Skeletal Impact of Spinal Cord Injury

South Carolina
Spinal Cord Injury Research Fund
Scientific Conference
3/10/2017
Support

• *FES-Rowing versus Zoledronic Acid to Improve Bone Health in SCI: A Comparative Clinical Trial*, Department of Defense W81XWH-10-1-1043 (Morse PI)

• *Adiposity and Bone Loss in Spinal Cord Injury*, NIH/NIAMS 1R01AR059270 (Morse PI)

• *Spaulding-Harvard SCI Model System*, the Administration for Community Living, NIDILRR, 90SI5007-02-01 (Morse PI)
Osteoporosis Following SCI

- Very rapid bone loss
  - Bed rest: 0.1%/week
  - Microgravity: 1%/month
  - Age related loss: 1%/year after age 30
  - Complete SCI: 1%/week
SCI-Induced Osteoporosis

- Motor complete SCI causes immediate, rapid bone loss
  - regardless of age, gender, hormone status
- 50-75% with complete SCI will fracture
  - distal femoral metaphysis
  - proximal tibial metaphysis
SCI-Induced Osteoporosis

- Bone loss progresses proximal to distal – hip, knee, ankle, calcaneus
- Trabecular bone loss rapid, plateaus at 1-3 years
- Cortical bone loss is slow but ongoing
Fragility Fracture Incidence

- 2%–6% per year
- increases with the duration of SCI
  - 14% incidence at 5 years post injury
  - 28% incidence at 10 years post injury
  - 39% incidence at 15 years post injury

Myth: Fractures in the paralyzed limb are irrelevant
SCI-induced osteocyte apoptosis

SCI-induced osteoblast apoptosis and suppressed proliferation

SCI-induced uncoupled bone remodeling

Pathophysiology: Severe trabecular wasting with cortical thinning

Therapeutic Goal: Restore Normal Cellular Activities in the Bone Microenvironment after SCI

- Promote osteocyte survival (bone mechanoreceptors)
- Promote coupled remodeling (not stimulate osteoclast apoptosis)
- Regenerate bone (is this possible after SCI?)

Anabolic bone agents: testosterone, PTH, mechanical loading
Limited literature in SCI
Mechanical Loading

- Required for normal skeletal growth
- Regulates bone mass and structure

- achieved during ambulation:
  - impacts such as heel strike
  - muscular contraction causing deformation of bone in tension, compression, and torsion
Adaptation

dynamic adjustment of skeletal strength in response to changing loading conditions
Hypothesis

- Robust rodent literature supporting mechanical loading as an osteogenic stimulus
  - Cyclical, dynamic loading with periods of rest
- Wolff’s Law: Repeated stress stimulates bone formation at the points of maximum stress
- FES-rowing is an osteogenic stimulus sufficient to promote new bone formation in the paralyzed legs

Adaptive and injury response of bone to mechanical loading, McBride et al, Bonekey, 2012
FES-Rowing versus Zoledronic Acid to Improve Bone Health in SCI: A Comparative Clinical Trial
Electrodes stimulate the paralyzed quadriceps and hamstrings, causing the legs to thrust and retract.

Shin braces keep the legs from flopping to the left or right.

Stimulation units powered by a 9-volt battery allows a technician to adjust the voltage delivered to muscles.

Trigger allows rowers to power their own leg muscles while pulling the handle.

Springs help rowers change the direction of their movement.

High-backed seat keeps the paralyzed rower sitting upright.
Study Design

- Randomized Trial (35 each arm)
  - Rowing alone
  - Rowing + Zoledronic Acid
    - one time 5mg infusion

Inclusion/Exclusion Criteria

- 18 or older
- C6-T12 AIS A-C SCI (non-ambulatory)
- Not pregnant or intending to becoming pregnant
- No active treatment for seizures
- No active use of bone medications
- Pressure ulcer grade 2 or greater in a rowing-sensitive location
Study Flow

Enrollment/Randomization

Screening
Vitamin d deficiency, cardiac clearance, renal function, atrial fibrillation, invasive dental work

Pre-rowing Training
(sustained rowing X 20 minutes)

Rowing only Arm
3 days per week X 12 months

Rowing plus ZA
5mg infusion ZA then rowing
3 days per week X 12 months
FES-protocol

- Stimulation parameters
  - four channel electrical stimulator
    - motor points of hamstrings and quadriceps
    - 6 second per contraction, no ramp, pulse width of 450ms, frequency of 40 Hz
Pre-row Training

• **Strength Training**
  • Stimulation intensity increased with fatigue to maintain full knee extension

• Training in the FES-row technique
  • proper timing of the stimulation in the rowing stroke

• **Endurance Training**
  • intervals of FES-rowing that continually increased with the goal of 30 minutes of continuous FES-rowing 3 days/week
FES row-training

- 3 days/week
  - intensity of 75-85% of maximal heart rate
- 30 minutes per session
- FES-rowing with intervals of 3-5 minutes arms-only rowing
  - rest for the leg muscles as they fatigued
  - individualized based on the responses to FES
- FES-strength training at home on days not rowing
Outcomes

