Animal Models & Bone Histomorphometry:

Translational Research for the Human Research Program

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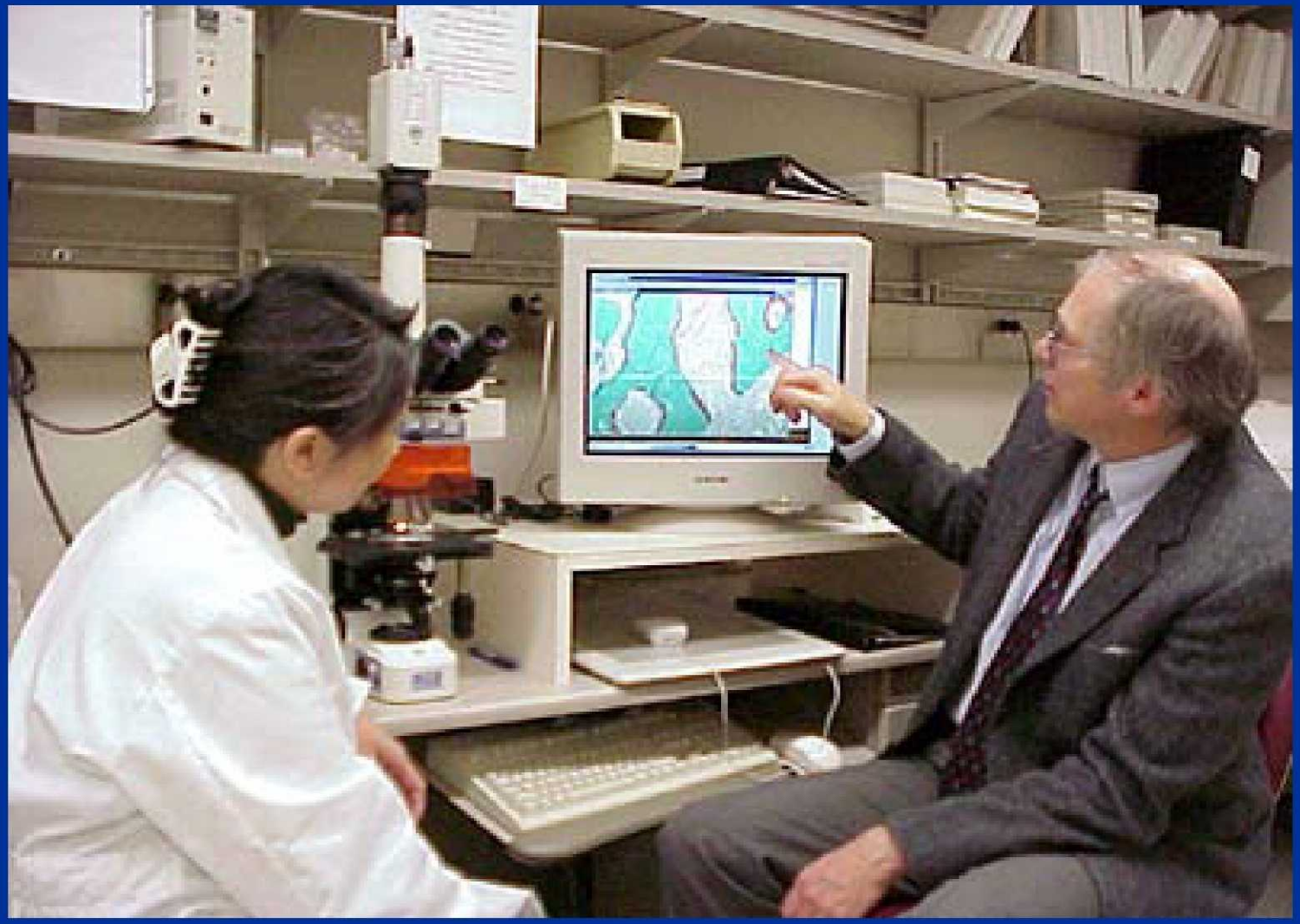
Bone Scientist, Human Health Countermeasures
NASA Johnson Space Center
June 1, 2010

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

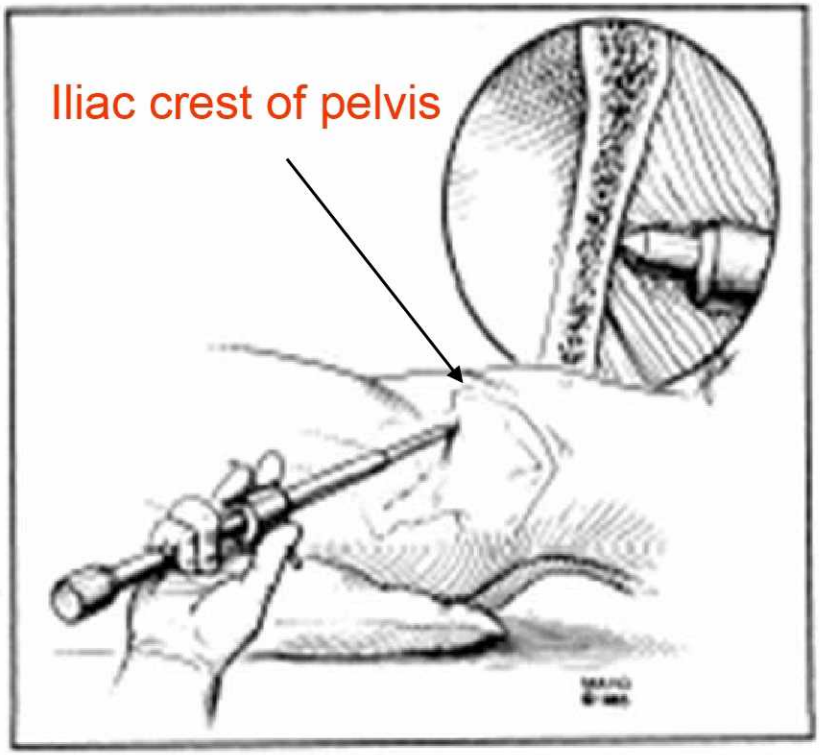
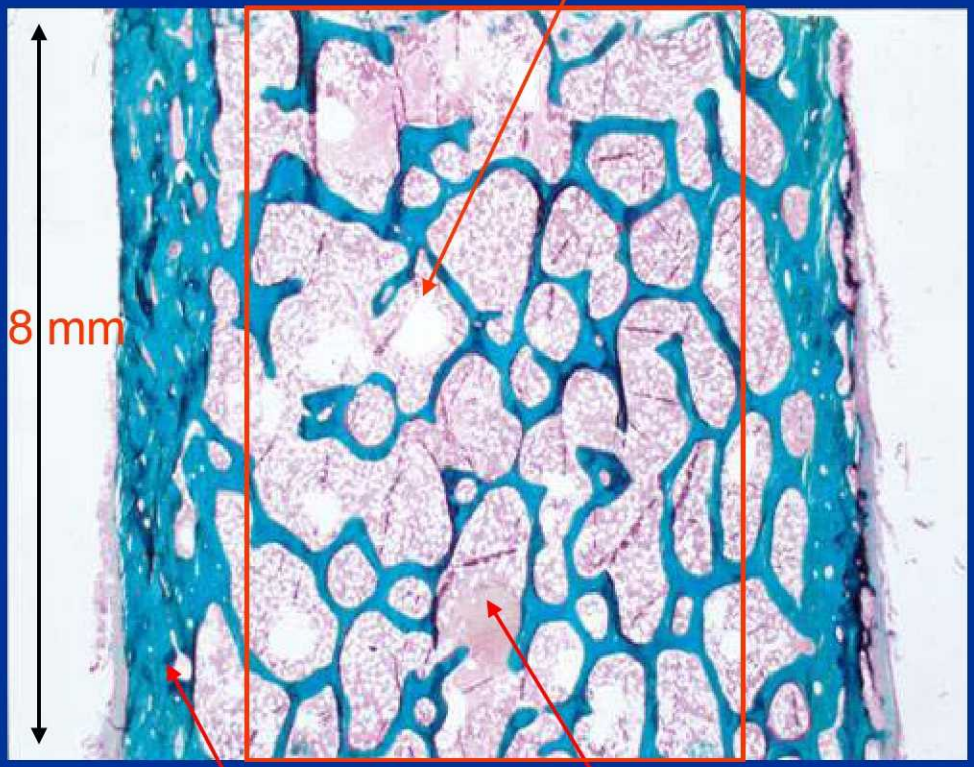


Fig. 5. Positioning of guide sleeve and obturator at iliac biopsy site.



Mayo Clinic Proceedings 61:28-31, 1986.

