2011

Nitric Oxide and Osteoporosis: What a Gas!

Sophie Jamal

University of Toronto

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Nitric Oxide and Osteoporosis: What a Gas!

Sophie Jamal, MD, PhD, FRCPC
Associate Professor of Medicine
University of Toronto
Outline

- Pathophysiology of osteoporosis
- Nitric oxide and bone turnover
- The effects of isosorbide mononitrate on markers of bone turnover
- The effects of nitroglycerin on bone turnover geometry and strength
### Effect of estrogen and transdermal NTG on BMD (Wimalawansa et al Bone 1996)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Percent increase in BMD (L2-L4) over 6 weeks</th>
</tr>
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<tbody>
<tr>
<td>Sham operated</td>
<td>25% ± 2%</td>
</tr>
<tr>
<td>Ovariectomized rats</td>
<td>8% ± 3%</td>
</tr>
<tr>
<td>Ovariectomized + Estrogen</td>
<td>27% ± 5%*</td>
</tr>
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<td>Ovariectomized + NTG</td>
<td>20% ± 3%**</td>
</tr>
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<td>Ovariectomized + E + NTG</td>
<td>22% ± 2%*</td>
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*different than ovariectomized rats at p<0.005
** different than ovariectomized rats at p<0.02
Low bone mineral density (BMD): a risk factor for osteoporotic fractures

- Relationship between hip fracture and hip BMD: 2.6 RR/SD
- BMD = bone formation - bone resorption
- Increased bone resorption at menopause
- Nitric oxide may contribute to postmenopausal bone loss
Sources of nitric oxide

L-arginine + O₂ → Nitric Oxide

Nitric Oxide Synthase (NOS)

Nitrate & Nitrite

Endogenous

Exogenous

- Isosorbide Mononitrate
- Isosorbide Dinitrate
- Nitroglycerin
Bone cells make nitric oxide

- Two cell types involved in bone remodeling:
  - Osteoclasts → bone resorption
  - Osteoblasts → bone formation
- Osteoblasts produce nitric oxide synthase
- Estrogen and mechanical strain produce nitric oxide synthase
NO influences osteoclasts

- Nitric oxide has a biphasic effect on osteoclasts
  - Low levels enhance osteoclast activity and differentiation
  - High levels inhibit osteoclast activity and differentiation
NO may mediate effects via OPG

- High levels of nitric oxide activate OPG
- Low levels of nitric oxide activate OPGL
Bone resorption depends on the balance of OPG and OPGL.
The OPG/OPGL/RANK axis

Hormones → OPG Ligand → RANK → Osteoclast Precursor

OPG Ligand

OPG

Bone

Osteoblasts

RANK

NO

Osteoclast
What about osteoblasts?

- Limited data
- Biphasic activity:
  - High levels of NO stimulate osteoblast activity
  - Low levels of NO inhibit osteoblast activity
In vitro data: a summary

- Nitric oxide stimulates OPG
  - OPG binds to OPGL
  - Prevents binding of OPGL to RANK
  - Decreased osteoclast activity (bone resorption)

- Issues
  - Is the effect similar with nitric oxide donors?
  - Does the decrease in bone resorption lead to increased bone mineral density?
# Effect of estrogen and transdermal NTG on BMD

(Wimalawansa et al Bone 1996)

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** different than ovariectomized rats at p<0.02
## Effect of frequency of administration

<table>
<thead>
<tr>
<th>Treatment Group (n = 5)</th>
<th>Change in Spine BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>6.3 % ± 5.3 *</td>
</tr>
<tr>
<td>Ovariectomy (OVX)</td>
<td>– 2.5 % ± 2.0</td>
</tr>
<tr>
<td>OVX + estrogen</td>
<td>5.9 % ± 3.4*</td>
</tr>
<tr>
<td>OVX + 0.2 mg NTG once a day</td>
<td>6.2 % ± 2.8*</td>
</tr>
<tr>
<td>OVX + 0.2 mg NTG twice a day</td>
<td>1.9 % ± 2.1</td>
</tr>
<tr>
<td>OVX + 0.2 mg NTG three times a day</td>
<td>– 0.2 % ± 3.3</td>
</tr>
</tbody>
</table>

Wimalawansa SJ et al. JBMR 2000
Does NO play a role in menopause?