• Δ Axial stiffness at knee (CT)
  – finite element analysis
  – 3 time points
    • Baseline
    • 6 months of rowing
    • 12 months of rowing

• Secondary outcome
  – Δ BMD at knee
Assessed for eligibility (n=143)
- Excluded (n=74)
  - Not meeting inclusion criteria (n=37)
  - Declined to participate (n=18)
  - Other reasons (n=19)
Randomized (n=69)
Allocation
- Allocated to intervention [Exercise Only] (n=35)
  - Began Rowing (n=18)
  - Did not begin rowing (n=17)
    - Failure to progress to rowing: 16
    - Withdrew: 1
- Allocated to intervention [Exercise + ZA Infusion] (n=34)
  - Began rowing (n=20)
    - Received Infusion (n=18)
    - Did not begin rowing (n=16)
      - Failure to progress to rowing: 16
Follow-Up
- Lost to follow-up (n=0)
- Discontinued intervention (n=2)
  - Injury: 2
- Lost to follow-up (n=2)
  - Could not reach by phone to schedule: 2
  - Discontinued intervention (n=3)
    - Could not reach by phone to schedule: 2
    - Injury: 1
Analysis
- Analyzed (n=16)
  - Excluded from analysis (n=0)
- Analyzed (n=13)
  - Excluded from analysis (n=0)
### Results: Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Exercise only (n=16)</th>
<th>Exercise + ZA (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>15/1</td>
<td>12/1</td>
</tr>
<tr>
<td>Age</td>
<td>38 ± 3</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 ± 1.4</td>
<td>24.0 ± 1.5</td>
</tr>
<tr>
<td>Age at injury</td>
<td>25 ± 2</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Injury duration (years)</td>
<td>13 ± 3</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Injury severity (A/B/C)</td>
<td>9/3/4</td>
<td>9/3/1</td>
</tr>
<tr>
<td>25OH Vitamin D</td>
<td>26.4 ± 2.3</td>
<td>34.3 ± 2.5*</td>
</tr>
<tr>
<td>Tibia BMD</td>
<td>0.79 ± 0.07</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td>Femur BMD</td>
<td>0.77 ± 0.08</td>
<td>0.76 ± 0.09</td>
</tr>
</tbody>
</table>

* p=0.03
Rowing Compliance

- On average, volunteers exercised 1.6 + 0.1 times a week
- Total exercise varied from none to ~2000 watts x minutes
Adverse Events

- No serious, study related adverse events
- 45% in ZA arm experienced an acute phase reaction
- No one developed atrial fibrillation due to the infusion
- The majority of active rowers experienced non-serious, expected musculoskeletal symptoms (back, shoulder, wrist, elbow pain, increased spasticity) or minor skin irritation
- 1 participant reported spontaneous ejaculation with electrical stimulation
Change in Fibular Stiffness: ZA Effect

- **Fibula**
  - Stiffness (MPa)
  - Time (days)
  - Treatment x Time p = 0.01

- **ΔStiffness (MPa)**
  - Time (days)
  - Treatment x Time p < 0.01
FES-rowing as Effective as ZA
Dose Effect of FES-row training on bone stiffness

Mild: 0 – 27 watts x min
Moderate: 27 – 344 watts x min
High: 344 – 2000 watts x min

Bone x exercise interaction
$p = 0.03$
50% Increase Tibial Axial Stiffness

Total Watt Minutes

Months of Rowing

Tibial Stiffness kN/mm^2

Baseline
End of Study
9.7% Decrease Tibial Axial Stiffness

Baseline  | End of Study

Total Watt Minutes vs. Months of Rowing

Tibial Axial Stiffness kN/mm²
A 10 mm section of the proximal tibia was segmented from the surrounding tissue by thresholding followed by region growing. The model was meshed with tetrahedral elements and linear elastic material properties for bone were assigned to the model (Young’s Modulus, E, of 17kGPa and a Poisson’s ratio of 0.3).
Conclusions

• Zoledronic Acid prevents decline in bone strength (axial stiffness) of the paralyzed lower extremity

• FES-rowing is as effective as zoledronic acid at preventing this decline

• FES-rowing alone increases bone strength in a dose-dependent fashion

Regenerate bone
(YES, this is possible after SCI)
Need for novel bone biomarkers/therapeutic targets in SCI

- Bone density by DXA is gold standard
  - Few facilities are accessible
  - Few have knee software
- Fracture risk prediction
- Monitor response to therapy
Technically limited scans
Identification of bone biomarkers in SCI

- Which molecule/s mediate the bone response to mechanical loading?
- Is one/more a candidate biomarker of osteoporosis/ fracture risk in SCI?
Bone-fat interactions

Adipokines as biomarkers/therapeutic targets in SCI?