Bone Histomorphometry as a Clinical Test

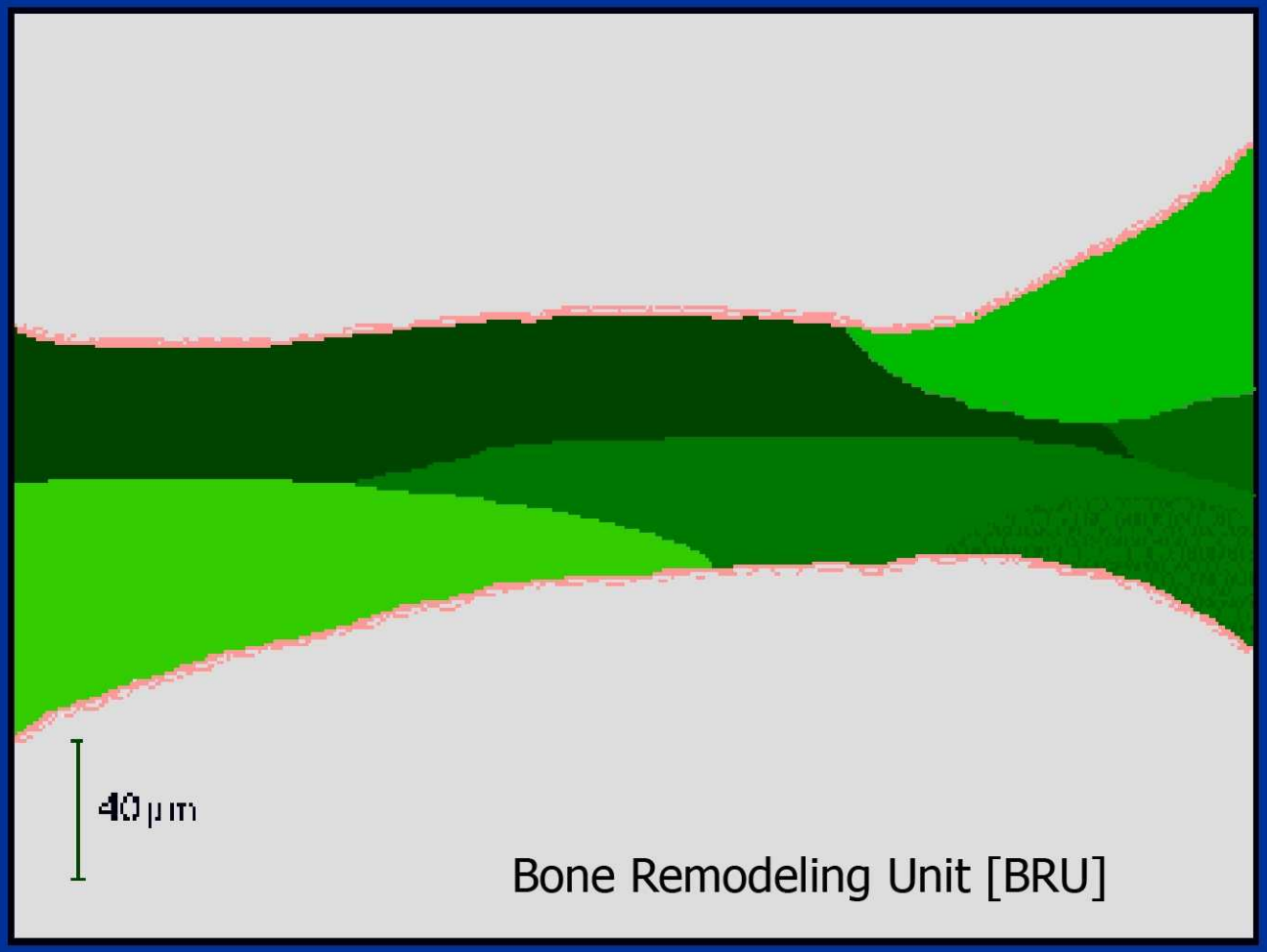
- Invasive - Iliac crest easily accessible
- Metabolically active site - earlier detection of metabolic events and diagnosis of metabolic bone disease
- “Pattern of numbers” referenced to normal values

…as a Clinical Test

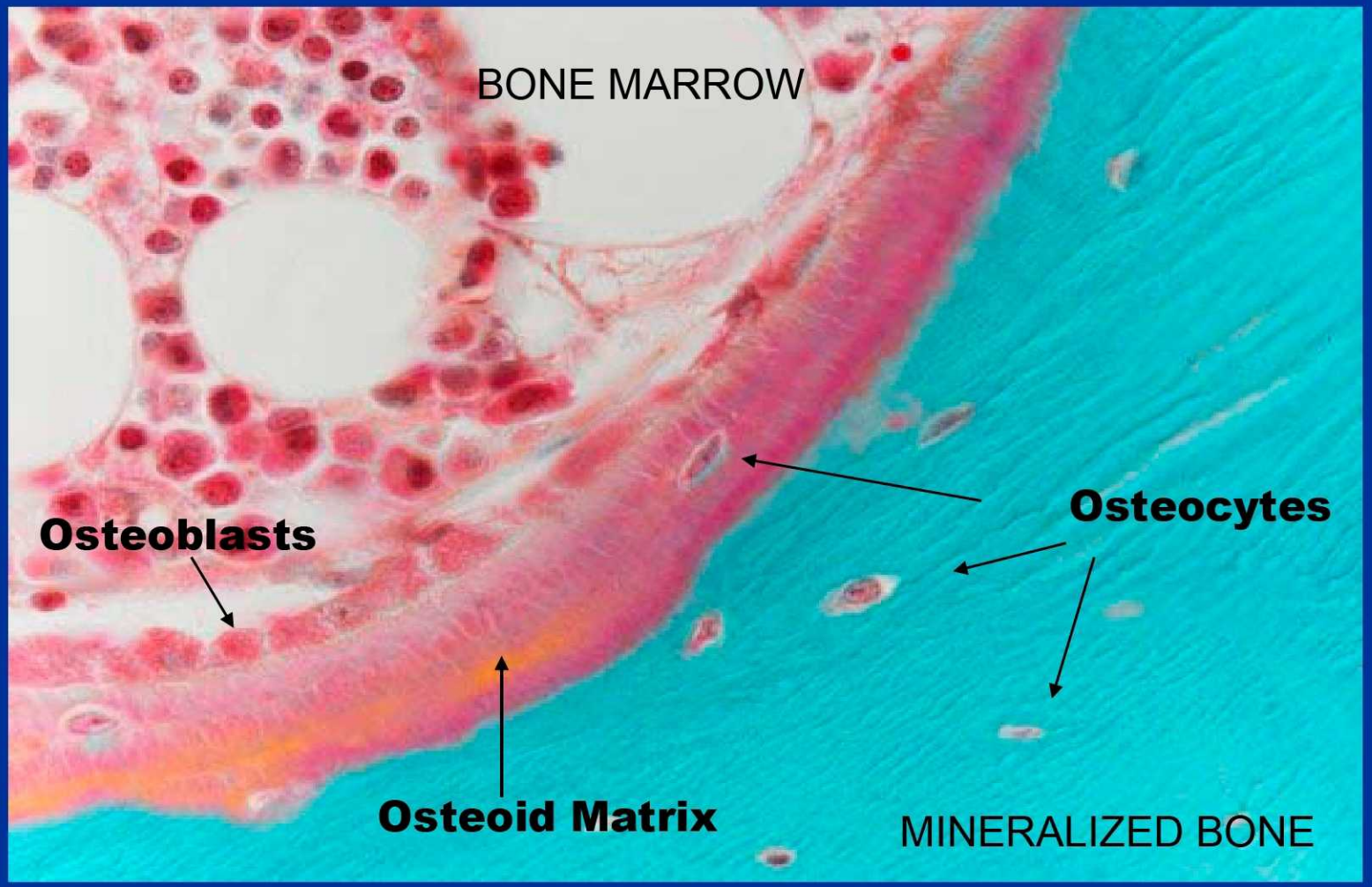
- Not prescribed much (can treat based upon biochemistry)
- Gold standard for osteomalacia and renal osteodystrophy diagnosis
- Measures bone remodeling *directly* in tissue – *valuable* research tool. (Lord Kelvin)

Bone remodeling is the process by which the adult skeleton renews and repairs itself.

