Program and Abstracts

Advances in Skeletal Anabolic Agents for the Treatment of Osteoporosis

May 24-25, 2004

Hyatt Regency Bethesda
Bethesda, Maryland, USA

A Scientific Meeting Sponsored by

The American Society for Bone and Mineral Research (ASBMR)

Co-Sponsored by

The American Academy of Orthopaedic Surgeons (AAOS)
The Endocrine Society (ENDO)
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The National Institute of Dental and Craniofacial Research (NIDCR)
The National Osteoporosis Foundation (NOF)
The Osteogenesis Imperfecta Foundation (OIF)

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The following Institutes from the U.S. National Institutes of Health provided funding for this meeting through an unrestricted educational grant (R13DK/AR/HD/DE/AG67034):

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
The National Institute on Aging (NIA)
The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
The National Institute of Child Health and Human Development (NICHD)
The National Institute of Dental and Craniofacial Research (NIDCR)

SUPPORTERS

This conference was supported by unrestricted educational grants from the following companies:

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Welcome!

On behalf of the organizers of this ASBMR-NIH co-sponsored meeting on the current and future status of anabolic skeletal agents for the treatment of osteoporosis, we welcome you and thank you for participating. The widespread availability of the anti-resorptive class of osteoporosis drugs has advanced the world of osteoporosis therapy dramatically over the past decade. This has led to the ability to increase bone mass reliably and progressively in patients at risk of osteoporotic fracture, and to reduce markedly their future risk of fracture, with its attendant morbidity and mortality.

CONTENTS

As was the case approximately 15 years ago for anti-resorptives, we are now at the dawn of an entirely new, and complementary, approach to future therapies for osteoporosis. The first of the therapeutically useful skeletal anabolic agents has now been on the market for more than a year. It is very clear that additional members of this class will be in the hands of physicians treating osteoporosis in the next few years. This is exciting because it means that we can now approach osteoporosis therapeutically in two completely different ways: by reducing osteoclastic bone loss, and by stimulating osteoblastic new bone formation.

There are currently an estimated 30 million people in the U.S. with osteoporosis, a demographic that will be highlighted in an upcoming U.S. Surgeon General's Report. As the population of the U.S. ages, osteoporosis will become an even larger problem.

This symposium includes an overview of the current anabolic approaches to increasing bone mass, both in the clinic and in the laboratory. Our goal is to provide an opportunity for the participants in the field – researchers, clinicians, health policy personnel, regulatory personnel, marketing personnel and others – to interact, to think collegially, to hypothesize, to argue constructively, and to plan together the future of anabolic skeletal therapy for osteoporosis and other metabolic bone diseases.

The organizers want to thank the innumerable people who help to organize this meeting, beginning with Dr. Mehrdad Tondravi at NIH who was instrumental in helping to plan this meeting in its initial phases. We also thank NIAMS Director Drs. Stephen Katz, NIDDK Director Allen Spiegel, Senior Advisor for Molecular Endocrinology Ronald Margolis and Director of the Musculoskeletal Diseases Branch at NIAMS Joan McGowan for their unwavering support. We are also grateful for the co-sponsorship of our sister organizations – the American Academy of Orthopaedic Surgeons, the Endocrine Society, the International Society of Clinical Densitometry, the National Osteoporosis Foundation, and the Osteogenesis Imperfecta Foundation. We also want to thank the commercial sponsors who have helped to support this meeting (please note that commercial support was invited only after the scientific program was developed). Finally, we want to thank the ASBMR staff who provided continuous, intense and infallible organizational support.

Sincerely,

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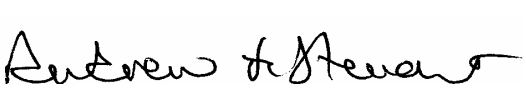
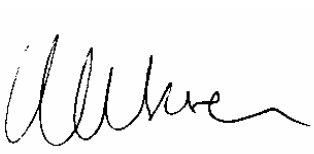


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Advances in Skeletal Anabolic Agents for the Treatment of Osteoporosis

ORGANIZING COMMITTEE

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*This awardee donated his travel grant funds to the ASBMR.

General Information

VENUE

This meeting will take place in the Crystal Ballroom of the Hyatt Regency Bethesda located at One Bethesda Metro Center (corner of Wisconsin Ave. and Old Georgetown Rd.), Bethesda, Maryland, USA.

REGISTRATION

All registration services will take place in the Foyer of the Crystal Ballroom at the Hyatt Regency Bethesda.

