Animal Models & Bone Histomorphometry:

Translational Research for the Human Research Program

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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.
<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></td>
<td>915.6 um</td>
<td>722.3</td>
<td>-0.678</td>
</tr>
<tr>
<td>Cancellous Bone Volume (BV/TV)</td>
<td></td>
<td>19.7 %</td>
<td>19.5</td>
<td>-0.028</td>
</tr>
<tr>
<td>Osteoid Volume (OV)</td>
<td></td>
<td>0.98 %</td>
<td>0.22</td>
<td>-1.334</td>
</tr>
<tr>
<td>Osteoid Width (O.Wi)</td>
<td></td>
<td>11.1 um</td>
<td>11.5</td>
<td>0.131</td>
</tr>
<tr>
<td>Osteoid Surface (OS)</td>
<td></td>
<td>6.53 %</td>
<td>7.25</td>
<td>0.183</td>
</tr>
<tr>
<td>Osteoblast-osteoid interface (Ob.s/OS)</td>
<td></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></td>
<td>3.5 /100 mm</td>
<td>7.1</td>
<td>0.691</td>
</tr>
<tr>
<td>Eroded Surface (ES)</td>
<td></td>
<td>1.48 %</td>
<td>0.34</td>
<td>-2.144</td>
</tr>
<tr>
<td>Single-LS (eL.Pm)</td>
<td></td>
<td>2.44 %</td>
<td>2.94</td>
<td>0.327</td>
</tr>
<tr>
<td>Double-LS (dL.Pm)</td>
<td></td>
<td>3.03 %</td>
<td>11.64</td>
<td>1.574</td>
</tr>
<tr>
<td>Mineral Apposition Rate (MAR)</td>
<td></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></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></td>
<td>0.131 mm²/mm²/yr</td>
<td>0.575</td>
<td>1.904</td>
</tr>
<tr>
<td>Adjusted Apposition Rate (AjAR)</td>
<td></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></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

Risks

Gaps

IOM Review

Standing Review Panels Review

Integrated Research Plan

Results and Deliverables

Customer Review

Solicitations & Directed Research

Customer Review

NASA Spaceflight Human System Standards

Exploration Missions & Architectures

Peer Review
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
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.

Stimulation of periosteal bone occurs when bones are loaded, i.e., Periosteal (P)

Image from Dr. C. Turner, Clin Review Bone Miner Metab
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Evaluating “Interactive Effects” with Animal Models

Examples of Experimental Designs
Mayo Clinic Bone Cell Biology & Physiology Laboratory
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 a, Urszula T. Iwaniec b,*, Kristen L. Shogren c, Clifford J. Rosen d,c, Russell T. Turner b

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

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. Turnball, Satoko Inoue, Theresa E. Hefferan, and Emily Morey-Holton

1Department of Neurobiology, University of Oregon; 2Department of Orthopedics, University of Oregon; 3Department of Aeronautics and Space Administration.

Disuse in adult male rats attenuates the bone anabolic response to a therapeutic dose of parathyroid hormone

- Parathyroid hormone (PTH) is used to prevent bone loss induced by hind-limb elevation.
- 6-month old rats were treated with PTH to prevent bone loss.
- Two-weeks of hind-limb elevation decreases bone formation rates in both cortical and trabecular bone.
- PTH and HLE treatments were compared for adverse effects.
Results

- PTH prevents bone loss induced by mechanical unloading.

- PTH stimulates bone formation in mechanically loaded environment.

Fig. 4. Effects of HLU and PTH on cancellous bone histomorphometry. A: MAR. B: double-labeled perimeter/bone perimeter (dL.Pm/B.Pm). 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.
HRP 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?
Effect of gender on bone turnover in adult rats during simulated weightlessness

T. E. Hefferan, G. L. Evans, S. Lotinun, M. Zhang, E. Morey-Holton, and R. T. Turner

Departments of Orthopedics, Biochemistry, and Molecular Biology, Mayo Clinic, Rochester, Minnesota 55905; and National Aeronautics and Space Administration-Ames Research Center, Moffett Field, California 94035

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• 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.

Fig. 3. Effects of gender and hindlimb unloading on bone formation rate (BFR). Measurements were taken 1 mm from the growth plate in the proximal tibial metaphysis. Values are means ± SE. Two-way ANOVA indicates significant effects of gender ($P = 0.036$) and hindlimb unloading ($P = 0.0005$), with no interaction between the 2 variables.
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?
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