TEAM SCIENCE AT A PROGRAMMATIC LEVEL

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APPRAUCHE TO TRANSLATIONAL RESEARCH IN OSTEOPOOROSIS

Epidemiological Studies → Clinical Investigation → Mouse and Cellular Models

Translation into Practice
FRACTURES IN MEN

Cooper & Melton, Trends Endocrinol Metab 8:225, 1992
### LIFETIME RISK OF FRACTURE AT AGE 50 YEARS (%)

<table>
<thead>
<tr>
<th></th>
<th>White women</th>
<th>White men</th>
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<tbody>
<tr>
<td>Hip fracture</td>
<td>17.5</td>
<td>6.0</td>
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<tr>
<td>Vertebral fracture</td>
<td>15.6</td>
<td>5.0</td>
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<tr>
<td>Forearm fracture</td>
<td>16.0</td>
<td>2.5</td>
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<tr>
<td>Any of the 3</td>
<td>39.7</td>
<td>13.1</td>
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Annual healthcare costs ~ $17B

Cummings & Melton, Lancet 359:1761, 2002
• Oophorectomy/menopause in women →
  loss of E → ↑↑BR/ ↑BF → Rapid bone loss
Therefore, E regulates bone metabolism in women
EFFECT OF ORCHIDECTOMY ON BMD IN MEN

Stepan et al. JCEM 69:523, 1989
ESTROGEN VERSUS ANDROGEN EFFECTS ON THE SKELETON – TRADITIONAL VIEW

• Oophorectomy/menopause in women →
  loss of E →↑BR/↑BF → Rapid bone loss
Therefore, E regulates bone metabolism in women

• Orchidectomy in men →
  loss of T →↑BR/↑BF → Rapid bone loss
Therefore, T regulates bone metabolism in men
ROLE OF ESTROGEN IN THE MALE SKELETON
Evidence from “experiments of nature”

- An estrogen receptor (ER) mutant male (Smith et al. NEJM 331:1056, 1994) and two aromatase deficient males (Morishima et al. JCEM 80:3689, 1995; Carani Et al. NEJM 337:91, 1997) were described

- All three individuals had unfused epiphyses, high rates of bone resorption (and formation), and osteopenia
• E is required for epiphyseal closure

• Presence of E is necessary for the acquisition of bone mass during puberty in boys
ISSUES LEFT UNRESOLVED BY THE ERα AND AROMATASE DEFICIENT MALES

• What if any, is the role of E in regulating bone remodeling in adult men with mature skeletons?

• What is the role of E or T deficiency in mediating “age-related” bone loss in men
APPROACH TO TRANSLATIONAL RESEARCH IN OSTEOPOROSIS

- Epidemiological Studies
- Clinical Investigation
- Mouse and Cellular Models

Translation into Practice
DISSECTING RELATIVE CONTRIBUTIONS OF E VS T TO BONE TURNOVER IN MEN

Visit  Entry  Baseline  Final
Weeks  0    1    2    3    4    5    6    7    8

Leuprolide  Leuprolide  Leuprolide

Letrozole

T + E Patch

A: No Patch  B: E Patch  C: T Patch  D: T + E Patch  T Patch

Falahati-Nini et al. JCI 106:1553-1560, 2000
IN VIVO EFFECTS OF SEX STEROIDS ON BONE RESORPTION MARKERS

**P < 0.001
** P < 0.005
* P < 0.05

Dpd

NTx

<table>
<thead>
<tr>
<th>Group</th>
<th>Effect</th>
<th>% Change</th>
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<tr>
<td>A (-T, -E)</td>
<td>**</td>
<td>30</td>
</tr>
<tr>
<td>B (-T, +E)</td>
<td>***</td>
<td>40</td>
</tr>
<tr>
<td>C (+T, -E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (+T, +E)</td>
<td></td>
<td>-10</td>
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IN VIVO EFFECTS OF SEX STEROIDS ON BONE RESORPTION MARKERS

