Bone Loss After SCI: What Do Animal Studies Tell Us About the Cause and Potential Treatments

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Disclosures

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Learning Objectives

At the conclusion of this activity, the participant will be able to:

• A. Discuss the key changes in molecular signals pertinent to bone loss after SCI.
• B. Discuss the potential role of androgens in treatment of bone loss after SCI suggested by findings from animal studies.
• C. Discuss acute benefits to bone health of mechanical reloading of bone by FES that are suggested by animal studies.
• Identify two potential targets for drugs in bone loss after SCI.
Utility of Animal Models

• Examine the temporal sequence of changes in bone mass and structure
• Through genetic manipulations, determine the underlying cellular and molecular events
• Evaluate candidate therapies
Wnt Signaling (Canonical)

- sFRP
- Wnt
- Frizzled Receptor
- LRP5 (Co-receptor)
- Sclerostin
- DKK1
- Cytoplasmic Membrane
Key Elements in Bone Biology

- **Osteoblast**
- **Osteoclast**
- **Mesenchymal Stem Cells**
- **Hematopoietic Stem Cells**

**Cyclical Loading**

**Wnt**

**Unloading**

**Sclerostin**

**DKK1**

**RANKL**

**OPG**

**Bone Resorption**

**Bone Formation**

**Osteocyte**
Effect of Unloading on SOST/Sclerostin
Robling et al. 2008 J Biol Chem 283:5866-75/

• In the rat ulna, mechanical loading reduced SOST and increased bone formation rates.
• These changes correlated with loading and strain gradients.
• Unloading increased SOST expression in-vivo at 3 and 7 days.
Ex-vivo Culture of Marrow Cells

Bone Marrow Stromal Cells

- RANKL

Osteoclast
- TRAP+
- Multinucleated
- Bone Resorption

Pre-osteoblast
- Alk. Phos +
- ascorbic acid-2-phosphate

Osteoblast
- Bone Nodules
Male rats had hindlimbs unloaded for 5 days by tail suspension.

Osteoblast numbers in bone and bone formation rate were reduced by 34% and 39%.

Numbers of osteoblasts present in ex-vivo cultures of bone marrow stromal cells was reduced and these OB exhibited reduced bone forming activity.

Unloading stimulated increased IL-6 release from bone marrow stromal cells.
• Male Rats (juvenile) with a severe (10gx50 mm) contusion injury studied at 10 days after SCI
• BMD at the proximal metaphysis reduced by 34%.
• 3-fold increase in osteoclast numbers at the growth plate.
• There appeared to be a reduction in bone formation rate at the distal metaphysis and a mineralization defect of newly formed bone.
• Thinning and disorganization of chondrocytes was noted at the growth plate.
Evidence that Androgens Influence Bone

Animal Model

• Male Wistar rats with complete transection at T9-T10.
• Nandrolone plus testosterone (replacement dose) administered beginning day 29 after SCI and continued through day 56.
• Bones harvested for analysis at day 56.
Nandrolone Reduces Bone Loss After SCI

Sham
SCI
SCI+Nandrolone

Femur

BMD (g/cm²)

Tibia

BMD (g/cm²)
Nandrolone Reversed Upregulation of Osteoclast Markers after SCI

Osteoclast Differentiation Markers

- TRAP
- Calc R

[Graph showing relative expression levels of TRAP and Calc R for Sham, SCI, and SCI+Nandrolone groups, with statistical significance indicated by asterisks (*) and triple asterisks (***)]
Differentiation Pathway of Osteoblasts

Mesenchymal Stem Cell → Runx2 → Pre-osteoblast → Osterix → Osteoblast

Bone Sialoprotein
Osteocalcin
Collagen
Nandrolone Partially Reversed Reductions in Osteoblast Differentiation Markers

Osteoblast Differentiation Markers

- **Runx2**: Relative Expression (% of control)
  - ****: p < 0.001
  - *: p < 0.05

- **Osteocalcin**: Relative Expression (% of control)
  - ****: p < 0.001
  - *: p < 0.05

- **BSP**: Relative Expression (% of control)
  - ****: p < 0.001
  - *: p < 0.05
Nandrolone Increased OPG Expression

A. OB OPG

B. OB RANKL

C. OB OPG/RANKL

Sham
SCI
SCI+Nandrolone
Nandrolone Increased Wnt Signaling Genes

A. OB Wnt3a

B. OB LRP5

C. OB Fzd5

D. OB ENC1

- **Sham**
- **SCI**
- **SCI+Nandrolone**

*Relative Expression (% of control)*
FES&Bone: Experimental Design

- **Spinal cord transection** $T_{9-10}$
- **Sciatic N**
- **Common peroneal N**
- **Anterior tibial N**
- **FES electrodes**
- **Gastrocnemius**
- **Plantaris**
- **Soleus**
- **Stimulator ON**
- **Stimulator OFF**

### Timeline
- **SCI**
- **FES implanted**
- **FES Initiated**
- **Euthanize**
  - 0 weeks
  - 14 weeks
  - 16 weeks
  - 17 weeks
Effects on the Plantaris Muscle of FES for 7 Days

- **Weight**
- **MAFbx mRNA**
- **MurF1 mRNA**
SCI Led to Reductions in Bone Mass