Osteocalcin stimulates expression of adiponectin

Adipocyte derived hormones regulate bone cell activities
Adiponectin Is a Candidate Biomarker of Lower Extremity Bone Density in Men With Chronic Spinal Cord Injury

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3Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA, USA
4VA Cooperative Studies Program, VA Boston Healthcare System, Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA
5Primary Care and Rheumatology Sections, VA Boston Healthcare System and Boston University School of Medicine, Boston, MA, USA
6Pulmonary and Critical Care Medicine Section, Medical Service, VA Boston Healthcare System, Boston, MA, USA
7Channing Division of Network Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA
8Department of Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA, USA

ABSTRACT

Adipose tissue is a major regulator of bone metabolism and in the general population obesity has generally been associated with greater bone mineral density (BMD). However, bone-fat interactions are multifactorial, and may involve pathways that influence both bone formation and resorption with competing effects on the skeleton. One such pathway involves adipocyte production of adipokines that regulate bone metabolism. In this study we determined the association between BMD, walking status, and circulating adipokines (adiponectin and leptin) in 149 men with chronic spinal cord injury (SCI). Although adipokine levels did not vary significantly based on walking status, there was a significant inverse association between adiponectin and BMD in wheelchair users independent of body...
Boston SCI-Health Study

• enrollment began 2009
  • testing performed every 1½ years
• through May 2014 enrolled 356 subjects with chronic SCI
  • 13 withdrawn from the study
  • 10 have moved away
  • 3 are lost to follow-up
  • 8 have died
  • 3 were unable to participate further due to memory loss
• 50 participants without SCI
  • same testing as those with SCI.
Boston SCI-Health Study

- Biorepository
- Image archive
- Participant characteristics
Study Design

Boston SCI-Health Study
N=196
22 years or older
One year post injury or more
Not ventilator dependent
No tracheostomy
No other neurological condition

Exclusions:
Female (n = 29)
SCI < 5 years post-injury (n= 18)

Adiponectin & Bone Health Sub Study
N=149
BMD by DXA
Circulating adiponectin, leptin, vitamin d, markers of bone turnover
Association between Adiponectin and BMD by Walking Status

<table>
<thead>
<tr>
<th>Adiponectin (ng/ml)</th>
<th>Bone Mineral Density (g/cm²)</th>
<th>B ± SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Walkers</strong></td>
<td></td>
<td></td>
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<tr>
<td>Unadjusted</td>
<td>-7.64 x 10⁻⁷ ± 5.32 x 10⁻⁶</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted*</td>
<td>-4.6 x 10⁻⁶ ± 4.96 x 10⁻⁶</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td><strong>Wheelchair Users</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-1.79 x 10⁻⁵ ± 4.94 x 10⁻⁶</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Fully adjusted**</td>
<td>-1.97 x 10⁻⁵ ± 4.97 x 10⁻⁶</td>
<td>0.0004</td>
<td></td>
</tr>
</tbody>
</table>

* adjusted for lean mass (kg)
** adjusted for age

Doherty et al, JBMR. 2014
<table>
<thead>
<tr>
<th>Adiponectin Quartiles (ng/ml)</th>
<th>Walkers</th>
<th>Wheelchair Users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean BMD (SE)</td>
<td>P for trend</td>
</tr>
<tr>
<td>Quartile 1 (≤3350)</td>
<td>0.87 ± 0.032</td>
<td>0.87*</td>
</tr>
<tr>
<td>Quartile 2 (3350 ≤ 4916)</td>
<td>0.901 ± 0.031</td>
<td></td>
</tr>
<tr>
<td>Quartile 3 (4916 ≤7336)</td>
<td>0.852 ± 0.031</td>
<td></td>
</tr>
<tr>
<td>Quartile 4 (&gt;7336)</td>
<td>0.910 ± 0.035</td>
<td></td>
</tr>
</tbody>
</table>

* adjusted for lean mass (kg)
** adjusted for age

Doherty *et al*, JBMR. 2014
In this study of 149 adults with chronic SCI:

- Adiponectin levels did not vary based on walking status
- Fat-bone associations varied based on walking status
- In the absence of mechanical loading, adiponectin contributes to ongoing bone loss
Adiponectin as biomarker of bone strength or fracture risk?

- **Hypothesis**
  - circulating adiponectin levels will be negatively associated with axial stiffness at the knee
  - People with a history of Post-SCI osteoporotic fracture will have greater adiponectin levels
Adiponectin is associated with bone strength and fracture history in paralyzed men with spinal cord injury

C. O. Tan · R. A. Battaglino · A. L. Doherty · R. Gupta · A. A. Lazzari · E. Garshick · R. Zafonte · L. R. Morse

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Abstract
Summary We explored the association between adiponectin levels and bone strength in paralyzed men with spinal cord injury. We found that bone strength was inversely associated with circulating adiponectin levels. Thus, strength estimates and adiponectin levels may improve fracture risk prediction and detection of response to osteogenic therapies following spinal cord injury.
Purpose Previous research has demonstrated an inverse relationship between circulating adiponectin and bone mineral density, suggesting that adiponectin may be used as a biomarker for bone health. However, this relationship may reflect indirect effects on bone metabolism via adipose-mediated mechanical pathways rather than the direct effects of adipokines on bone metabolism. Thus, we explored the association between circulating adiponectin levels and bone strength in 27 men with spinal cord injury.
Methods Plasma adiponectin levels were quantified by ELISA assay. Axial stiffness and maximal load to fracture of the distal femur were quantified via finite element analysis using reconstructed 3D models of volumetric CT scans. We
Study Design