** Group A: (-T, -E) ** Group B: (-T, +E) ** Group C: (+T, -E) ** Group D: (+T, +E)

*** P < 0.001
** P < 0.005
* P < 0.05

Dpd

NTx
IN VIVO EFFECTS OF SEX STEROIDS ON BONE RESORPTION MARKERS

**Group A** (-T, -E)  **Group B** (-T, +E)  **Group C** (+T, -E)  **Group D** (+T, +E)

**Dpd**

ANOVA
E effect: P = 0.005
T effect: P = 0.232

**NTx**

ANOVA
E effect: P = 0.0002
T effect: P = 0.085
IN VIVO EFFECTS OF SEX STEROIDS ON SERUM OSTEOCALCIN LEVELS

![Bar chart showing the effects of different groups on serum osteocalcin levels. Group A (-T, -E), Group B (-T, +E), Group C (+T, -E), and Group D (+T, +E). The x-axis represents the groups, and the y-axis represents the serum osteocalcin level as a percentage change. Group D shows a significant increase compared to the other groups, indicated by *** P < 0.001.}
IN VIVO EFFECTS OF SEX STEROIDS ON SERUM OSTEOCALCIN LEVELS

Serum Osteocalcin, % change

Group A (-T, -E)  Group B (-T, +E)  Group C (+T, -E)  Group D (+T, +E)

*** P < 0.001
IN VIVO EFFECTS OF SEX STEROIDS ON SERUM OSTEOCALCIN LEVELS

ANOVA  E effect: P = 0.002; T effect: P = 0.013

* * * P < 0.001
In men, E is the dominant sex steroid regulating bone resorption, although T (in the absence of aromatization to E) may make a smaller contribution (< 30%).

Both E and T contribute to the maintenance of bone formation.
E DEFICIENCY IS THE PRIMARY MEDIATOR OF BONE LOSS IN HYPOGONADAL MEN

- Healthy men (age 20-50 yrs) given GnRH and aromatase blocker for 16 weeks
- Treated with 5 doses of T (0, 1.25 g, 2.5 g, 5 g, 10 g)
- All subjects had suppressed E₂ levels (1.1-3.6 pg/mL)
- T levels: 43, 234, 378, 497, and 985 ng/dL
- Regardless of the T level, in the setting of low E₂ levels, bone loss (total, cortical, trabecular density, cortical area and thickness) was identical in all the T groups

Yu et al. ASBMR, 2012 (#1199)
RALOXIFENE IN MEN

Study Design

- 50 elderly men, mean age 69 years
- Randomized to placebo or raloxifene, 60 mg/d, for 6 months
- Baseline and 6 month assessment of bone turnover markers

Doran et al. JBMR 16:2118-2125, 2001
## RALOXIFENE IN MEN

Changes in PTH and bone turnover markers

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<tr>
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<th>Placebo</th>
<th>Raloxifene</th>
<th>P-value</th>
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<tr>
<td>PTH, pmol/L</td>
<td>+0.2 ± 0.3</td>
<td>+0.3 ± 0.2</td>
<td>0.439</td>
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<tr>
<td>BSAP, U/L</td>
<td>-1 ± 1</td>
<td>0 ± 0</td>
<td>0.100</td>
</tr>
<tr>
<td>Urine NTx, nmol/d</td>
<td>-17 ± 12</td>
<td>3 ± 11</td>
<td>0.109</td>
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RALOXIFENE IN MEN
Change in Urine NTx Excretion as a Function of Baseline E₂ Levels

Δ Urinary NTx Excretion, nmol/24 hr

Baseline Serum Estradiol, pg/mL

Raloxifene

Placebo

R = 0.57
P < 0.01

R = -0.15
P = 0.49
RALOXIFENE IN MEN
Change in Urine NTx Excretion as a Function of Baseline E$_2$ Levels

Raloxifene

R = 0.57
P < 0.01

26 pg/mL

Placebo

R = -0.15
P = 0.49
APPROACH TO TRANSLATIONAL RESEARCH IN OSTEOPOROSIS

Epidemiological Studies

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Translation into Practice
RELATIONSHIP OF $E_2$ VS. T LEVELS TO FRACTURE RISK IN OLDER MEN

