Animal Models & Bone Histomorphometry:

Translational Research for the Human Research Program

Jean D. Sibonga, Ph.D.
Lead, Bone Discipline, Human Research Program
Bone Scientist, Human Health Countermeasures
NASA Johnson Space Center
June 1, 2010
Overview

• Bone Histomorphometry as a Research Tool
• Recommendation to Human Research Program by Standing Review Panel [SRP]
• Translational Research – Why animal models?
• Examples & Relevance to HRP Gaps
• Closing Remarks
What’s a histomorphometrist and how did one end up in the space program?
Bone Histo-morphometry as an Analysis Tool

"...when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; ..."

Lord Kelvin, engineer and mathematical physicist

"To measure is to know."
Iliac crest bone biopsy

Fig. 5. Positioning of guide sleeve and obturator at iliac biopsy site.

Bone Marrow

Cortical or “compact” bone

Cancellous “trabecular” bone

Bone Histomorphometry as a Clinical Test

- Invasive - Iliac crest easily accessible
- Metabolically active site - earlier detection of metabolic events and diagnosis of metabolic bone disease
- “Pattern of numbers” referenced to normal values
...as a Clinical Test

- Not prescribed much (can treat based upon biochemistry)

- Gold standard for osteomalacia and renal osteodystrophy diagnosis

- Measures bone remodeling directly in tissue – valuable research tool. (Lord Kelvin)
Bone remodeling is the process by which the adult skeleton renews and repairs itself.
Bone Remodeling: Removing and replacing bone in discrete “packets” on the same bone surface in a specific sequence of cell activities.
Histology: Bone Remodeling Unit

- Osteoblasts
- Osteoid Matrix
- Osteocytes
- Bone Marrow
- Mineralized Bone
Static Histomorphometry: Direct measures of cells in tissue.

Bone Formation

Bone Resorption
Dynamic histomorphometry: Tetracycline labeling to calculate rates of remodeling

High Rate of Remodeling with Metabolic Bone Disease

Normal Rate of Remodeling
Gold Std for Osteomalacia Diagnosis – "Adult Rickets"

Mineralization defect not detectable by x-ray based imaging devices.
# Bone Histomorphometry Report (V2)

**Mayo Clinic, Rochester, Minnesota 55905, Telephone (507) 255-5946**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Difference from Normal Mean (in standard deviations)</th>
<th>Male normal mean values</th>
<th>Results</th>
<th>Z-Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Width (Cl.Wi)</td>
<td>&lt;--&gt; lower higher</td>
<td>915.6 um</td>
<td>722.3</td>
<td>-0.678</td>
</tr>
<tr>
<td>Cancellous Bone Volume (BV/TV)</td>
<td>&lt;--&gt; lower higher</td>
<td>19.7 %</td>
<td>19.5</td>
<td>-0.028</td>
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<tr>
<td>Osteoid Volume (OV)</td>
<td>&lt;--&gt; lower higher</td>
<td>0.98 %</td>
<td>0.22</td>
<td>-1.334</td>
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<tr>
<td>Osteoid Width (O.Wi)</td>
<td>&lt;--&gt; lower higher</td>
<td>11.1 um</td>
<td>11.5</td>
<td>0.131</td>
</tr>
<tr>
<td>Osteoid Surface (OS)</td>
<td>&lt;--&gt; lower higher</td>
<td>6.53 %</td>
<td>7.25</td>
<td>0.183</td>
</tr>
<tr>
<td>Osteoblast-osteoid interface (Ob.s/OS)</td>
<td>&lt;--&gt; lower higher</td>
<td>14.35 %</td>
<td>5.28</td>
<td>-0.800</td>
</tr>
<tr>
<td>Osteoclast per Length (N.Oc/B.Pm)</td>
<td>&lt;--&gt; lower higher</td>
<td>3.5/100 mm</td>
<td>7.1</td>
<td>0.691</td>
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<tr>
<td>Eroded Surface (ES)</td>
<td>&lt;--&gt; lower higher</td>
<td>1.46 %</td>
<td>0.34</td>
<td>-2.144</td>
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<tr>
<td>Single-LS (sL.Pm)</td>
<td>&lt;--&gt; lower higher</td>
<td>2.44 %</td>
<td>2.94</td>
<td>0.327</td>
</tr>
<tr>
<td>Double-LS (dL.Pm)</td>
<td>&lt;--&gt; lower higher</td>
<td>3.03 %</td>
<td>11.64</td>
<td>1.574</td>
</tr>
<tr>
<td>Mineral Apposition Rate (MAR)</td>
<td>&lt;--&gt; lower higher</td>
<td>0.89 um/day</td>
<td>0.65</td>
<td>-0.021</td>
</tr>
<tr>
<td>Bone Formation Rate - Surface Based (BFR/BS)</td>
<td>&lt;--&gt; lower higher</td>
<td>0.009 mm²/mm²/yr</td>
<td>0.041</td>
<td>1.530</td>
</tr>
<tr>
<td>Bone Formation Rate - Volume Based (BFR/BV)</td>
<td>&lt;--&gt; lower higher</td>
<td>0.131 mm²/mm²/yr</td>
<td>0.575</td>
<td>1.904</td>
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<tr>
<td>Adjusted Apposition Rate (Ajar)</td>
<td>&lt;--&gt; lower higher</td>
<td>0.16 mm²/mm²/yr</td>
<td>0.56</td>
<td>2.389</td>
</tr>
<tr>
<td>Mineralization Lag Time (MLT)</td>
<td>&lt;--&gt; lower higher</td>
<td>27.6 days</td>
<td>7.5</td>
<td>-1.908</td>
</tr>
</tbody>
</table>
Histomorphometry in Animal Models