FES-rowing clinical trial in SCI
N=33
October 2010–October 2013
18 years or older
C4 or lower SCI (ASIA A, B or C)
Non-ambulatory

Exclusions:
Females (n=5)
Bisphosphonate use within 1 year of testing (n=1)

Adiponectin & Bone Health Sub Study
N=27
Bone strength by CT
Circulating adiponectin, leptin, vitamin d, markers of bone turnover
### Association between Adiponectin and Bone Strength

<table>
<thead>
<tr>
<th></th>
<th>β ± SE</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Distal femur stiffness (MPa</em>)</em>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>-0.013 ± 0.005</td>
<td>0.44</td>
</tr>
<tr>
<td>Injury duration (years)</td>
<td>-1.707 ± 0.756</td>
<td></td>
</tr>
<tr>
<td><strong>Distal femur maximal load (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>-0.00719 ± 0.003</td>
<td>0.58</td>
</tr>
<tr>
<td>Injury duration (years)</td>
<td>-0.995 ± 0.55</td>
<td></td>
</tr>
<tr>
<td>Lower extremity lean mass (kg)</td>
<td>3.855 ± 1.74</td>
<td></td>
</tr>
</tbody>
</table>

* MPa=megapascal
## Factors Associated with History of Post-SCI Osteoporotic Fracture

<table>
<thead>
<tr>
<th></th>
<th>Fracture (n=6)</th>
<th>No fracture (n=21)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone mineral density (BMD) (g/cm²)</strong> [mean ± SD]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCI-specific skeletal sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Distal femur</td>
<td>0.458 ± 0.10</td>
<td>0.797 ± 0.25</td>
<td>0.007</td>
</tr>
<tr>
<td>• Proximal tibia</td>
<td>0.453 ± 0.10</td>
<td>0.809 ± 0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Traditional skeletal sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Total hip</td>
<td>0.621 ± 0.15</td>
<td>0.835 ± 0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>• Femur neck</td>
<td>0.646 ± 0.15</td>
<td>0.856 ± 0.24</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Hip bone density classification</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal BMD</td>
<td>0 (0.0%)</td>
<td>10 (47.6%)</td>
<td>0.06</td>
</tr>
<tr>
<td>• Osteopenia/Osteoporosis/BMD lower than expected for age</td>
<td>6 (100.0%)</td>
<td>11 (52.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Distal femur strength</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Distal femur stiffness (MPa**)</td>
<td>99.5 ± 56.3</td>
<td>160.6 ± 49.0</td>
<td>0.01</td>
</tr>
<tr>
<td>• Distal femur maximal load (kg)</td>
<td>38.4 ± 14.6</td>
<td>91.1 ± 40.8</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>5656.7 ± 3003.3</td>
<td>3802.2 ± 1380.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>20.47 ± 3.1</td>
<td>22.46 ± 1.6</td>
<td>0.57</td>
</tr>
<tr>
<td>CTX (ng/mL)</td>
<td>0.307 ± 0.09</td>
<td>0.398 ± 0.05</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*based on Z- or T-score at the total hip or femoral neck

** MPa=megapascal
Summary

- In this study of 27 non-ambulatory males with SCI:
  - Inverse relationship between adiponectin and distal femur axial stiffness/maximal load
  - Adiponectin, injury duration, and leg lean mass (maximal load) explain 44-58% variation in bone strength at the distal femur
  - Adiponectin levels were significantly greater in subjects with a history of post-SCI osteoporotic fracture
Sclerostin mediates bone response to mechanical unloading

Qualities of a good biomarker

Accessible

Quantifiable

Specific
Hypothesis

- In humans, mechanical unloading of bone occurs in conditions that cause paralysis.

- The association between sclerostin and bone loss is expected to be strongest in conditions like spinal cord injury.