Registration Hours

Monday, May 24	6:30 am – 2:00 pm
Tuesday, May 25	7:00 am – 2:00 pm

SPEAKER READY ROOM

All speakers must check into the Speaker Ready Room a minimum of one hour prior to their presentation, but preferably the day before their presentation, if possible. At that time, speakers may review their slides. The Speaker Ready Room is located in the Susquehanna Room on the Y level of the Hyatt Regency Bethesda. We encourage speakers to review their slides in the Speaker Ready Room to ensure all Greek characters and graphs transferred successfully. The Speaker Ready Room will be open during the following times:

Speaker Ready Room Hours

Sunday, May 23, 2004	4:30 pm – 9:00 pm
Monday, May 24, 2004	6:30 am – 6:30 pm
Tuesday, May 25, 2004	7:00 am – 2:00 pm

POSTER INFORMATION

All poster sessions will be held in the Crystal Ballroom of the Hyatt Regency Bethesda. Presenters must be at their posters during their designated poster sessions on Monday or Tuesday, from 1:00 pm to 1:45 pm and must be available to answer questions during this period.

POSTER SCHEDULE		
	Monday **May 24, 2004**	**Tuesday** **May 25, 2004**
Poster Presenter Information		
Poster Set-Up	7:00 am – 8:00 am	7:00 am – 8:00 am
Presentation Time	**Poster Session I (M1-M35)** 1:00 pm – 1:45 pm	**Poster Session II (T1-T35)** 1:00 pm – 1:45 pm
Poster Dismantle Period	7:00 pm – 7:15 pm	6:00 pm – 6:15 pm
Poster Viewing Schedule		
Morning Break	10:20 am – 10:35 am	10:20 am – 10:35 am
Lunch Break	12:35 pm – 1:45 pm	12:35 pm – 1:45 pm
Afternoon Break	4:00 pm – 4:15 pm	4:20 pm – 4:35 pm
Post-Meeting	5:55 pm – 7:00 pm	5:05 pm – 6:00 pm

CONFERENCE MEALS

Your registration for the conference includes a continental breakfast and boxed lunch on Monday and Tuesday, May 24th and 25th, 2004. The meals will be served in the Foyer of the Crystal Ballroom at the Hyatt Regency Bethesda.

ASBMR'S EXPECTATIONS OF PRESENTERS

Through ASBMR meetings, the Society wishes to promote excellence in bone and mineral research. Toward that end, ASBMR expects that all presenters participating in the ASBMR Meeting on Advances in Skeletal Anabolic Agents for the Treatment of Osteoporosis will provide informative and fully accurate scientific and other information. Furthermore, the ASBMR expects that all presentations at this meeting will reflect the highest level of scientific rigor and integrity.

The content of speaker presentations, slides, and reference materials must remain the ultimate responsibility of the faculty. The planning, content and execution of speaker presentations, slides, abstracts and reference materials should be free from corporate influence or control. Industry-based and supported presenters should provide full disclosure of their relationship with the respective company(ies).

DISCLOSURE/CONFLICT OF INTEREST

The ASBMR is committed to ensuring the balance, independence, objectivity and scientific rigor of all its educational activities. ASBMR desires for audiences at its educational programs to be informed of a presenter's academic and professional affiliations and the existence of any significant financial interest or other relationship a presenter has with the manufacturer(s) of any commercial product(s) discussed in an educational presentation. This policy allows the listener/attendee to be fully informed in evaluating the information being presented.

The following key was used to identify the potential conflicts which are listed at the end of each abstract.

1. stock options or bond holdings in a for-profit corporation or self-directed pension plan
2. research grants
3. employment (full* or part-time)
4. ownership or partnership
5. consulting fees or other remuneration (payment)
6. non-remunerative positions of influence such as officer, board member, trustee, or public spokesperson
7. receipt of royalties
8. speakers bureau

For full-time employees of industry or government, the affiliation listed in the biographical information will constitute full disclosure.

DISCLAIMER

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AUDIO- AND VIDEOTAPING

ASBMR expects that attendees will respect each presenter's willingness to provide free exchange of scientific information without the abridgement of his or her rights or privacy and without the unauthorized copying and use of the scientific data shared during his or her presentation. Cameras or recording devices will not be permitted in the Oral Scientific Sessions or the Poster Sessions, **without the prior written permission of the ASBMR Convention Management.**

The use of cameras, audiotaping devices, and videotaping equipment is strictly prohibited within all Oral Scientific Sessions, the Exhibit Halls, and the Poster Sessions without the express written permission of the ASBMR Convention Management. Unauthorized use of this taping equipment may result in the confiscation of the equipment or the individual may be asked to leave the Scientific Session. These rules will be strictly enforced.