Sensitive tool, statistical comparisons and in vivo evaluations
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Program Reviews

Evidence Base – Flight and Ground
• Science
• Clinical
• Operational experience

IOM Review

Risks

Gaps

Standing Review Panels Review

Evidence Base – Flight and Ground
• Science
• Clinical
• Operational experience

Exploration Missions & Architectures

NASA Spaceflight Human System Standards

Results and Deliverables

Customer Review

Solicitations & Directed Research

Peer Review

Integrated Research Plan
SRP Comments

- Evaluate new drugs that have improved safety profile, acceptance, efficacy, and convenience.
- Evaluate interactions among pharmaceuticals and exercise interventions
- Evaluate ... efficacy of various anti-resorptive medications, and their interaction with reduced mechanical loading.
- Are there gender differences in the time course of bone loss?
Translational Research – Why animal models as a tool for HRP?

- **Time-efficient** – results sooner, informs clinical studies, validate drug countermeasures

- **Cost-effective** – relatively less expensive, manipulate experimental design to model operations and constraints, greater statistical power

- **Invasive measures** – research measures/designs not readily applied clinically (e.g., cell signaling)

- **Predictability** – some models predictive for drug effects (e.g., FDA - OVX rat for Type 1 Op), may require multiple models to address different aspects of spaceflight

The following images courtesy of Mayo Clinic Bone, Orthopedic Research, Cell Biology & Physiology Lab.
High fidelity animal models – postmenopausal and senile osteoporosis

Animal Tissue from OVX’d rat

Clinical Bx from postmenopausal woman
Animal Model for Parathyroid Bone Disease

Tissue rat continuous infused with PTH

Clinical Bx from patient with hyperparathyroidism
Animal models - unique research observations of tissue and cells.

Fibroblast Expression of cbfa-1 Osteoblast Transcription Factor

Osteoclasts on outside (periosteal) surface of bone. Novel?
Cast immobilization in adult beagle (40 wks) predicts the changes suggested by measures from QCT imaging in astronauts.
Animal Studies: Evidence for stimulated periosteal bone apposition with mechanical stimulus.
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Evaluating “Interactive Effects” with Animal Models

Examples of Experimental Designs

Mayo Clinic Bone Cell Biology & Physiology Laboratory
Ovarian status influences the skeletal effects of tamoxifen in adult rats

Jean D. Sibonga,¹ Glenda L. Evans,¹ Eric R. Hauck,¹ Norman H. Bell² and Russell T. Turner¹²
Department of ¹Orthopedic Research and ²Biochemistry and Molecular Biology, Mayo Clinic, Rochester
MN 55905, and ³Research Service, Ralph H. Johnson Department of Veterans Affairs Medical Center
Charleston, S.C. 29401, USA

- Tamoxifen – competitive inhibitor suppresses proliferation of ER-positive breast cancer cells

- Clinical relevance of study: Should cancer therapy be given as a preventative measure to pre-menopausal females at high risk for developing breast cancer?