- *Motor Complete SCI will be associated with high sclerostin levels compared to incomplete SCI and No SCI.*
Association Between Sclerostin and Bone Density in Chronic Spinal Cord Injury

Leslie R Morse,¹,²,³,⁴ Supreetha Sudhakar,² Valery Danilack,⁵ Carlos Tun,⁶ Antonio Lazzari,⁷ David R Gagnon,⁸ Eric Garshick,⁹,¹⁰ and Ricardo A Battaglino³,¹¹

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E-mail: rbattaglino@forsyth.org
DOI: 10.1002/jbmr.546
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Study Design

Boston SCI-Health Study
N=196
SCI: 22 years or older
one year post injury or more
no other neurological condition

Sclerostin pilot study (N=49)
convenience sample of men
39 with SCI
10 No SCI

BMD by DXA
Circulating sclerostin levels

Morse et al, JBMR. 2011 Oct 17
Circulating Sclerostin increases with age in chronic SCI

Morse et al, JBMR. 2011 Oct 17
<table>
<thead>
<tr>
<th></th>
<th>SCI (wheelchair)</th>
<th>SCI (no wheelchair)</th>
<th>No SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=30</td>
<td>n=9</td>
<td>n=10</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted Sclerostin (pmol/L)</td>
<td>49.60</td>
<td>77.23</td>
<td>79.17</td>
</tr>
</tbody>
</table>
Positive association between BMD and sclerostin

Morse et al, JBMR. 2011 Oct 17
Conclusions

• Sclerostin maybe a *biomarker* of osteoporosis severity, not a *mediator* of ongoing bone loss, in *chronic* paraplegia

• This is in contrast to the *acute* sclerostin-mediated bone loss demonstrated in animal models of mechanical unloading where high sclerostin levels suppress bone formation

Morse *et al*, JBMR. 2011 Oct 17
Circulating sclerostin is elevated in short-term and reduced in long-term SCI☆

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c Spaulding-Harvard SCI Model System, Spaulding Rehabilitation Hospital, Boston, MA, USA
d Primary Care and Rheumatology Sections, VA Boston Healthcare System and Boston University School of Medicine, Boston, MA, USA
e Pulmonary and Critical Care Medicine Section, Medical Service, VA Boston Healthcare System, Boston, MA, USA
f Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA
g Department of Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA, USA
Study Design

Boston SCI-Health Study
N=196
SCI: 22 years or older
one year post injury or more
no other neurological condition

Excluded:
29 women
12 men taking bisphosphonates

Sclerostin Sub Study (N=155)

BMD by DXA
Circulating sclerostin, vitamin d, markers of bone turnover
Sclerostin is high in *acute* SCI

Battaglino *et al*, Bone. 2012
Clinical factors associated with sclerostin

- Varied based on chronicity of injury
  - Short term ($\leq 5$ years)
  - Long term ($>5$ years)
Short-term SCI: Vitamin D is associated with sclerostin

Association between sclerostin and vitamin D status, adjusted for injury duration, in short-term SCI (≤5 years).

<table>
<thead>
<tr>
<th></th>
<th>$R^2$</th>
<th>$\beta \pm SE$</th>
<th>$e^\beta$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury duration (years)</td>
<td>0.49</td>
<td>$-0.33 \pm 0.14$</td>
<td>0.72</td>
<td>0.03</td>
</tr>
<tr>
<td>ln sclerostin</td>
<td></td>
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<tr>
<td>LS means ± SE</td>
<td></td>
<td></td>
<td></td>
<td>p</td>
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</tbody>
</table>

Vitamin D status

- Normal ($\geq 20$ ng/mL)  
  $4.51 \pm 0.18$  
  91.32  
  0.02

- Deficient (<20 ng/mL)  
  $3.76 \pm 0.22$  
  42.78

Battaglino et al, Bone. 2012
Long-term SCI: BMD is associated with sclerostin

Association between sclerostin and bone density or osteoporosis diagnosis, adjusted for age, in long-term SCI (≥5 years).

<table>
<thead>
<tr>
<th>Model A (R² = 0.35)</th>
<th>N</th>
<th>β ± SE</th>
<th>eβ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>134</td>
<td>0.01 ± 0.003</td>
<td>1.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tibia BMD (g/cm²)</td>
<td></td>
<td>0.70 ± 0.14</td>
<td>2.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model B (R² = 0.40)</th>
<th>N</th>
<th>β ± SE</th>
<th>eβ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ln sclerostin</td>
<td>129</td>
<td>0.01 ± 0.003</td>
<td>1.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Osteoporosis diagnosis</th>
<th>e(ln sclerostin)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.505</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>6.628</td>
<td>0.51</td>
</tr>
<tr>
<td>Osteoporosis/BMD lower than expected for age/gender</td>
<td>4.585</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Battaglino et al, Bone. 2012
Conclusions

- Sclerostin is *increased* after SCI in response to mechanical unloading

- This response is time limited
A dual role for sclerostin after SCI?

- Therapeutic target in acute SCI
  - anti-sclerostin antibody on the horizon

- Biomarker of osteoporosis severity in chronic SCI
Could sclerostin be a good biomarker?