Menopause is characterized by:
- Increased bone resorption
- Low circulating estrogen
- Low levels of nitric oxide

Treatment with HRT increases nitric oxide and decreases bone resorption
Nitrates and bone mineral density - 1996

- SOF study (n = 6201) (Jamal SA et al JBMR 1998)
  - BMD at heel and spine
    - Daily users = nonusers
    - Intermittent users higher
- Open label trial (n = 16) (Wimalawansa SJ, JBMR 2000).
- Conjugated estrogen or transdermal nitroglycerin
- Equal BMD at one year
Percent difference in BMD, nitrate users compared with nonusers

<table>
<thead>
<tr>
<th></th>
<th>Daily vs. Nonusers (n = 317, 5810) (95% CI)</th>
<th>Intermittent vs. Nonusers (n=74,5810) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hip BMD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0 (-2.7 to 1.4)</td>
<td>0 (-4.1 to 4.1)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.3 (0.14 to 4.1)</td>
<td>2.6 (0.4 to 6.8)</td>
</tr>
<tr>
<td><strong>Heel BMD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-2.6 (-5.3 to 0)</td>
<td>0 (-5.3 to 7.9)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0 (-2.6 to 2.6)</td>
<td>5.3 (2.6 to 11)</td>
</tr>
</tbody>
</table>

Nitrates and Fractures

- 124,655 subjects with fractures (cases)
- 373,962 controls
- Self-reported nitrate use 1995-200
- Nitrate use associated with decreased fracture risk:
  - Any fracture: OR = 0.89 (0.86 to 0.92)
  - Hip fracture: OR = 0.85 (0.79 to 0.92)

Rejnmark L et al, JBMR 2006
Findings so far...

- Use of nitrates may be associated with increases in BMD
- Use of nitrates may decrease risk of fractures
- Tolerance to nitrates might exist
The second study - 1998

- To determine the effects of isosorbide on markers of bone turnover
- To assess if there is a dose response
- Applied for funding 1998, received funding 1999
- Completed study 2002
- Published 2004
Study design

**Phase I**
- Calcium 500 mg
- Vitamin D 400 IU
- ISMO 5 mg or ISMO 20 mg or Placebo

**Phase II**
- Blood Urine

Months 0 to 3

Blood Urine
Study subjects

- **Inclusion Criteria:**
  - Postmenopausal women, 50 to 80 yrs
  - Osteopenia or normal BMD at femoral neck

- **Exclusion Criteria:**
  - Low trauma fracture (hip, wrist, spine)
  - Active bone disease
  - Treatment for osteoporosis
  - Treatment with steroids
  - Heart disease
Markers of bone turnover

- 2 classes of biochemical markers:
  - Bone formation (osteoblast activity)
  - Bone resorption (osteoclast activity)
- Resorption and formation are coupled
- May be different with ISMO
Why biochemical markers?

- Markers of bone turnover are correlated with bone mineral density
- Substantial changes in markers can be seen within 3 months of treatment
- Minimizes costs
- Minimizes adverse events
451 women screened → 206 ineligible

245 eligible → 99 did not participate

146 women: calcium and vitamin D → 2 did not return

144 women randomized

48: placebo
- 4 drop outs
  - no samples
  - 44: placebo

49: 5 mg ISMO
- 11 drop outs
  - 6 samples
  - 44: 5 mg ISMO

47: 20 mg ISMO
- 10 drop outs
  - 5 samples
  - 42: 20 mg ISMO
### Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 48)</th>
<th>5 mg ISMO (n = 49)</th>
<th>20 mg ISMO (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>62 ± 7.0</td>
<td>59 ± 7.5</td>
<td>59 ± 5.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 ± 16</td>
<td>70 ± 15</td>
<td>74 ± 15</td>
</tr>
<tr>
<td>Caucasian</td>
<td>45 (94%)</td>
<td>44 (90%)</td>
<td>44 (94%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>5 (10%)</td>
<td>5 (10%)</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>13 ± 8</td>
<td>12 ± 10</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>NTX (nmol BCE/mmol Cr)</td>
<td>62 ± 65.9</td>
<td>79 ± 61.8</td>
<td>82 ± 71.5</td>
</tr>
<tr>
<td>BSAP (IU/L)</td>
<td>25 ± 6.9</td>
<td>23 ± 6.3</td>
<td>22 ± 6.6</td>
</tr>
</tbody>
</table>

