 years follow-up

↑ stroke
↑ coronary heart disease
↑ invasive breast cancer
↓ fractures at all sites
↓ colorectal cancer

E2
6.8 years follow-up

↑ stroke
– coronary heart disease
– colorectal cancer
↓ fractures at all sites
↓ invasive breast cancer

HRT should not be initiated or continued for primary prevention of CHD

AGING: Food-drug synergy

Flaxseed + Low dose estrogen

- $\alpha$-linolenic acid
- lignan

Flaxseed: Lignan Metabolism

SDG → Enterodiol

Colonic microflora

17β-Estradiol

Oxidation

Enterolactone
Bone Immune System

Proinflammatory Cytokines/Eicosanoids

- TNF-α
- IL-1, IL-6
- PGE₂

Proinflammatory Cytokines/Eicosanoids

Estrogen Action on Bone

Estrogen

Lignans?

Bone

- Osteoclasts
- Osteoblasts

Immune System

- Proinflammatory Cytokines/Eicosanoids

Metabolism of n-3 and n-6 PUFA

n-3 PUFA

- α-linolenic acid (ALA)
  - desaturase
  - elongase
  - desaturase
  - Inhibit bone loss
  - Eicosapentaenoic Acid (EPA)

n-6 PUFA

- Linoleic Acid (LA)
  - Arachidonic Acid (AA)
  - Eicosanoids (PGE₂)
  - Promote bone loss
  - Docosahexaenoic Acid (DHA)
Flaxseed and Bone Health

? Biochemical Markers of Bone Resorption\(^1,7,10\)

? BMD\(^2,3,4,8,11,12\)

? Bone strength \(^4,5,6,9,12\)

In vitro effects: ↑ transcription of alkaline phosphatase, osteonectin, type 1 collagen

AGING: *Food-drug synergy*

Sprague-Dawley rats, 12 week intervention

- Sham (POS)
- Ovx (NEG)
- Flaxseed
- Low dose estrogen
- Flaxseed + low dose estrogen

*Sacco et al. Menopause 2009; in press.*
AGING: Food-drug synergy

Bars with different letters, p<0.05
Sacco et al. Menopause 2009;in press.

AGING: Food-drug synergy

Bars with different letters, p<0.05
Sacco et al. Menopause 2009;in press.
AGING: Food-drug synergy

Fatty acids mediating the effect on BMD and vertebral strength?

- no relationship between bone outcomes and fatty acids
- ALA, LA, EPA, AA, total n-3 PUFA, total n-6 PUFA, n-6/n-3 ratio

Sacco et al. Menopause 2009;in press.
AGING: Food-drug synergy

Role of lignan?

- isolation from bone tissue
- localization within bone tissue

Sacco et al. Menopause 2009;in press.

BONE DEVELOPMENT

- peak bone mass
DEVELOPMENT

Investigation of foods or food components that favorably modulate bone development

- peak bone mass
- BMD
- stronger bones

DEVELOPMENT

In healthy male & female weanling mice:

- fish oil
- flaxseed oil
DEVELOPMENT

In healthy male & female weanling mice:
  - fish oil
  - flaxseed oil \[ Modest\ effects \]

DEVELOPMENT

Does neonatal exposure to *soy isoflavones* favorably program bone metabolism?
  - ↑ BMD
  - stronger bones \[ adulthood \]
Early Programming of Bone

Early life nutrition may influence later health

- adaptive process
- alter expression of specific genes but without directly altering DNA sequence

*Does soy and its isoflavones with potential estrogen-like activity program bone?*

---

**Early Programming of Bone**

Exposure of female mice to diethylstilbestrol (DES) from day 1 through 5 of life programmed bone at adulthood:

- ↓ osteoclast number
- ↓ osteoclastic activity
- ↑ bone mineral density

Migliaccio et al., *Bone*. 2000;27(1):47-52
Early Programming of Bone

<table>
<thead>
<tr>
<th>Isoflavone per kg</th>
<th>Avg. isofoavone intake per liter of formula</th>
<th>Isoflavone per kg body weight*</th>
</tr>
</thead>
<tbody>
<tr>
<td>China (1990 survey)¹,²</td>
<td>3 mg</td>
<td>0.05 mg</td>
</tr>
<tr>
<td>Japan (1998 survey)³</td>
<td>25 mg</td>
<td>0.42 mg</td>
</tr>
<tr>
<td>FDA recommended amount²,⁴</td>
<td>24 mg</td>
<td>0.40 mg</td>
</tr>
<tr>
<td>Soy-based formula⁵</td>
<td>32 - 45 mg</td>
<td>5.30 - 7.50 mg</td>
</tr>
<tr>
<td>Cow's milk formula</td>
<td>5 mg</td>
<td>0.83 mg</td>
</tr>
<tr>
<td>Breast milk</td>
<td>3 - 13 mg</td>
<td>0.05 - 2.17 mg</td>
</tr>
</tbody>
</table>

* Assume 60 kg for adults and 6 kg for 4 month old infants


Development

CD-1 mice, 5 day intervention, 4 month study

- Control (- CON)
- DES (+ CON)
- Daidzein
- Genistein
- Daidzein + Genistein

Early Programming of Bone

**TABLE 1** Circulating isoflavones of 5-d-old CD-1 mice compared with human infants fed soy protein-based infant formula

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>DAI (μmol/L)</th>
<th>GEN (μmol/L)</th>
<th>Equol (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAI</td>
<td>6</td>
<td>1.07 ± 0.19</td>
<td>&lt;0.02</td>
<td>ND²</td>
</tr>
<tr>
<td>GEN</td>
<td>4</td>
<td>&lt;0.02</td>
<td>2.61 ± 0.97</td>
<td>ND</td>
</tr>
<tr>
<td>DAI+GEN</td>
<td>4</td>
<td>1.18 ± 0.21</td>
<td>2.66 ± 1.78</td>
<td>ND</td>
</tr>
<tr>
<td>Human infants³</td>
<td>7</td>
<td>1.16 ± 0.09</td>
<td>2.53 ± 0.62</td>
<td>ND, n = 3; &lt;0.02, n = 4</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM.
² ND. Not detected.
³ Plasma samples from 4-mo-old infants who were exclusively fed soy protein-based infant formula from the first week of life (22).


Early Programming of Bone

**LV1-LV3 BMD (mg/em²)**

- Con -ve
- DAI -ve
- GEN -ve
- DAI+ -ve

**LV2 Peak Load (N)**

- Con -ve
- DAI -ve
- GEN -ve
- DAI+ -ve

Gender x treatment (p < 0.001)  Gender x treatment (p = 0.008)

DAI and DES had greater (p<0.001) trabecular number and lower (p<0.001) trabecular separation than the CON.

GEN and DAI+GEN had greater (p<0.031) trabecular thickness.


Early Programming of Bone

Gender x treatment (p = 0.023) Gender x treatment (p = 0.039)

All treatments resulted in greater (p<0.001) cortical area than the CON, with DAI having the greatest (p<0.05) effect.

Cortical and trabecular thickness at the femur midpoint was greater (p<0.05) with DAI than CON.

Early Programming of Bone

- No effect on trabecular parameters at femur neck
- Cortical area and thickness at the femur neck were greater (p<0.05) with DAI than CON


Early Programming of Bone

- Mechanism: epigenetics?
- Protection beyond 4 months of age and post-ovariectomy?
Future directions.....

- Food synergy
- Food-drug synergy
- Long-term programming
- Inflammation

Acknowledgments

Mentors:
- Dr. Stephanie Atkinson
- Dr. Sharon Donovan
- Dr. Lilian Thompson

Students:
- Sandra Sacco
- Jovana Kaludjerovic
- Beatrice Lau
- Kellie Welch
- Pearl Breitman
- Isabelle Sirois
- Stacey Cohen
- Debbie Fonseca
- Ana Piekarz
- Susie Kim
- Shira Tenenbaum
- Jessica Jiang

Collaborators:
- Dr. Aideen Moore
- Dr. Michael Archer
- Dr. Kafi Ealey
- Dr. David Ma

Funding Agencies:
- CIHR
- NSERC
- ILSI North America
- National Institute of Nutrition
- Flax Council of Canada
- Saskatchewan Flax Development Commission
- Bickell Foundation
- Cummings Foundation
- Hospital for Sick Children Foundation