- Sclerostin is present in blood
- It can be detected and quantified reproducibly
- Circulating level is associated with BMD and BMC
- Sclerostin is exclusively made by osteocytes
  - Osteocytes compose 90% to 95% of all bone cells in adult bone
  - Osteocytes are the longest lived bone cell: up to decades within the mineralized matrix
Hypothesis

• Sclerostin is a good biomarker of osteoporosis severity

• Produced only by osteocytes, it will be a better biomarker than other circulating proteins modulating bone metabolism or markers of bone turnover
  – RANKL, OPG, CTX, osteocalcin
Sclerostin: a candidate biomarker of SCI-induced osteoporosis

L. R. Morse • S. Sudhakar • A. A. Lazzari • C. Tun • E. Garshick • R. Zafonte • R. A. Battaglino

Received: 12 April 2012 / Accepted: 25 May 2012
© International Osteoporosis Foundation and National Osteoporosis Foundation 2012

Abstract
Summary We assessed several circulating proteins as candidate biomarkers of bone status in men with chronic spinal cord injury. We report that sclerostin is significantly associated with bone mineral content and bone density at all skeletal sites tested. We found no association between bone

Methods We assessed the relationship between bone mineral content or bone density and the following circulating bone-related proteins: sclerostin, DKK-1, soluble receptor activator of nuclear factor kappa B ligand, osteoprotegerin, osteocalcin, and e-telopeptide in 39 men with chronic SCI and 10 men with no SCI.
RANKL and OPG

Morse et al, Osteoporosis International, 2012
Sclerostin and DKK-1

LEG

ARM

R^2=0.33  
p=0.0002  

R^2=0.09  
p=0.004  

R^2=0.000025  
p=0.97  

R^2=0.00050  
p=0.88  

Morse et al, Osteoporosis International, 2012
### Association between BMD and sclerostin (adjusted for age)

<table>
<thead>
<tr>
<th>Skeletal Site</th>
<th>$R^2$</th>
<th>$\beta \pm SE$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCI-specific sites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg BMC (n=48)</td>
<td>0.33</td>
<td>187.25 ± 46.96</td>
<td>0.0002</td>
</tr>
<tr>
<td>Arm BMC (n=48)</td>
<td>0.18</td>
<td>52.28 ± 17.14</td>
<td>0.0038</td>
</tr>
<tr>
<td>Distal femur (n=47)</td>
<td>0.23</td>
<td>0.13 ± 0.04</td>
<td>0.0057</td>
</tr>
<tr>
<td>Proximal tibia (n=47)</td>
<td>0.28</td>
<td>0.19 ± 0.05</td>
<td>0.0008</td>
</tr>
<tr>
<td><strong>Traditional sites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hip (n=46)</td>
<td>0.30</td>
<td>0.11 ± 0.04</td>
<td>0.0094</td>
</tr>
<tr>
<td>Femoral neck (n=46)</td>
<td>0.26</td>
<td>0.10 ± 0.04</td>
<td>0.0071</td>
</tr>
<tr>
<td>Radius (n=39)</td>
<td>0.13</td>
<td>0.04 ± 0.02</td>
<td>0.0262</td>
</tr>
</tbody>
</table>

Morse *et al*, Osteoporosis International, 2012
Sclerostin-Mediated Bone Loss Following SCI

- α-Sclerostin antibodies
- Physical therapies

Thank you
Future Directions/Other Active Projects

Dental Pulp Stem Cells Promote Neurorecovery after SCI

- Severe SCI + dental pulp stem cell transplantation (n=15)
- Severe SCI (n=9)
Clinical Vignette

22 YO male with C5 ASIA B SCI due to ATV accident 18 months prior to visit

Uses manual wheelchair with power assist

Does no walking, reports no weight-bearing therapies

Takes alendronate 70 mg weekly since injury, but reports poor compliance (taking every 2-3 weeks instead of weekly)
## Clinical Vignette

<table>
<thead>
<tr>
<th>Left Femur</th>
<th>Date</th>
<th>Age</th>
<th>Z-score</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>07/09/2015</td>
<td>22.4</td>
<td>0.4</td>
<td>1.152 g/cm²</td>
</tr>
<tr>
<td>Total</td>
<td>07/09/2015</td>
<td>22.4</td>
<td>-0.4</td>
<td>1.055 g/cm²</td>
</tr>
<tr>
<td>Radius 33%</td>
<td>07/09/2015</td>
<td>22.4</td>
<td>-1.7</td>
<td>0.821 g/cm²</td>
</tr>
</tbody>
</table>

**Fracture risk factors in disuse osteoporosis:**
- Age at injury less than 16: no
- Female gender: no
- Motor complete injury: yes
- Paraplegia: no
- Duration of injury 10 years or more: no
- Alcohol use more than 5 units per day: no
- BMI less than 19: no
- Prior fragility fracture: no
- Parental history of fragility fracture: no
- Risk factors: 1
Hybrid-FES Exercise to Prevent Cardiovascular Declines in Acute Spinal Cord Injury  
NIH/ NHLBI  R01HL117037-01A1 (Taylor, PI)

Had been rowing 3X/week for 1 year, high compliance
CTX and Osteocalcin

**LEG**

- $R^2=0.0007$
- $p=0.86$

**ARM**

- $R^2=0.04$
- $p=0.18$

- $R^2=0.01$
- $p=0.48$

Morse *et al*, Osteoporosis International, 2012
SCI-Induced Autoimmunity

**Acute SCI**
- B cell infiltration
- Release of CNS antigens (myelin, axon debris)
- B cell activation
- B cell proliferation and differentiation