- Sprague Dawley Rat model for estrogen replete/deficient status; 4 groups: + ovariectomy, + tamoxifen treatment (5 months)

- 2x2 Design – test interaction between drug & menopausal status (young pre- vs. mature post-). Evaluated static and dynamic histomorphometry
Results

Significant Interaction Effects ($p < 0.05$), i.e., drug effect depends upon estrogen status.

Acts "like estrogen" in estrogen-deficient ovx'd rats and protects bone

Acts as estrogen antagonist in ovary-intact rats and fails to prevent bone loss
HRP Relevance: Use of animal model to demonstrate a side-effect of a clinical therapy, used “off-label” as a preventative, in a healthy target population.*

* Doesn’t replace validation in Flight Analog Test Bed but can inform clinical validation.
Effects of parathyroid hormone (1–34) on tibia in an adult rat model for chronic alcohol abuse

Jean D. Sibonga, Urszula T. Iwaniec, Kristen L. Shogren, Clifford J. Rosen, Russell T. Turner

- Next two reports: Parathyroid hormone (Forteo™) given in intermittent fashion will stimulate bone formation (new and only anabolic drug approved for treatment of Primary osteoporosis).
- Expensive; peptide injected sc daily* (transdermal patch, on horizon)
- Alcohol-induced bone loss - Secondary osteoporosis.
FYI: Categories of Osteoporosis

- **Primary Osteoporosis** (natural decline with aging)
  Two types:
  Type I – Postmenopausal Osteoporosis
  Type II – Senile “Age-related” Osteoporosis

- **Secondary Osteoporosis** (induced decline)
  Examples of Risk Factors:
  Glucocorticoid-induced
  Alcohol-induced
  Spaceflight-induced*
Effects of parathyroid hormone (1-34) on tibia in an adult rat model for chronic alcohol abuse.

- Clinical relevance of study: What is the effectiveness of a drug therapy in *reversing* bone loss in the continued presence of bone loss inducer?

<table>
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<tr>
<th>GROUP DESIGNATIONS</th>
<th>BONE LOSS PHASE</th>
<th>RECOVERY PHASE</th>
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<tbody>
<tr>
<td>Baseline Alcohol-Fed</td>
<td></td>
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<tr>
<td>Baseline Pair-Fed</td>
<td></td>
<td></td>
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<tr>
<td>Alcohol Withdrawal</td>
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<tr>
<td>Alcohol Withdrawal + PTH</td>
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<tr>
<td>Alcohol-Fed</td>
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<tr>
<td>Alcohol-Fed + PTH</td>
<td></td>
<td></td>
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<tr>
<td>Pair-Fed Control</td>
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</tbody>
</table>

Week #

Treatment in a Rehabilitation Clinic vs. Treatment on the street.
Results

- Drug reverses alcohol-induced loss in bone area (p<0.05), but Significant Interactive Effects (p<0.05), i.e., drug effect is attenuated in the presence of alcohol (similar response pattern in BMD).
Results

- Alcohol similarly attenuates response to PTH in cortical bone (also on BMD) by its effects on bone cells.
HRP Relevance: Use of animal model to demonstrate how a *restorative* therapy is influenced in the continued presence of risk factor for bone loss.*

* Could drug potency be reduced in mechanically unloaded state, i.e., space?
Disuse in adult male rats attenuates the bone anabolic response to a therapeutic dose of parathyroid hormone

Russell T. Turner; Satoshi Itoh; Theresa F. Hefferon; and Emily Morey-Holton

1Department of Neurology, Oregon Health & Science University, Portland, Oregon; 2Department of Orthopedics, Oregon Health & Science University, Portland, Oregon; 3Department of Aeronautics and Space Administration, National Aeronautics and Space Administration.