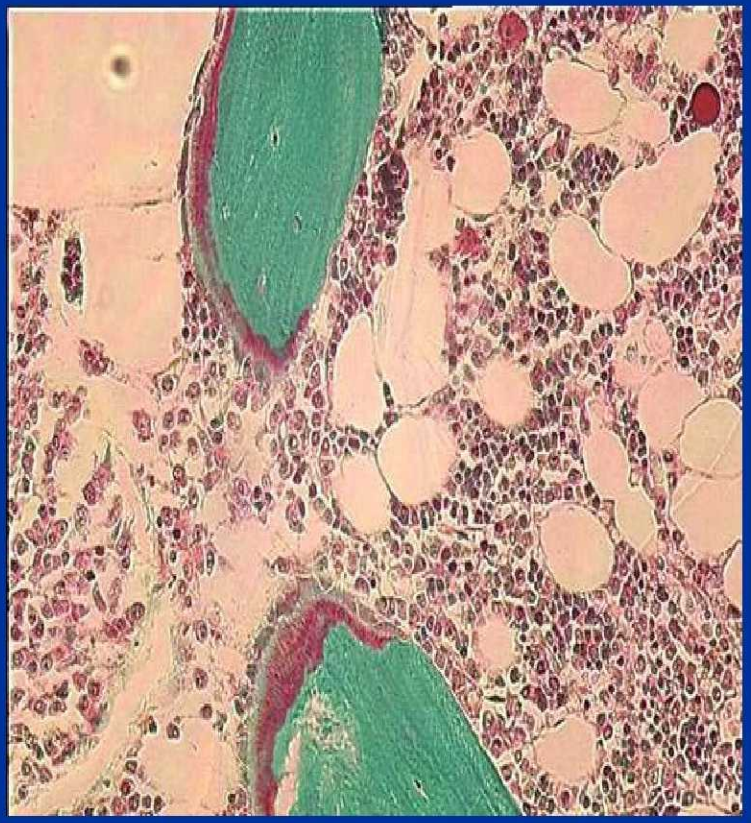
Bone Remodeling: Removing and replacing bone in discrete "packets" on the same bone surface in a specific sequence of cell activities.



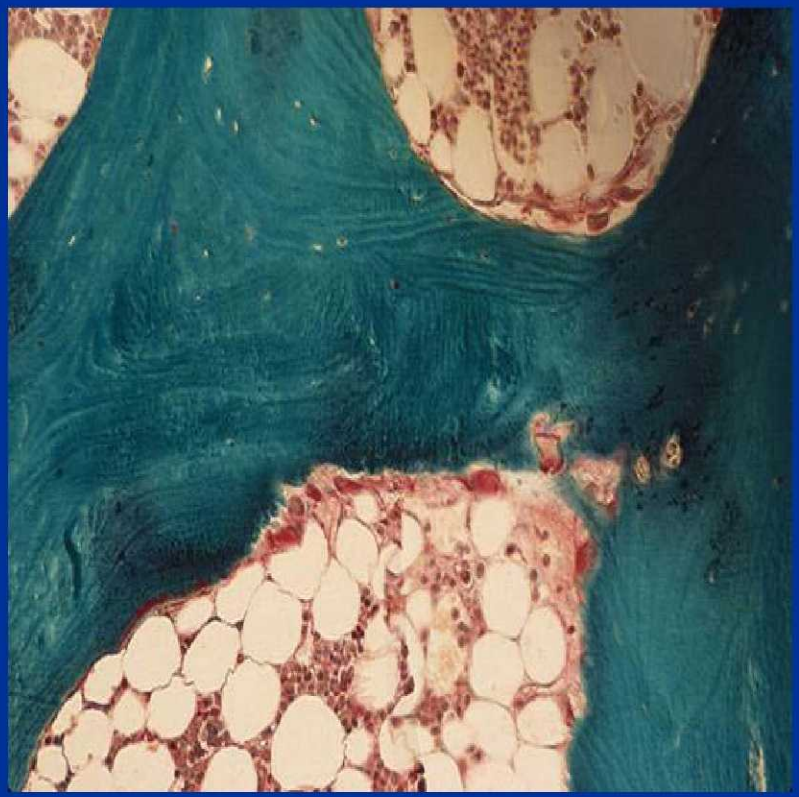
Histology: Bone Remodeling Unit



Static Histomorphometry: Direct measures of cells in tissue.

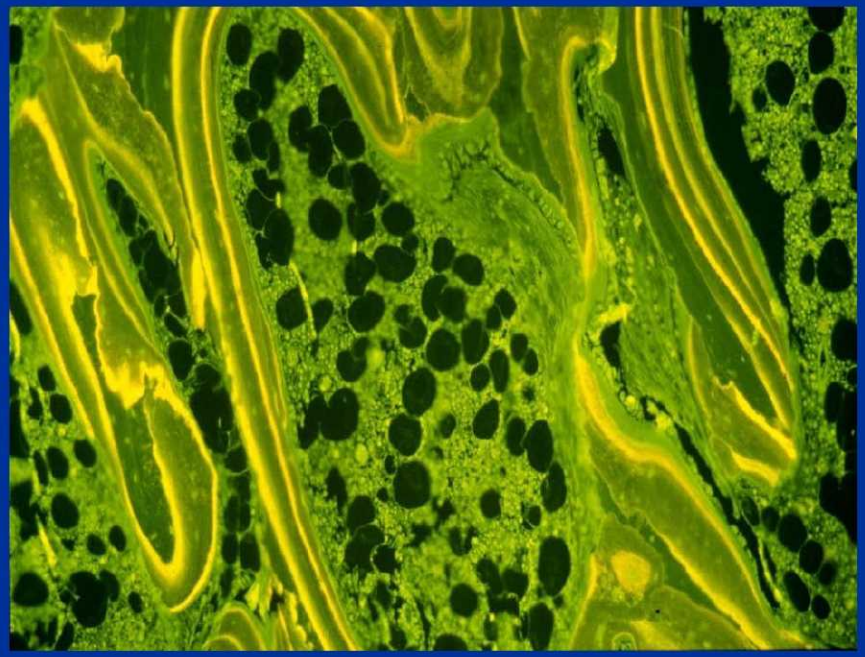


Bone Formation

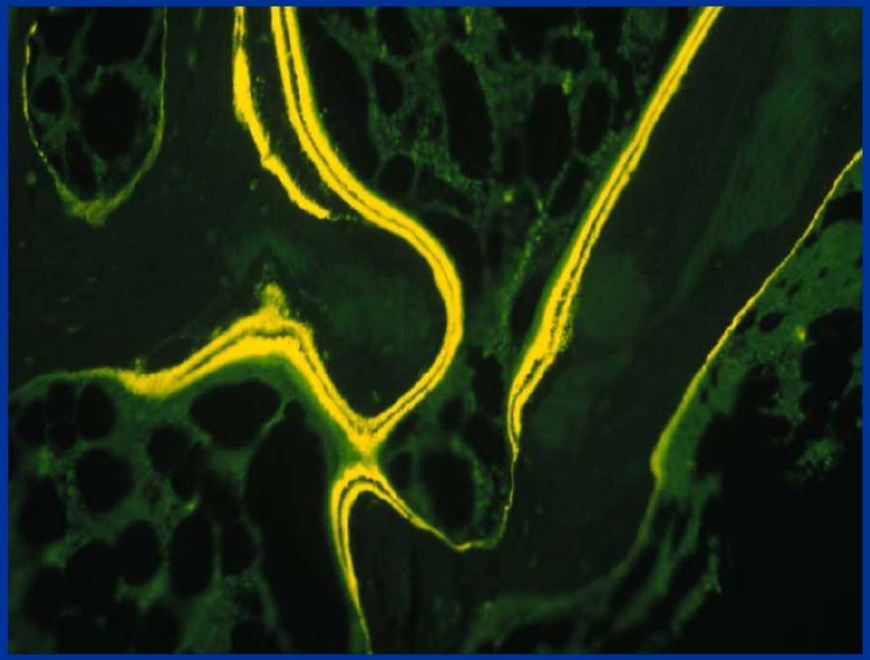


Bone Resorption

*Dynamic histomorphometry: Tetracycline labeling to calculate rates of remodeling

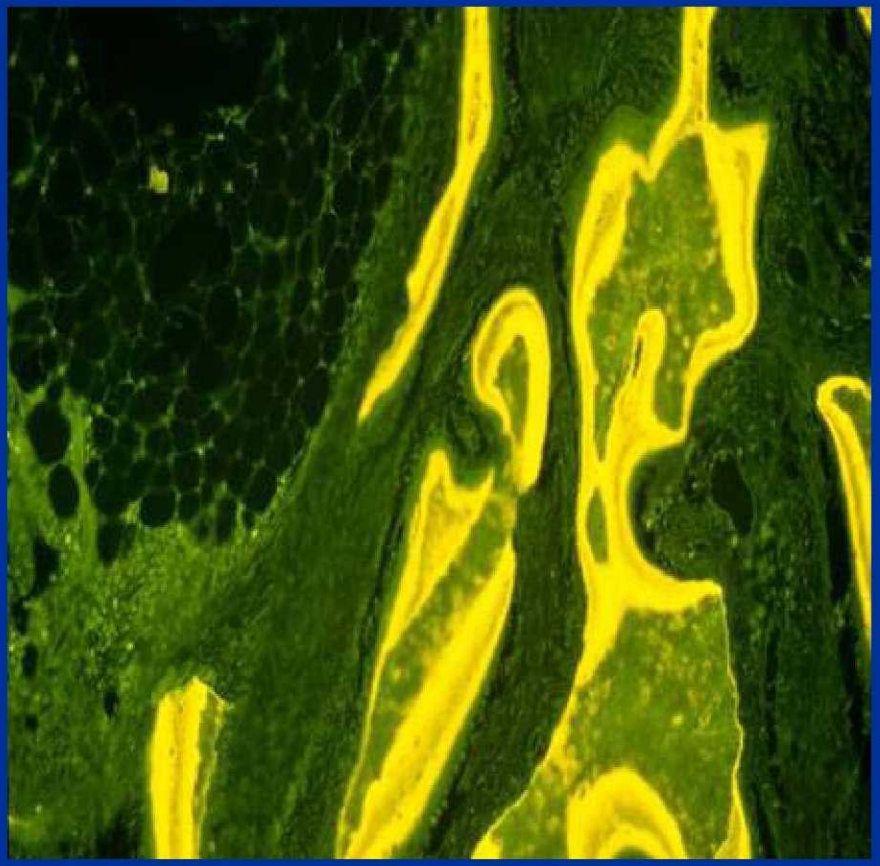


High Rate of Remodeling with Metabolic Bone Disease



Normal Rate of Remodeling

Gold Std for Osteomalacia Diagnosis – "Adult Rickets"



Mineralization defect not detectable by x-ray based imaging devices.