**Chronic SCI**
- Lesion expansion
- B cells accumulation
- B cell re-infiltration
- Ig flooding
- IgG and IgM production

*Trends Immunol. 2010 September; 31(9): 332–338*
B cell mediated neurotoxicity

- B cell activation leads to increased synthesis of auto-antibodies
- B cell-deficient mice experience greater locomotor recovery and reduction in lesion pathology than WT mice
- Intra-spinal injection of antibodies purified from SCI mice causes hind limb paralysis and neuropathology in uninjured mice

- **Production of auto-antibodies by B cells mediates neurological outcomes after SCI**
B-Cell Maturation Antigen, A Proliferation-Inducing Ligand, and B-Cell Activating Factor Are Candidate Mediators of Spinal Cord Injury-Induced Autoimmunity

Jonah W. Saltzman, Ricardo A. Battaglino, Lois Salles, Prateek Jha, Supreetha Sudhakar, Eric Garshick, Helen L. Stott, Ross Zafonte, and Leslie R. Morse

Abstract

Autoimmunity is thought to contribute to poor neurological outcomes after spinal cord injury (SCI). There are few mechanism-based therapies, however, designed to reduce tissue damage and neurotoxicity after SCI because the molecular and cellular bases for SCI-induced autoimmunity are not completely understood. Recent groundbreaking studies in rodents indicate that B cells are responsible for SCI-induced autoimmunity. This novel paradigm, if confirmed in humans, could aid in the design of neuroprotective immunotherapies. The aim of this study was to investigate the molecular signaling pathways and mechanisms by which autoimmunity is induced after SCI, with the goal of identifying potential targets in therapies designed to reduce tissue damage and inflammation in the chronic phase of SCI. To that end, we performed an exploratory microarray analysis of peripheral blood mononuclear cells to identify differentially expressed genes in chronic SCI. We identified a gene network associated with lymphoid tissue structure and development that was composed of 29 distinct molecules and five protein complexes, including two cytokines, a proliferation-inducing ligand (APRIL) and B-cell–activating factor (BAFF), and one receptor, B-cell maturation antigen (BMCA) involved in B cell development, proliferation, activation, and survival. Real-time polymerase chain reaction analysis from ribonucleic acid samples confirmed upregulation of these three genes in SCI. To our knowledge, this is the first report that peripheral blood mononuclear cells produce increased levels of BAFF and APRIL in chronic SCI. This finding provides evidence of systemic regulation of SCI-autoimmunity via APRIL and BAFF mediated activation of B cells through BMCA and points toward these molecules as potential targets of therapies designed to reduce neuroinflammation after SCI.
A network of proteins related to B cell proliferation and activation
Correlation Between Bone Density and Distal Femur Stiffness or Maximal Load

<table>
<thead>
<tr>
<th>Bone Density (g/cm²)</th>
<th>Distal Femur Stiffness (MPa*)</th>
<th>Distal Femur Maximal Load (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$-value</td>
</tr>
<tr>
<td>Distal femur</td>
<td>0.58</td>
<td>0.002</td>
</tr>
<tr>
<td>Proximal tibia</td>
<td>0.52</td>
<td>0.007</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.40</td>
<td>0.04</td>
</tr>
<tr>
<td>Total hip</td>
<td>0.35</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* MPa=megapascal
### Univariate Predictors of Bone Strength

<table>
<thead>
<tr>
<th>Variables</th>
<th><strong>β ± SE</strong></th>
<th><strong>p-value</strong></th>
<th><strong>β ± SE</strong></th>
<th><strong>p-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.049 ± 0.972</td>
<td>0.96</td>
<td>0.110 ± 0.741</td>
<td>0.88</td>
</tr>
<tr>
<td>Injury duration (years)</td>
<td>-2.487 ± 0.815</td>
<td>0.005</td>
<td>-1.967 ± 0.613</td>
<td>0.004</td>
</tr>
<tr>
<td>25 OH Vitamin D (ng/mL)</td>
<td>-0.096 ± 1.145</td>
<td>0.93</td>
<td>0.434 ± 0.870</td>
<td>0.62</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>2.653 ± 1.734</td>
<td>0.14</td>
<td>2.823 ± 1.263</td>
<td>0.03</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>1.331 ± 1.028</td>
<td>0.21</td>
<td>1.905 ± 0.710</td>
<td>0.01</td>
</tr>
<tr>
<td>Total mass (kg)</td>
<td>0.533 ± 0.532</td>
<td>0.33</td>
<td>0.706 ± 0.388</td>
<td>0.08</td>
</tr>
<tr>
<td>Lower extremity lean mass (kg)</td>
<td>6.310 ± 2.5</td>
<td>0.02</td>
<td>6.711 ± 1.7</td>
<td>0.0005</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>1.136 ± 1.481</td>
<td>0.45</td>
<td>1.118 ± 1.120</td>
<td>0.33</td>
</tr>
<tr>
<td>C-telopeptide (ng/mL)</td>
<td>36.34 ± 49.61</td>
<td>0.47</td>
<td>36.81 ± 37.54</td>
<td>0.34</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>-0.016 ± 0.005</td>
<td>0.002</td>
<td>-0.013 ± 0.004</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* MPa=megapascal
### B cell-related gene network