Jamal SA et al, JBMR 2004
ISMO decreased bone resorption markers

NTX Percent change vs. placebo

5 mg: -36.3, p = 0.0002
20 mg: -45.4, p = 0.0001
ISMO increased bone formation markers

BSAP Percent change vs. placebo

5 mg  20 mg

15.9  23.3

p = 0.005  p = 0.001
Headaches were common

<table>
<thead>
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<th>Placebo (n = 48)</th>
<th>5 mg ISMO (n = 49)</th>
<th>20 mg ISMO (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discontinued study</td>
<td>4</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>D/C due to headache</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>
Limitations

- Generalizability
  - Study population healthy Caucasians
- Duration of study was 12 weeks
- Studies with BMD and fracture end points are needed
Implications of our findings

- Decrease in NTx similar to bisphosphonates
- 40% decrease in NTx: 30% decrease in fracture risk
- We also found an increase in formation markers -
- Greater fracture reduction than with antiresorptives
- Headache may limit the use of this drug:
  - 10% of women had headaches, 75% of drop outs
  - 4X more common in women randomized to ISMO
  - Higher than reported in CV literature
  - Less headaches with transdermal NTG
The third study

- Applied for funding March 2004 - rejected
- Reapplied for funding March 2005
- Funded October 2005
- Completed Trial March 2010
- 2 part randomized controlled trial:
  - 3 week cross over study to compare tolerability of NTG and ISMO
  - 2 year study of best tolerated nitrate
- Outcomes in 2 year study:
  - Change in lumbar spine and hip BMD
  - Change in bone turnover markers
  - Changes in trabecular bone with pQCT
Study Hypothesis

15 mg of Nitroglycerin, applied once daily, will lead to uncoupling of bone turnover, increases in bone mineral density and bone geometry, compared with placebo.
Study Design

- One week run in phase
- 24 month duration
- End points:
  - Bone turnover markers
  - Bone mineral density
  - pQCT
Study Subjects

- **Inclusion:**
  - Postmenopausal $\geq 50$ years
  - L spine T score between 0 and -2.0

- **Exclusion:**
  - Migraine headaches
  - Already taking nitrates
  - SBP $\leq 100$ mm Hg, DBP $\geq 110$ mm Hg
Statistical Analyses

- **Primary Endpoint:**
  - Lumbar spine BMD

- **Secondary Endpoints:**
  - Hip BMD
  - pQCT at radius and tibia
  - B-ALP, NTX (log transformed data)
Assessed for eligibility (n=1526)

- Excluded (n = 1283)
  Did not meet inclusion criteria (541)
  Declined to participate (585)

- Discontinued study (n= 157)
  Headaches (93)
  Headaches and nausea (11)

Enrolled in 1 week run in phase (n = 400)

Randomized (n = 243)

- Nitroglycerin ( n =126)
  Discontinued intervention ( n = 30)
    Headaches ( n =7)
    No follow up data ( n = 8)
  Analysed:
  Data on 116 at 24 m

- Placebo ( n = 117)
  Discontinued intervention ( n =15)
    Headaches ( n = 2)
    Lost to follow up ( n = 6)
  Analysed:
  Data on 109 at 24 m
<table>
<thead>
<tr>
<th></th>
<th>NTG Ointment</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>126</td>
<td>117</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>61.3</td>
<td>61.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.3</td>
<td>70.9</td>
</tr>
<tr>
<td>Vitamin D (IU/day)</td>
<td>783.2</td>
<td>753.2</td>
</tr>
<tr>
<td>Calcium (mg/day)</td>
<td>1548.8</td>
<td>1565.6</td>
</tr>
<tr>
<td>L Spine T-score</td>
<td>-0.9</td>
<td>-1.1</td>
</tr>
</tbody>
</table>
B-ALP at 12 months