Pattern of Numbers

mayo

Bone Histomorphometry Report (V2)

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

Patient's Name:

Registration Number:

Patient's Age: 33 Sex M Biopsy Date: 12/2/2003

☑ Ilium ☐ Other

Referring Physician:

Referring Physician's Tel: (520) 297-0404 Biopsy # 5439

Physician's Address: 6050 North Corona Road, Suite #1, Tuscon AZ 85704

Parameter	Difference from Normal Mean (in standard deviations) <---lower higher---> -3 -2 -1 mean +1 +2 +3	Male normal mean values	Results	Z-Scores
Cortical Width (Ct.Wi)		915.6 um	722.3	-0.678
Cancellous Bone Volume (BV/TV)		19.7 %	19.5	-0.028
Osteoid Volume(OV)		0.98 %	0.22	-1.334
Osteoid Width(O.Wi)		11.1 um	11.5	0.131
Osteoid Surface(OS)		6.53 %	7.25	0.183
Osteoblast-osteoid interface(Ob.s/OS)		14.35 %	5.28	-0.800
Osteoclast per Length (N.Oc/B.Pm)		3.5 /100 mm	7.1	0.891
Eroded Surface(ES)		1.48 %	0.34	-2.144
Single-LS (sL.Pm)		2.44 %	2.94	0.327
Double-LS (dL.Pm)		3.03 %	11.64	1.574
Mineral Apposition Rate(MAR)		0.89 um/day	0.85	-0.021
Bone Formation Rate - Surface Based (BFR/BS)		0.009 $mm^3/mm^2/yr$	0.041	1.530
Bone Formation Rate - Volume Based (BFR/BV)		0.131 $mm^3/mm^3/yr$	0.575	1.904
Adjusted Apposition Rate (AjAR)		0.16 $mm^3/mm^2/y$	0.56	2.389
Mineralization Lag Time (MLT)		27.6 days	7.5	-1.908

Histomorphometry in Animal Models

Sensitive tool,
statistical comparisons
and in vivo evaluations

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- Translational Research – Why animal models?
- Examples & Relevance to HRP Gaps
- Closing Remarks

Program Reviews

IOM Review

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

Risks

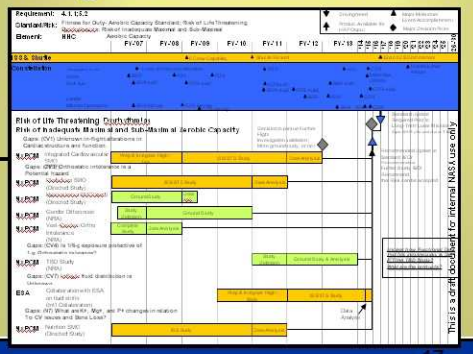
Gaps

Standing Review Panels Review

Exploration Missions & Architectures

NASA Spaceflight Human System Standards

Integrated Research Plan



Solicitations & Directed Research

Peer Review

Results and Deliverables

Customer Review

Slide courtesy of C. Kundrot 11 Feb 2008

SRP Comments

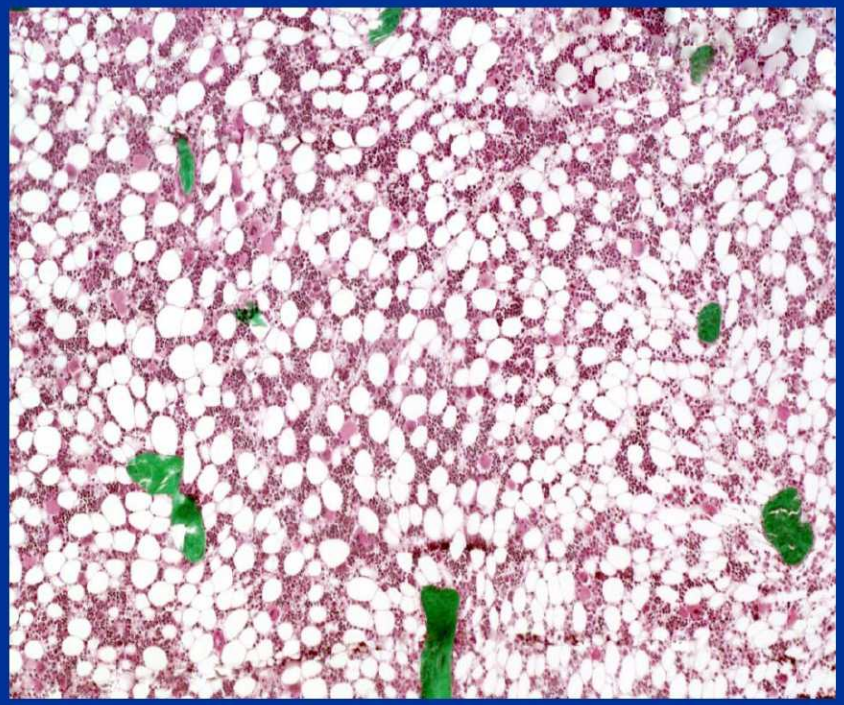
- Evaluate new drugs that have improved safety profile, acceptance, efficacy, and convenience.
- Evaluate interactions among pharmaceuticals and exercise interventions
- Evaluate … efficacy of various anti-resorptive medications, and their interaction with reduced mechanical loading.
- Are there gender differences in the time course of bone loss?

Translational Research – Why animal models as a tool for HRP?

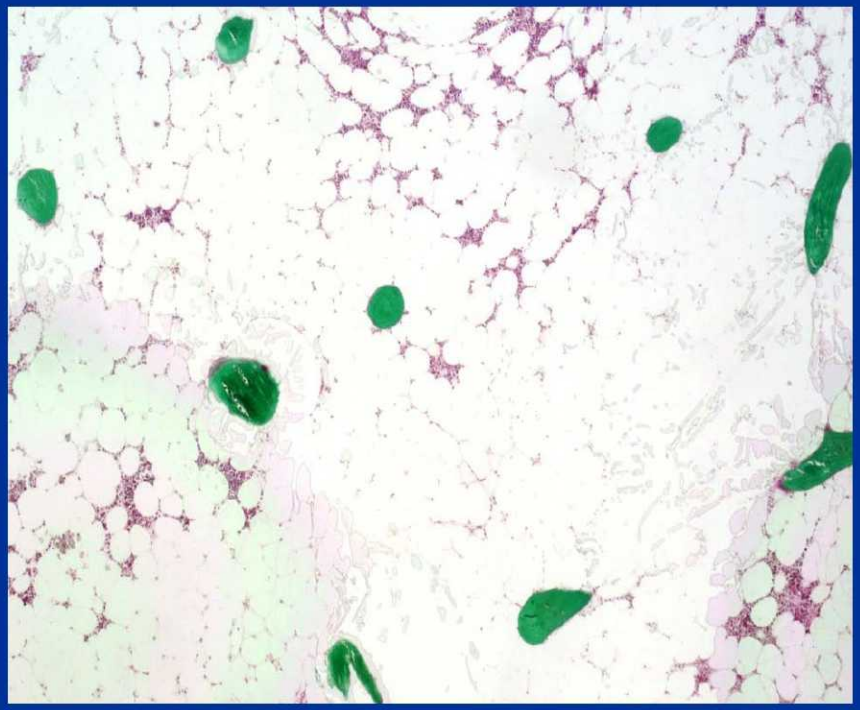
- Time-efficient – results sooner, informs clinical studies, validate drug countermeasures
- Cost-effective - relatively less expensive, manipulate experimental design to model operations and constraints, greater statistical power
- Invasive measures – research measures/designs not readily applied clinically (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