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Fold Change</th>
<th>p-value</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAB3C</td>
<td>RAB3C, member RAS oncogene family</td>
<td>-1.086</td>
<td>1.454E-02</td>
<td>enzyme</td>
</tr>
<tr>
<td>RHCE/RHD</td>
<td>Rh blood group, D antigen</td>
<td>-1.389</td>
<td>3.200E-02</td>
<td>transporter</td>
</tr>
<tr>
<td>SCLY</td>
<td>selenocysteine lyase</td>
<td>1.119</td>
<td>4.896E-02</td>
<td>enzyme</td>
</tr>
<tr>
<td>SPTB</td>
<td>spectrin, beta, erythrocytic</td>
<td>-1.193</td>
<td>1.134E-02</td>
<td>other</td>
</tr>
<tr>
<td>STK10</td>
<td>serine/threonine kinase 10</td>
<td>-1.198</td>
<td>2.931E-02</td>
<td>kinase</td>
</tr>
<tr>
<td>TK2</td>
<td>thymidine kinase 2, mitochondrial</td>
<td>1.245</td>
<td>1.057E-02</td>
<td>kinase</td>
</tr>
<tr>
<td>TNFRSF10A</td>
<td>tumor necrosis factor receptor superfamily, member 10a</td>
<td>-1.180</td>
<td>1.851E-02</td>
<td>transmembrane receptor</td>
</tr>
<tr>
<td>TNFRSF10B</td>
<td>tumor necrosis factor receptor superfamily, member 10b</td>
<td>1.198</td>
<td>1.090E-02</td>
<td>transmembrane receptor</td>
</tr>
<tr>
<td>TNFRSF17</td>
<td>tumor necrosis factor receptor superfamily, member 17</td>
<td>3.129</td>
<td>3.863E-03</td>
<td>transmembrane receptor</td>
</tr>
<tr>
<td>TNFRSF25</td>
<td>tumor necrosis factor receptor superfamily, member 25</td>
<td>-1.172</td>
<td>4.633E-02</td>
<td>transmembrane receptor</td>
</tr>
<tr>
<td>TNFSF13</td>
<td>tumor necrosis factor (ligand) superfamily, member 13</td>
<td>1.329</td>
<td>2.769E-02</td>
<td>cytokine</td>
</tr>
<tr>
<td>TNFSF13B</td>
<td>tumor necrosis factor (ligand) superfamily, member 13b</td>
<td>1.234</td>
<td>1.131E-03</td>
<td>cytokine</td>
</tr>
<tr>
<td>Trail-R</td>
<td>pathway</td>
<td>n/a</td>
<td>n/a</td>
<td>group</td>
</tr>
<tr>
<td>tyrosine kinase</td>
<td>group</td>
<td>n/a</td>
<td>n/a</td>
<td>group</td>
</tr>
<tr>
<td>WNT10A</td>
<td>wingless-type MMTV integration site family, member 10A</td>
<td>-1.322</td>
<td>1.154E-02</td>
<td>other</td>
</tr>
</tbody>
</table>
qPCR validation

- BCMA: 6.7
- APRIL: 2.6
- BAFF/BLyS: 2.8

* p < 0.05
Summary

• B cell-related mechanism of post-SCI autoimmunity is mediated in part by up-regulation of genes coding for BCMA, BAFF, and APRIL proteins.

• Molecules participating in this mechanism, including BCMA, BAFF, and APRIL, should be investigated as novel therapeutic targets to minimize chronic antibody mediated neurotoxicity following SCI.
Circulating BAFF varies by time post-injury.

p = 0.03

$r^2 = 0.21$
Fenbendazole improves pathological and functional recovery following traumatic spinal cord injury

Abstract During a study of spinal cord injury (SCI), mice in our colony were treated with the anthelmintic fenbendazole to treat pinworms detected in other mice not involved in the study. As this was not part of the original experimental design, we subsequently compared ...

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Support

- NICHD [R21HD057030 and R21HD057030-02S1, PI Morse]
- NIAMS [1R01AR059270, PI Morse]
- Department of Defense [W81XWH-10-1-1043, Morse PI]
- Office of Research and Development, Rehabilitation Research and Development [Merit Review Grant B6618R, PI Garshick]
• Develop evidence based clinical paradigm for SCI-induced osteoporosis
  – Improve diagnosis
  – Improve fracture risk prediction
• Develop effect therapies to prevent and treat SCI-induced osteoporosis
• Improve understanding of basic pathophysiology leading to SCI-induced osteoporosis
  – Develop novel mechanism based biomarkers and therapeutic targets