Areal BMD by DEXA Scan:
Distal Femur and Prox. Tibia

MicroCT Studies: Prox. Tibia

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>SCI</th>
<th>SCI+FES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV(%)</td>
<td>0.65±0.04</td>
<td>0.34±0.01</td>
<td>0.30±0.02 NS</td>
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<tr>
<td>Tb.N (µm⁻¹)</td>
<td>6.09±0.32</td>
<td>4.77±0.05</td>
<td>4.42±0.09 NS</td>
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<tr>
<td>Tb.Th (µm)</td>
<td>0.13±0.01</td>
<td>0.09±0.02 **</td>
<td>0.08±0.01 NS</td>
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<tr>
<td>Tb.Sp (µm)</td>
<td>0.11±0.01</td>
<td>0.18±0.01 ***</td>
<td>0.19±0.01 NS</td>
</tr>
</tbody>
</table>
Effect of FES on Blood Markers of Bone Metabolism

Bone Resorption Marker

Bone Formation Marker

- Serum CTX (ng/ml)
- Serum Osteocalcin level (% of control)

Sham
SCI
FES+SCI

***
***

*
Properties of Ex-vivo Cultures of Osteoclasts

TRAP staining

Osteoclast Counts

OC Differentiation Markers

- Sham
- SCI
- FES+SCI

TRAP+ Osteoclasts (% of control)

Fold Change

- Calcitonin receptor
- Integrin beta3
- TRAP
Properties of Ex-vivo Cultured Osteoblasts

**CFU-OB staining**

**CFU-F staining**

**Osteoblast Colony Counts**

**Fold Change**

- **Sham**
- **SCI**
- **FES+SCI**

**Osteoblast Differentiation Markers**

- Runx2
- Osteocalcin
- BSP
Expression in Ex-vivo Cultures of Osteoblasts (OB) of RANKL and OPG

![Bars showing expression levels of OB OPG, OB RANKL, and OB OPG/RANKL with statistical significance indicated by asterisks (*) and double asterisks (**) for different groups: Sham, SCI, and FES+SCI.](image-url)
Expression in Osteoblasts of Wnt-Signaling Molecules

- **OB DKK1**
  - Sham: *
  - SCI: **
  - FES+SCI: ***

- **OB SOST**
  - Sham: ***
  - SCI: **
  - FES+SCI: *

- **OB sFRP2**
  - Sham: *
  - SCI: **
  - FES+SCI: *

- **OB Wnt 5a**
  - Sham: *
  - SCI: **
  - FES+SCI: *

- **OB Wnt 3a**
  - Sham: ***
  - SCI: *
  - FES+SCI: *
Key Molecular Signals in Bone

- **Sclerostin**
  - **DKK1**
  - **SCI Unloading**
- **Osteocyte**
- **Osteblast**
  - **Runx2**
  - **ENC1**
- **FES**
  - **Cyclical Loading**
- **Wnt**
  - **Bone Formation**
- **Nandrolone**
  - **Testosterone**
- **RANKL**
  - **OPG**
  - **Bone Resorption**

- **Osteoclast**
# Microarray Analysis of Gene Expression in Osteoblasts and Osteoclasts

Table 1. Selected results of a pathways analysis of the microarray data.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Osteoblasts</th>
<th>Osteoclasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hedgehog-PTH signaling</td>
<td>15/37 genes, p 8.1x10^-7</td>
<td>9/37 genes, p 1.62x10^-2</td>
</tr>
<tr>
<td></td>
<td>12/52</td>
<td>PTH Signaling, 12/59</td>
</tr>
<tr>
<td>ß-Adrenergic signaling</td>
<td>12/53 genes, p 3.85x10^-3</td>
<td>Vitamin D 12/59</td>
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<tr>
<td></td>
<td>9/40</td>
<td>Receptor 9/40</td>
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<tr>
<td>Wnt Signaling</td>
<td>4.55x10^-3</td>
<td>Oxytocin 9/40</td>
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<tr>
<td></td>
<td></td>
<td>Signaling 2.89x10^-3</td>
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<tr>
<td></td>
<td></td>
<td>FSH Signaling 2.89x10^-3</td>
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<tr>
<td></td>
<td></td>
<td>Calcium/NFAT 10/57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Signaling 1.11x10^-2</td>
</tr>
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Early Effects of Reloading by FES on Bone

• In a subacute model of SCI:
  – FES rapidly reduces the accelerated bone resorption that is a characteristic of SCI.
  – FES reduces expression of inhibitors of Wnt signaling (SOST, DKK1 and sFRP2).
  – FES increases expression of Wnts and the Wnt-responsive gene OPG.
  – Increased OPG explains in part the favorable effects of FES on bone resorption after SCI.
What Clinical Directions do Studies in Animal Models of SCI Support

- Androgens may be beneficial to bone and may reduce bone resorption to a clinically meaningful degree.
- Interventions targeted against Wnt inhibitors, such as sclerostin may be beneficial after SCI.
- Interventions targeting RANKL may reduce bone resorption during the subacute period after SCI.
- Animal models permit the study of acute and subacute periods after SCI.
- There is a lack of animal systems that model the chronic phase of SCI-related bone loss.
• James J. Peters VAMC
  – Yong Wu
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• University of Liverpool
  Jonathan Jarvis

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