USE OF ASBMR NAME AND LOGO

ASBMR reserves the right to approve use of its name in all material disseminated to the media, public and professionals. ASBMR's name, meeting name, and meeting logo may not be used without permission. Use of the ASBMR logo is prohibited. Materials should be directed to ASBMR Executive Director Joan Goldberg. All ASBMR corporate supporters should share their media outreach plans with the ASBMR Executive Director before release.

No abstract presented at the ASBMR Scientific Meeting on Advances in Skeletal Anabolic Agents for the Treatment of Osteoporosis may be released to the press before its official presentation date and time. Press releases must be embargoed until one hour after the presentation.

FUTURE ASBMR ANNUAL MEETING DATES

ASBMR 26th Annual Meeting
October 1-5, 2004
Seattle, Washington, USA

ASBMR 27th Annual Meeting
September 23-27, 2005
Nashville, Tennessee, USA

ASBMR 28th Annual Meeting
September 15-19, 2006
Philadelphia Convention Center, Philadelphia, Pennsylvania, USA

ASBMR 29th Annual Meeting
September 16-20, 2007
Hawaii Convention Center, Honolulu, Hawaii, USA

Advances in Skeletal Anabolic Agents for the Treatment of Osteoporosis

A Scientific Meeting

SPONSORED BY

The American Society for Bone and Mineral Research (ASBMR)

SCHEDULE-AT-A-GLANCE

MONDAY, MAY 24, 2004		
TIME	**SESSION**	**ABSTRACT**
7:00 am	Breakfast	
8:00 am	Introduction — Welcome & Opening Comments	
8:15 am	Overview: Anabolic Therapy for Osteoporosis: The Urgent Need for a Consensus on Nomenclature — B. Lawrence Riggs, M.D.	1
8:45 am	Session I: Exercise, Calcium and Vitamin D as Skeletal Anabolic Agents	2-5
10:20 am	Break and Poster Viewing	
10:35 am	Session II: Skeletal Anabolism: Transcriptional Regulation and Signaling	6-10
12:35 to 1:45pm	Lunch and Poster Viewing	
1:00 to 1:45 pm	Poster Session I	M1-M35
1:45 pm	Young Investigator Oral Session I	11-14
2:45 pm	Session III: LRP5, Wnt and High Bone Mass	15-17
4:00 pm	Break and Poster Viewing	
4:15 pm	Session IV: Estrogens and SERMS	18-21
5:55 to 7:00 pm	Poster Viewing	
7:00 pm	Adjourn	
TUESDAY, MAY 25, 2004		
7:00 am	Breakfast	
8:00 am	Session V: Growth Hormones and Growth Factors	22-27
10:20 am	Break and Poster Viewing	
10:35 am	Session VI: PTH and PTHrP	28-32
12:35 to 1:45 pm	Lunch and Poster Viewing	
1:00 to 1:45 pm	Poster Session II	T1-T35
1:45 pm	Young Investigator Oral Session II	33-36
2:45 pm	Session VII: Miscellaneous Anabolic Agents	37-40
4:20 pm	Break and Poster Viewing	
4:35 pm	Closing Remarks — Future Directions in Skeletal Anabolic Research (Basic and Clinical)	
5:05 to 6:00 pm	Poster Viewing	
6:00 pm	Adjourn	

INTRODUCTION

Moderator: Andrew Stewart

8:00 am – 8:30 am

8:00 am **Welcome and Opening Comments**
Andrew F. Stewart, M.D., *Chairperson*
Division of Endocrinology and Metabolism
University of Pittsburgh Medical Center
Pittsburgh, Pennsylvania, USA

8:05 am **Opening Comments on Behalf of The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)**
Allen M. Spiegel, M.D.
NIDDK
The National Institutes of Health
Bethesda, Maryland, USA

8:10 am **Opening Comments on Behalf of The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)**
Stephen I. Katz, M.D., Ph.D.
NIAMS
The National Institutes of Health
Bethesda, Maryland, USA

8:15 am **1**
Overview: Anabolic Therapy for Osteoporosis: The Urgent Need for a Consensus on Nomenclature
B. Lawrence Riggs, M.D.
Division of Endocrinology
Mayo Clinic
Rochester, Minnesota, USA

SESSION I

Exercise, Calcium and Vitamin D as Skeletal Anabolic Agents

Moderators: Daniel Bikle and Mary Bouxsein

8:45 am – 10:20 am

8:45 am **2**
Effects of Exercise on Bone
Clinton T. Rubin, Ph.D.
Department of Biomedical Engineering
State University of New York at Stony Brook
Stony Brook, New York, USA

9:05 am **3**
Calcium and Vitamin D: Basic Aspects
Marie B. Demay, M.D.
Endocrine Unit
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts, USA