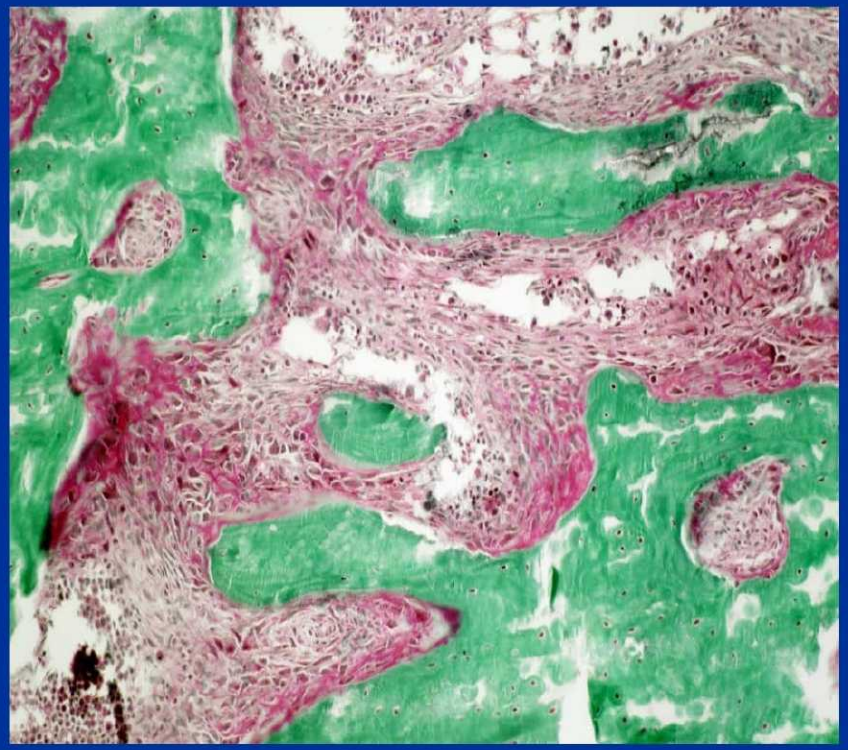


Animal Tissue from OVX'd rat

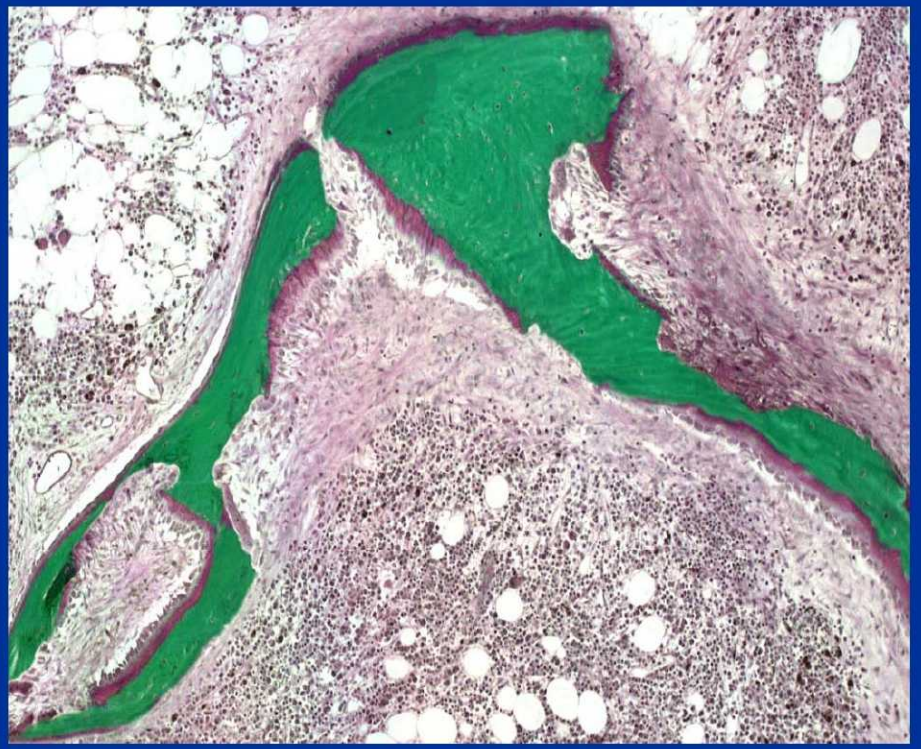


Clinical Bx from postmenopausal woman

Animal Model for Parathyroid Bone Disease

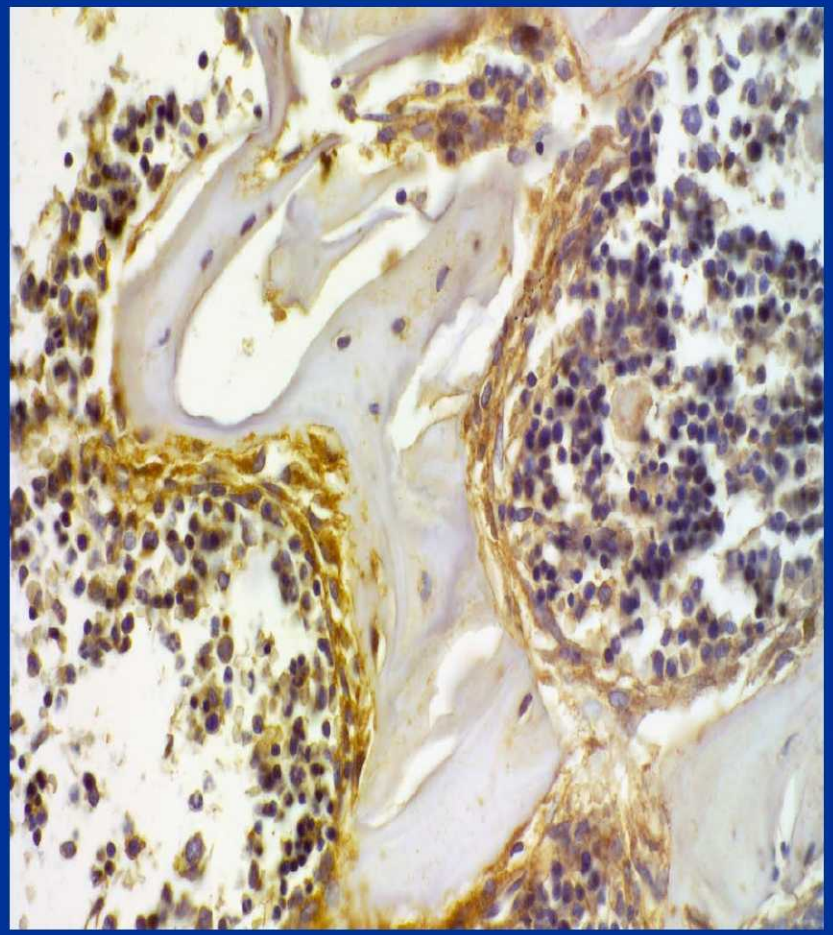


Tissue rat continuous infused with PTH

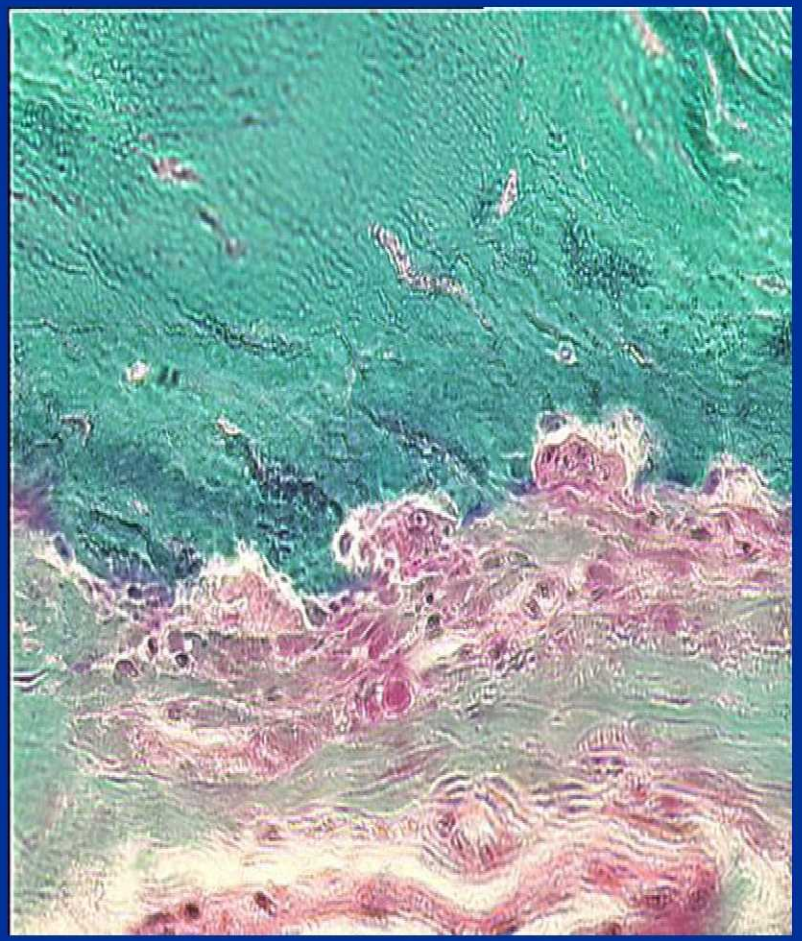


Clinical Bx from patient with hyperparathyroidism

Animal models - unique research observations of tissue and cells.