Nitrate = 126
Placebo = 117

Percentage of baseline

NTG
Placebo

21.3%
(21.6 to 22.1)

0 3 12
Months
Nitrate = 118
Placebo = 111
B-ALP at 24 months

Percentage of baseline

- NTG
- Placebo

35.9%
(34.4 to 37.4)
NTX at 12 months

- NTG
- Placebo

30.0% (23.9 to 39.6)
NTX at 24 months

- NTG
- Placebo

Percentage of baseline

Months

50.8%
(43.7 to 60.2)
Lumbar Spine BMD at 24 months

6.7% (5.2 to 8.2)
Femoral Neck BMD at 24 months

Percent Change

NTG
Placebo

7.0%
(5.5-8.5)

Months
Total Hip BMD at 24 months

NTG: 6.2% (5.2 to 7.3)
Placebo: 

Percent Change

Months

0 2 4 6 8 10
-2

12 24
### PQCT of the Radius

<table>
<thead>
<tr>
<th>Measurement</th>
<th>% Difference (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular density</td>
<td>11.9 (8.1 to 15.7)</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>13.9 (6.0 to 21.7)</td>
</tr>
<tr>
<td>Cortical density</td>
<td>2.2 (0.6 to 3.7)</td>
</tr>
<tr>
<td>Cortical area</td>
<td>10.6 (6.9 to 14.3)</td>
</tr>
<tr>
<td>Periosteal circumference</td>
<td>7.4 (4.3 to 10.4)</td>
</tr>
</tbody>
</table>
## Indices of Radial Bone Strength

<table>
<thead>
<tr>
<th>Measurement</th>
<th>% difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar Section Modulus</td>
<td>10.7 (7.5 to 13.8)</td>
</tr>
<tr>
<td>Polar Moment of Inertia</td>
<td>7.3 (4.6 to 10.1)</td>
</tr>
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</table>
## PQCT of the Tibia

<table>
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<th>Measurement</th>
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<tr>
<td>Trabecular density</td>
<td>8.5 (4.3 to 12.7)</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>24.6 (18.9 to 30.4)</td>
</tr>
<tr>
<td>Cortical density</td>
<td>1.5 (0.8 to 2.3)</td>
</tr>
<tr>
<td>Cortical area</td>
<td>10.0 (5.2 to 15.0)</td>
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<tr>
<td>Periosteal circumference</td>
<td>2.9 (1.0 to 6.8)</td>
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## Indices of Tibial Bone Strength

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<tr>
<td>Polar Section Modulus</td>
<td>9.8 (0.2 to 19.4)</td>
</tr>
<tr>
<td>Polar Moment of Inertia</td>
<td>14.5 (3.2 to 25.8)</td>
</tr>
<tr>
<td>Headaches</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Run In</td>
<td>400 enrolled, 104 did not continue</td>
</tr>
<tr>
<td>Phase</td>
<td>Treatment</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>Placebo</td>
</tr>
<tr>
<td>d/c at 12 months</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>1 month</td>
<td>35%</td>
</tr>
<tr>
<td>12 months</td>
<td>5.3%</td>
</tr>
<tr>
<td>24 months</td>
<td>2%</td>
</tr>
<tr>
<td>Event</td>
<td>Nitroglycerin</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Serious Adverse events</td>
<td>5 (4.2%)</td>
</tr>
<tr>
<td>-Death</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Fractures</td>
<td>2 (1.6%)</td>
</tr>
</tbody>
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Conclusions

- Nitroglycerin uncouples bone turnover
- Improves BMD, geometry, indices of strength
- Inexpensive and widely available
- Initiation of treatment limited by headache
- The efficacy of nitroglycerin to reduce fracture risk should be tested in a clinical trial