Abstract

Parathyroid hormone (PTH) is an important regulator of bone mass and is used in the prevention and treatment of osteoporosis. PTH stimulates bone formation by increasing osteoblast activity and inhibiting bone resorption. The effects of PTH on bone mass are enhanced by exercise, which likely occurs through the stimulation of the sympathetic nervous system. However, the effects of PTH on bone mass may be attenuated by disuse, as seen in spaceflight and gravitational loading. The current study investigated the effects of disuse on bone mass in adult male rats. The rats were subjected to hind-limb suspension or normal gravity for 2 weeks. The results showed that disuse attenuated the bone anabolic response to PTH, as evidenced by decreased bone formation rates in both cortical and cancellous bone. PTH and HU significantly increased bone formation rates in both groups, but the increase was greater in the normal gravity group. These findings have important implications for the use of PTH in the prevention and treatment of osteoporosis in astronauts.
Results

- PTH **prevents** bone loss induced by mechanical unloading.

- PTH **stimulates** bone formation in mechanically loaded environment.

![Graph showing effects of HLU and PTH on cancellous bone histomorphometry](image)

Fig. 4. Effects of HLU and PTH on cancellous bone histomorphometry. A: MAR. B: double-labeled perimeter/bone perimeter (dL/Pm/BPm). C: bone formation rate (BFR). Values are means ± SE (n = 7–10). *Bars marked differ from CON (P < 0.05). The results of the 2-way ANOVA are shown in the figure.
HPR Relevance: Use of animal model to demonstrate increased drug activity in a mechanically-loaded environment.

Is the responsiveness of bone cells to drugs affected by mechanical loading?
Does sex influence bone loss induced by simulated weightlessness (HLU)?

Six-month old Fisher 344 rats. HLU 2 weeks. Histomorphometry of tibia.

Definite sex differences in bone measures. Males had longer bones, greater cortical bone area and more separated trabeculae.

Females had greater bone formation rates, more cancellous bone and trabecular number.
Results

After 2 weeks HLU, in both males and females -
• cancellous bone was lost,
• trabecular number was decreased, and
• trabecular separation was increased.
• No change in trabecular thickness.

• In spite of the sex-specific pattern in bone loss.
Gender-specific effects on bone loss

- Weight-bearing females have greater indices of bone formation than males.
- Unloading induced significant reductions in bone formation indices regardless of sex.
HRP Relevance: Use of animal model to demonstrate the sex-specific effects of bone loss at the level of cellular mechanisms (does estrogen influence the mechanosensitivity of cells?).

Still need to consider the additive effect of risk factors that ARE sex-specific.
Summary

- Relative to spaceflight experiments, animal experiments can be manipulated to model operational issues, can provide greater n, can yield results sooner at less expense.

- Animal research can provide relevant preliminary data that can inform the design of efficient clinical experiments (e.g., reducing FAP overhead).

- Animal research enables invasive, direct measures (e.g., bone histomorphometry, mechanical testing) that can inform the interpretation of clinical results, especially those from indirect measures.
In closing, research applications for animal research for space program

- Currently: Models for Fracture Healing, Radiation Exposure, Partial Weight-bearing, Mechanical Testing

- Drug potency and efficacy in unloaded (space) condition vs. loaded (weight-bearing) conditions

- Gender effects – sex-specific pattern in age-related bone loss -- in weightless environment

- HRP “Integrative” Studies - Objective measures of combined countermeasures (drug + exercise; nutrition + drug; gonadal status)- synergistic?, additive? impaired cell signaling?
Acknowledgements

- Russell T. Turner, Ph.D. (Mayo Clinic)
- Emily Morey-Holton, Ph.D. (Ames Research Center)
- David J. Baylink, M.D. (Loma Linda University)
- Stephen A. Hodgson, M.D. (Mayo Clinic)
- Peter V. Hauschka, Ph.D. (Harvard School Dental Medicine)
- Mayo Bone Histo Lab: G. Evans, D. Jewison, J. Burgess, K. Shogren
- Judy Hayes (NASA) and Adrian LeBlanc, Ph.D. (USRA)

Thank you.