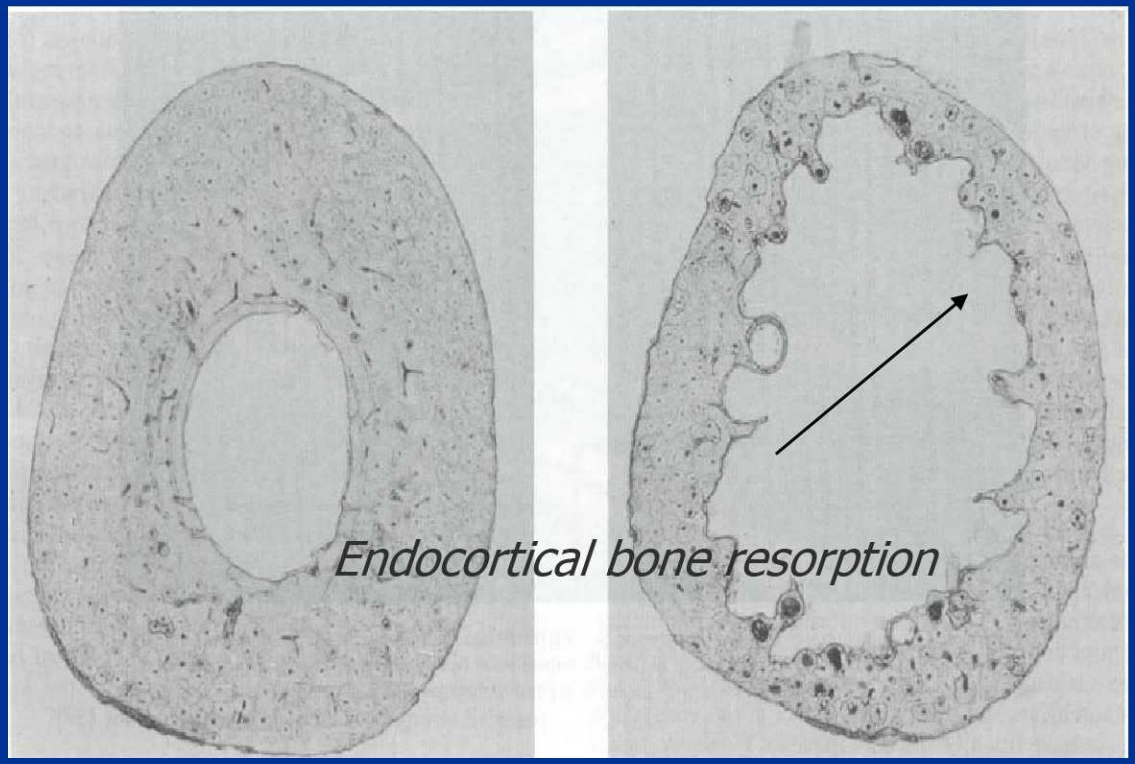


Fibroblast Expression of cbfa-1
Osteoblast Transcription Factor



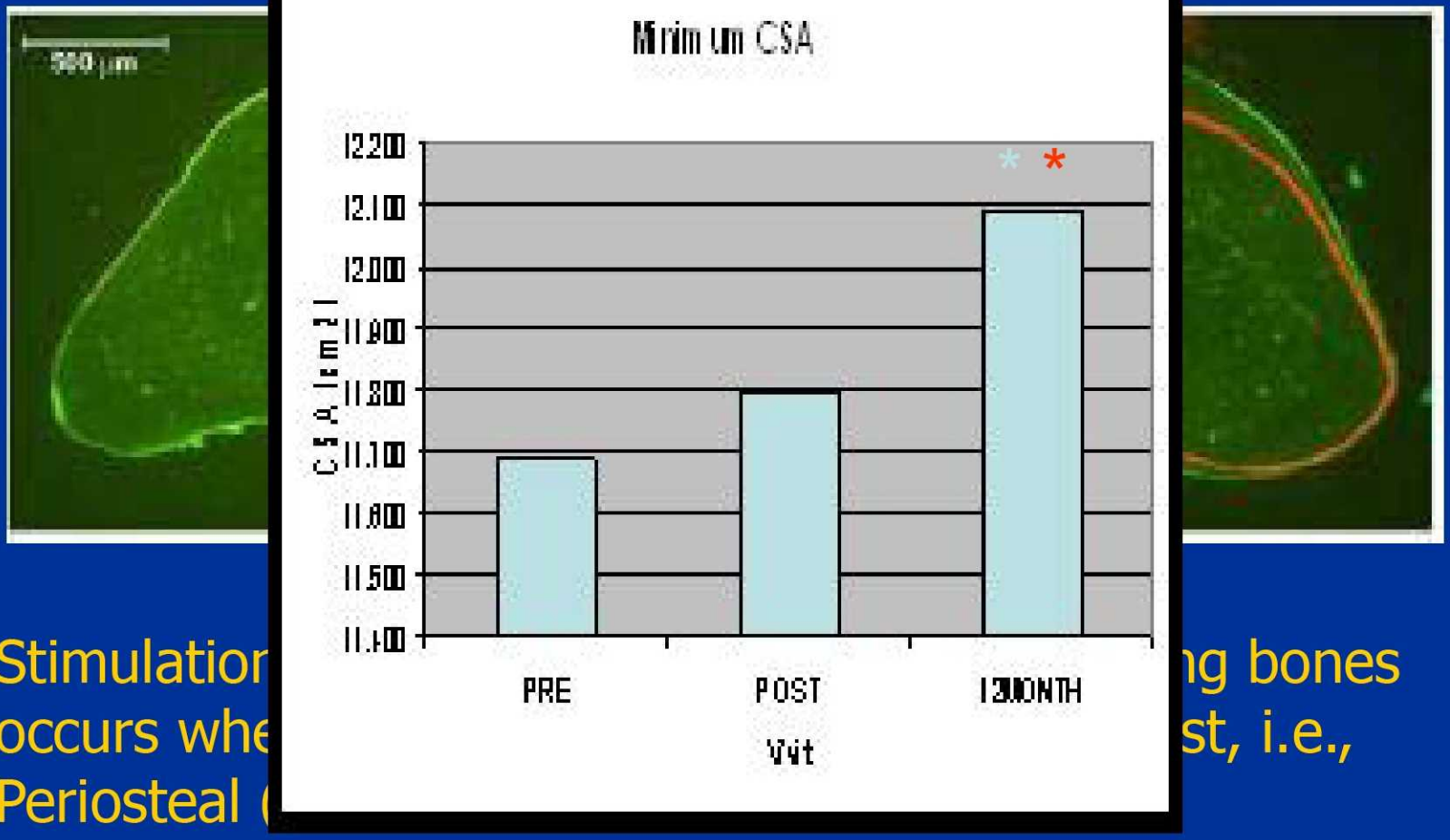
Osteoclasts on outside (periosteal) surface of bone. Novel?

Cast immobilization in adult beagle (40 wks) predicts the changes suggested by measures from QCT imaging in astronauts.



From J.W.Jaworski
Slide Courtesy of D Carter

Animal Studies: Evidence for stimulated periosteal bone apposition with mechanical stimulus



Stimulation
occurs whe
Periosteal (
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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

Breast Cancer Research and Treatment 41: 71–79, 1996.