- Serum sex steroids analyzed by GC-MS at baseline in older men ($n = 2639$, mean age 75 years)

- Fractures occurring after baseline were validated (average follow up of 3.3 years)

Mellström et al. JBMR 23:1552, 2008
RELATIONSHIP OF E₂ VS. T LEVELS TO FRACTURE RISK IN OLDER MEN (Cont’d)

- **I:** nl E₂, nl T
- **II:** low E₂, nl T
- **III:** nl E₂, low T
- **IV:** low E₂, low T

Low E₂: ≤ 16 pg/mL
Low T: ≤ 336 ng/dL
(Lowest quartiles for each)
RELATIONSHIP OF E$_2$ VS. T LEVELS TO FRACTURE RISK IN OLDER MEN (Cont’d)

Poisson regression model
BIOAVAILABLE VS SHBG-BOUND SEX STEROIDS

NON-BIOAVAILABLE

~50% SHBG (~90 kD)

E1

E2

BIOAVAILABLE

Free (1-3%)

~50% ALBUMIN (~66 kD)

T

E1

E2

E1

E2
SERUM TOTAL TESTOSTERONE AND ESTRADIOL LEVELS AS A FUNCTION OF AGE IN ROCHESTER, MN MEN AND WOMEN

Khosla et al. JCEM 83:2266, 1998
SERUM BIOAVAILABLE TESTOSTERONE AND ESTRADIOL LEVELS AS A FUNCTION OF AGE IN ROCHESTER, MN MEN AND WOMEN

Khosla et al. JCEM 83:2266, 1998
CHANGES IN SERUM SHBG IN MEN

Khosla et al. JCEM 83:2266, 1998
SUMMARY: AGE-RELATED CHANGES IN SEX STEROIDS

- Abrupt decrease in total and bioavailable estrogen levels in women at the time of menopause

- More gradual decreases in serum bioavailable testosterone and estrogen levels in men starting at age 50-60 years

- Marked increase in men in serum SHBG levels starting at age 50-60 years
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**Group A** (-T, -E)  
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**Group C** (+T, -E)  
**Group D** (+T, +E)

*** P < 0.001  
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Dpd

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IN VIVO EFFECTS OF SEX STEROIDS ON SERUM OSTEOCALCIN LEVELS

Serum Osteocalcin, % change

Group A (-T, -E)  Group B (-T, +E)  Group C (+T, -E)  Group D (+T, +E)

*** P < 0.001
DIFFERENTIAL EFFECTS OF E AND T ON BONE RESORPTION MARKERS IN YOUNG MEN

Leder et al. JCEM 88: 204, 2003
Differential effects of E and T on bone resorption markers in young men

Leder et al. JCEM 88: 204, 2003
DIFFERENTIAL EFFECTS OF E AND T ON BONE RESORPTION MARKERS IN YOUNG MEN

Leder et al. JCEM 88: 204, 2003

Phase II. "Re-coupling"

Percent Change

Time after GnRH, weeks

Urine Deoxypyridinoline

Serum N-telopeptide

Percent Change

Phase II. "Re-coupling"

Percent Change

Time after GnRH, weeks

Serum Osteocalcin

Serum PINP

Leder et al. JCEM 88: 204, 2003
ACUTE CHANGES IN BONE FORMATION AND OC #’s FOLLOWING OVX IN RATS

Sham

OVX

20
10
0
30

Days following OVX

Pre 2 5 8 11 14

BFR/BS, µm³/µm² per day (X10⁻²)

*P<0.02, **P<0.0002 vs Sham

OCs/BS (%)

*P<0.05

OSTEOBLAST REGULATION OF OSTEOCLAST FORMATION/FUNCTION

Stimulatory Factors

OC PRECURSORS

- IL-6
- IL-7
- PGE$_2$
- GM-CSF

Differentiation and activation

ACTIVE OC

- M-CSF
- RANKL
- OPG

Inhibitory Factors

OC APOPTOSIS

- IL-6
- IL-7
- PGE$_2$
- GM-CSF

OSTEOPROGENITOR/OSTEOBLASTS

RANKL

TGFβ

"coupling" factors
SCHEMATIC OF THE BONE REMODELING COMPARTMENT

Khosla, Westendorf, Oursler JCI 118:421, 2008
POTENTIAL MECHANISMS FOR COUPLING BONE FORMATION TO BONE RESORPTION