Report

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. Bell[3] and Russell T. Turner[1,2]
Department of [1]Orthopedic Research and [2]Biochemistry and Molecular Biology, Mayo Clinic, Rochester MN 55905, and [3]Research Service, Ralph H. Johnson Department of Veterans Affairs Medical Center Charleston, S.C. 29401, USA

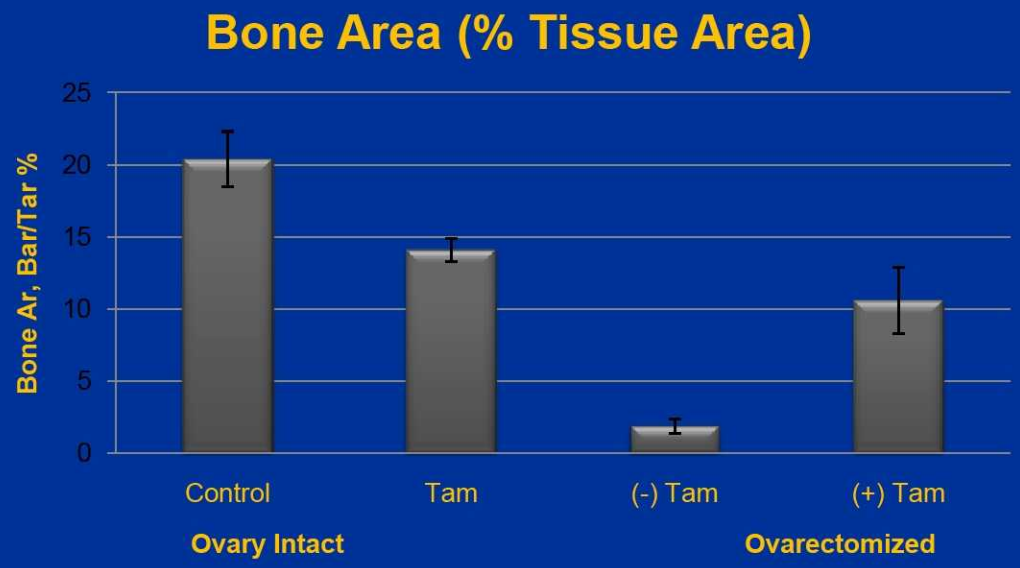
- Tamoxifen – competitive inhibitor suppresses proliferation of ER-positive breast cancer cells
- Clinical relevance of study: Should cancer therapy be given as a preventative measure to pre-menopausal females at high risk for developing breast cancer?
- Sprague Dawley Rat model for estrogen replete/deficient 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

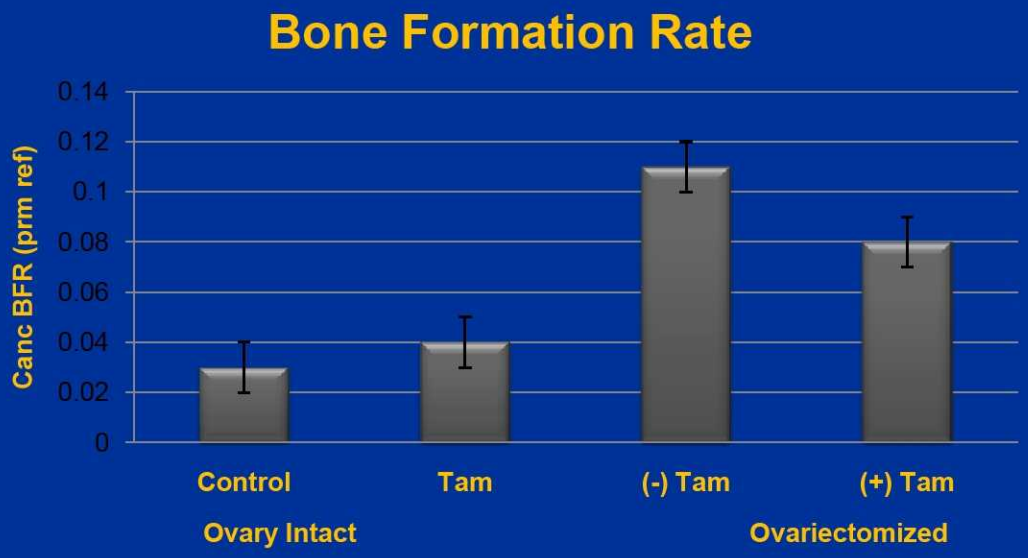
Results

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.

ELSEVIER

Bone 40 (2007) 1013–1020

BONE

www.elsevier.com/locate/bone

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,e], 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.

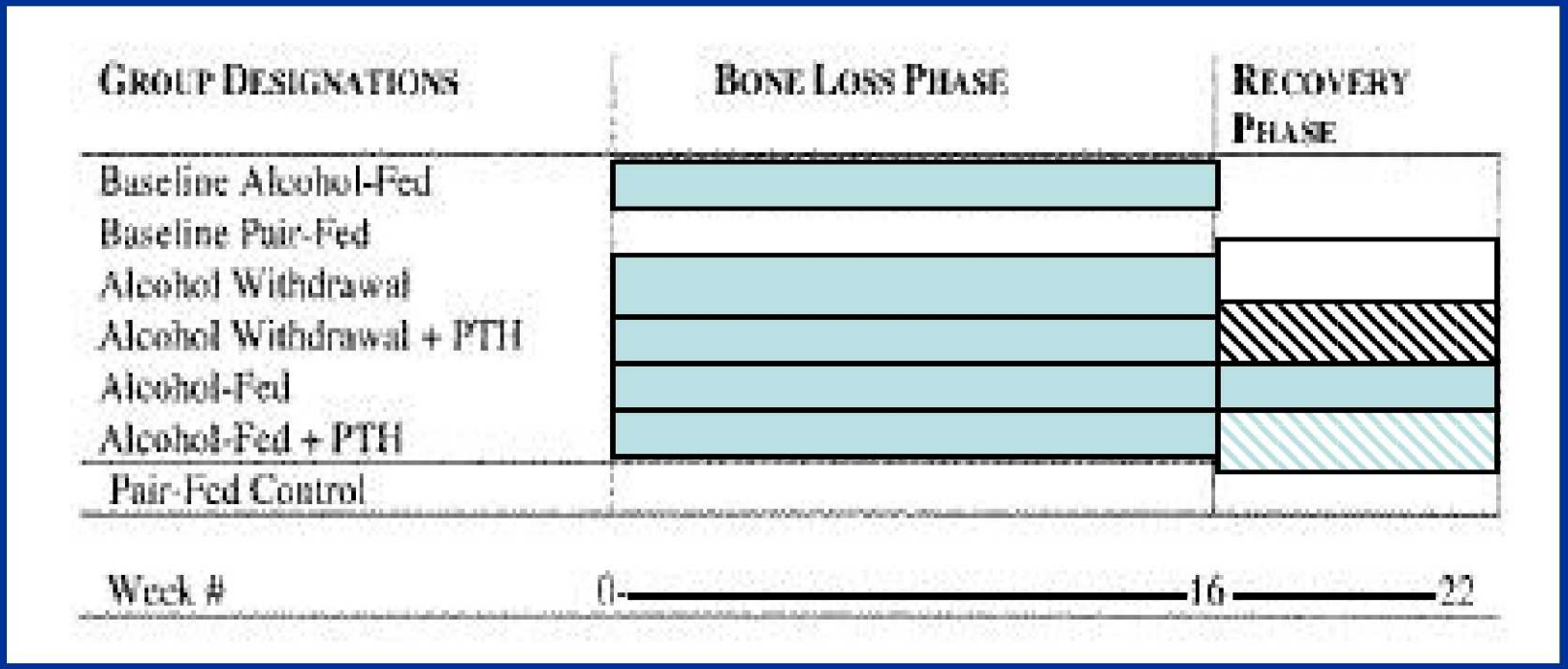
FYI: Categories of Osteoporosis

- **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.2

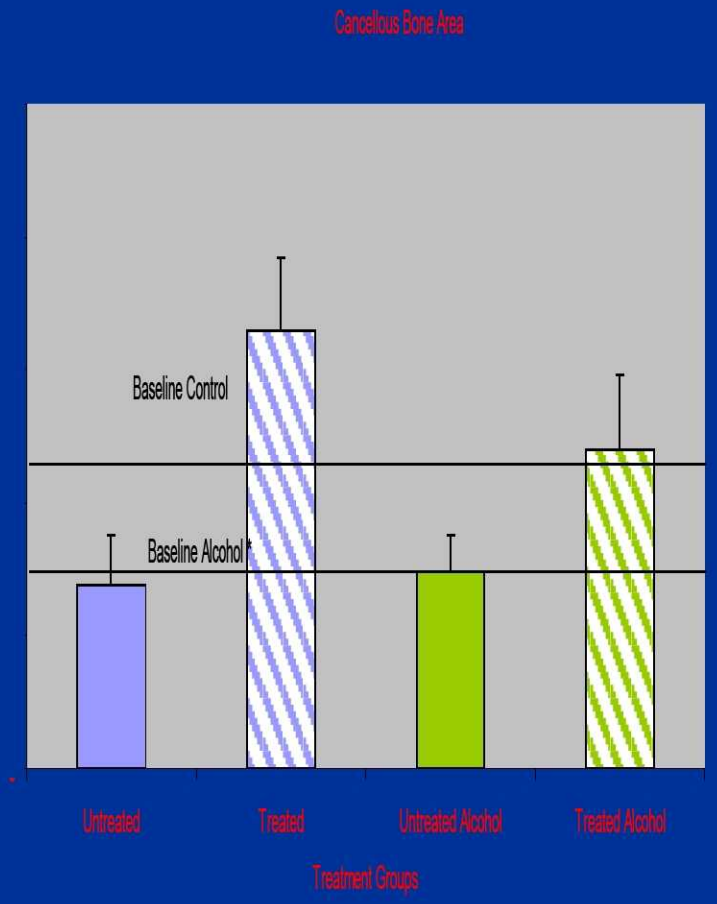
- Clinical relevance of study: What is the effectiveness of a drug therapy in *reversing* bone loss in the continued presence of bone loss inducer?