• Cell-cell contact
• Factors released from the bone matrix
• Osteoclast-derived secreted factors
BIDIRECTIONAL EPHRIN SIGNALING BETWEEN OSTEOCLASTS AND OSTEOBLASTS

ROLE OF TGFβ1 IN COUPLING BONE RESORPTION TO BONE FORMATION

SCHEMATIC OF THE BONE REMODELING COMPARTMENT
OSTEOCLAST CONDITIONED MEDIA STIMULATES MINERALIZATION

Pederson et al. PNAS 105:20764, 2008
MARROW-DERIVED OSTEOCLAST COUPLING FACTOR EXPRESSION

Pederson et al. PNAS 105:20764, 2008
COUPLING FACTOR HYPOTHESIS

Wnts

BMP6

S1P

SCLEROSTIN
APPROACH TO TRANSLATIONAL RESEARCH IN OSTEOPOROSIS

Epidemiological Studies -> Clinical Investigation -> Mouse and Cellular Models

Translation into Practice
CLINICAL IMPLICATIONS

- Use of serum estradiol levels in the evaluation of male osteoporosis
POTENTIAL CLINICAL APPROACH

• Male with low T (< 300 ng/mL), osteoporosis/increased fracture risk

• E₂ normal (> 16 pg/mL by mass spec): standard pharmacological therapy (e.g., bisphosphonate)

• E₂ low (< 16 pg/mL): Consider trial of T therapy and monitor BMD
CLINICAL IMPLICATIONS

• Use of serum estradiol levels in the evaluation of male osteoporosis
• Use of selective estrogen receptor modulators in male osteoporosis
RALOXIFENE IN MEN WITH PROSTATE CANCER ON GnRH
Smith et al. JCEM 89:3841, 2004

Lumbar spine

Percent change

P=0.07

Total hip

Percent change

P<0.001

Trochanter

Percent change

P<0.001

Femoral neck

Percent change

P=0.06

-3 -2 -1 0 1 2 3

0 3 6 9 12

Month

0 3 6 9 12

Month

-3 -2 -1 0 1 2 3

Raloxifene
n = 19

No raloxifene
n = 22
EFFECTS OF THE SERM, TOREMIFINE, ON VERTEBRAL FRACTURES IN MEN WITH PROSTATE CANCER

• 1284 men with prostate cancer on ADT randomized to receive 80 mg toremifine or placebo for up to 24 months

• 50% reduction noted in vertebral fractures in the toremifene treated group (P = 0.05)

CLINICAL IMPLICATIONS

- Use of serum estradiol levels in the evaluation of male osteoporosis
- Use of selective estrogen receptor modulators in male osteoporosis
- Cautionary note regarding possible efficacy of non-aromatizable selective androgen receptor modulators in preventing bone loss in men
CLINICAL IMPLICATIONS

- Use of serum estradiol levels in the evaluation of male osteoporosis
- Use of selective estrogen receptor modulators in male osteoporosis
- Cautionary note regarding possible efficacy of non-aromatizable selective androgen receptor modulators in preventing bone loss in men
- Based on osteoclast-derived osteoblast coupling factors, potential development of novel bone formation-stimulating agents
# ACKNOWLEDGEMENTS

<table>
<thead>
<tr>
<th>B. Lawrence Riggs</th>
<th>Louise McCready</th>
<th>Kristy Nicks</th>
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<tr>
<td>L. Joseph Melton III</td>
<td>Amanda Tweed</td>
<td>K. Chokalingam</td>
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<td>Shreyasee Amin</td>
<td>Margaret Holets</td>
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<td>Merry Jo Oursler</td>
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<td>Terry Therneau</td>
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<td>Elizabeth Atkinson</td>
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<td>Sara Achenbach</td>
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<td>Richard Robb/Jon Camp</td>
<td>Patrick Doran</td>
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