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

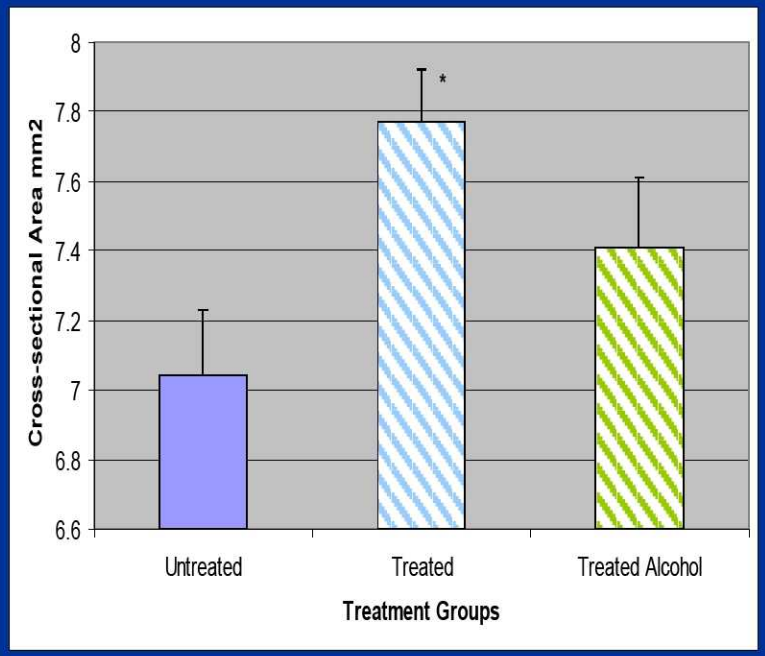
Results

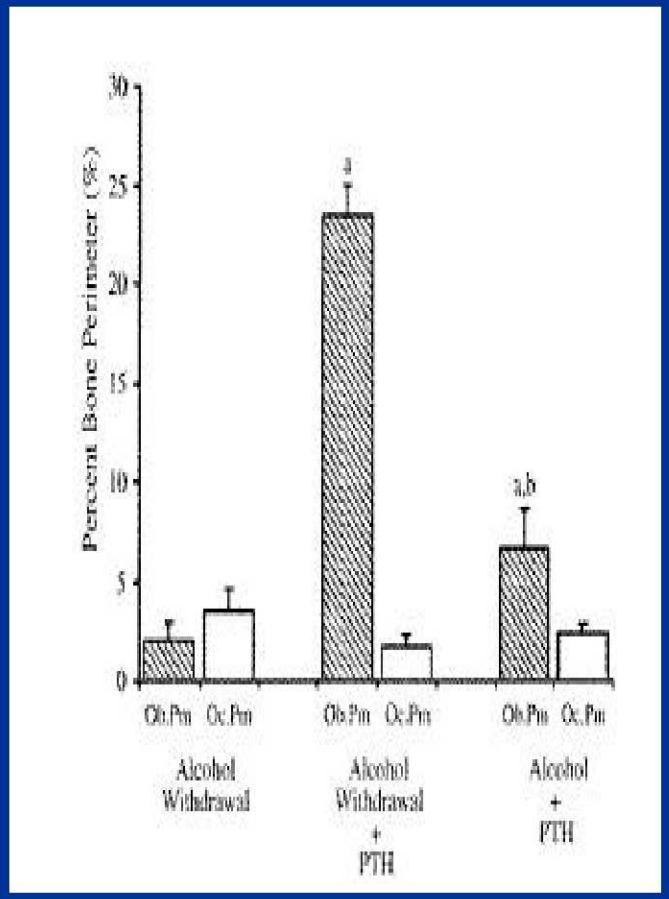
- Drug reverses alcohol-induced loss in bone area ($p<0.05$), but Significant Interactive Effects ($p< 0.05$), i.e., drug effect is attenuated in the presence of alcohol (similar response pattern in BMD).



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?

J Appl Physiol 101: 881–886, 2006.
First published May 4, 2006; doi:10.1152/japplphysiol.01622.2005.

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

Russell T. Turn[illegible] [illegible] Thorsen E. Hefferon [2] and Emily Morey-Holton[3]
[1]Department of Nu[illegible]gon; [2]Department of Orthopedics, M[illegible] Aeronautics and Space Adminis[illegible]

Submitted 27 Decem[illegible]

- Parathyroid [illegible] to prevent bone loss in [illegible] by hind-limb elevation.
- 6-month old [illegible] TH
- Two-weeks [illegible] rates in both cortical and [illegible]
- PTH and HL[illegible] site effects.

Results

- PTH prevents bone loss induced by mechanical **unloading**.
- PTH stimulates bone formation in mechanically **loaded** environment.

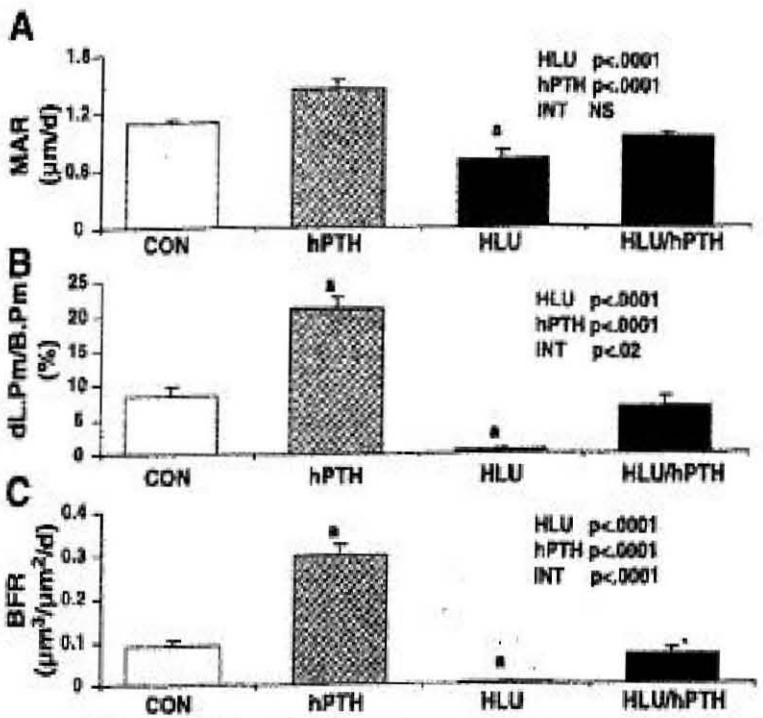


Fig. 4. Effects of HLU and PTH on cancellous bone histomorphometry. *A*: MAR. *B*: double-labeled perimeter/bone perimeter (dL.Pm/BPm). *C*: bone formation rate (BFR). Values are means + SE (n = 7–10). *Bars marked differ from CON ($P < 0.05$). The results of the 2-way ANOVA are shown in the figure.

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?

J Appl Physiol 95: 1775–1780, 2003.
First published July 25, 2003; 10.1152/japplphysiol.00455.2002.

Effect of gender on bone turnover in adult rats during simulated weightlessness

T. E. Hefferan,[1] G. L. Evans,[1] S. Lotinun,[1] M. Zhang,[1] E. Morey-Holton,[2] and R. T. Turner[1]
[1]*Departments of Orthopedics, Biochemistry, and Molecular Biology, Mayo Clinic, Rochester, Minnesota 55905; and* [2]*National Aeronautics and Space Administration-Ames Research Center, Moffett Field, California 94035*

Submitted 22 May 2002; accepted in final form 23 May 2003

- 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

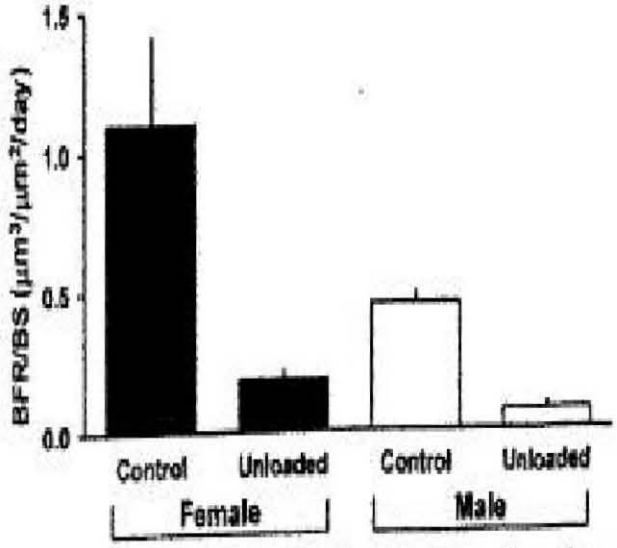


Fig. 3. Effects of gender and hindlimb unloading on bone formation rate (BFR). Measurements were taken 1 mm from the growth plate in the proximal tibial metaphysis. Values are means ± SE. Two-way ANOVA indicates significant effects of gender ($P = 0.036$) and hindlimb unloading ($P = 0.0005$), with no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

- 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.