9:25 am **4**
Calcium and Vitamin D: Clinical Aspects
Bess Dawson-Hughes, M.D.
Calcium and Bone Metabolism Laboratory
USDA Human Nutrition Research Center on Aging at Tufts University
Boston, Massachusetts, USA

9:45 am **5**
Mechanisms and Novel Anabolic Actions of Vitamin D and Its Analogs in Bone
J. Wesley Pike, Ph.D.
Department of Biochemistry
University of Wisconsin-Madison
Madison, Wisconsin, USA

10:05 am **Questions**

BREAK AND POSTER VIEWING

10:20 am – 10:35 am

SESSION II

Skeletal Anabolism: Transcriptional Regulation and Signaling

Moderators: Keith Hruska and Robert Jilka

10:35 am – 12:35 pm

10:35 am **6**
Anabolic Actions of PTH in Bone: Role of AP-1 and Osteoclastogenesis
Laurie K. McCauley, D.D.S., Ph.D
Departments of Periodontics/Prevention/Geriatrics
University of Michigan School of Dentistry
Ann Arbor, Michigan, USA

10:55 am **7**
Δ FosB, a Truncated Isoform of FosB Lacking Transactivation Domains Induces Osteosclerosis and Inhibits Adipogenesis in Transgenic Mice
Roldan Baron, D.D.S., Ph.D.
Department of Orthopaedics
and Department of Cell Biology
Yale University School of Medicine
New Haven, Connecticut, USA

11:15 am **8**
Runx/Cbfa1 Factors: Multifunctional Regulators of Skeletal Development
Jane B. Lian, Ph.D.
Department of Cell Biology
University of Massachusetts Medical School
Worcester, Massachusetts, USA

11:35 am **9**
PTH, Apoptosis and Bone Anabolism
Teresita M. Bellido, Ph.D.
Division of Endocrinology/Department of Medicine
Center for Osteoporosis and Metabolic Bone Diseases
University of Arkansas for Medical Sciences
Little Rock, Arkansas, USA

11:55 am **10**
PTH Responsive Genes and Novel Anabolic Targets
Nicola C. Partridge, Ph.D.
Department of Physiology and Biophysics
University of Medicine and Dentistry at New Jersey
Robert Wood Johnson Medical School
Piscataway, New Jersey, USA

12:15 pm **Questions**

LUNCH AND POSTER VIEWING

12:35 pm – 1:45 pm

POSTER SESSION I (M1 – M35)

1:00 pm – 1:45 pm

M1
YOUNG INVESTIGATOR ABSTRACT AWARD
Amphiregulin Is a Novel Growth Factor in Bone Stimulated by Parathyroid Hormone and Required for Normal Bone Development. L. Qin[1], J. Tamasi[2], L. Raggatt[1], X. Li[1], J. H. M. Feyen[2], D. Lee*[3], E. DiCicco-Bloom*[4], N. C. Partridge[1]. [1]Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [2]Bristol-Myers Squibb Pharmaceutical Research Institute, Pennington, NJ, USA, [3]Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC, USA, [4]Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.

M2
YOUNG INVESTIGATOR ABSTRACT AWARD
Endogenous PKIgamma Regulates Immediate-early Gene Expression Induced by PTH in Osteoblasts. X. Chen[1], J. Dai[1], S. A. Orellana*[2], E. M. Greenfield[1]. [1]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA, [2]Pediatrics, Case Western Reserve University, Cleveland, OH, USA.

M3
YOUNG INVESTIGATOR ABSTRACT AWARD
Anabolic Actions of PTH: Temporal Effects on Tissue-engineered Bone. G. J. Pettway*[1], A. J. Koh*[2], E. Widjaja*[3], M. Morris*[3], L. K. McCauley[2]. [1]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA, [2]Perio/Prev/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [3]Chemistry, University of Michigan, Ann Arbor, MI, USA.

M4
YOUNG INVESTIGATOR ABSTRACT AWARD
The Role of IGF-I in Regulating the Skeletal Response to PTH. Y. Wang[1], S. Nishida[1], H. Z. ElAlieh*[1], S. Majumdar[2], A. Burghardt*[2], T. L. Clemens[3], B. P. Halloran[1], D. D. Bikle[1]. [1]Endocrine Unit, Veterans Affairs Medical Center,University of California, San Francisco, San Francisco, CA, USA, [2]Radiology, University of California, San Francisco, San Francisco, CA, USA, [3]Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA.

M5
YOUNG INVESTIGATOR ABSTRACT AWARD
The Mechanism for IGF-I Resistance Induced by Skeletal Unloading Is Not Shared by Other Growth Factors. S. Nishida, Y. Wang, H. Z. ElAlieh*, B. P. Halloran, D. D. Bikle. Endocrine Unit, University of Calfornia, Veterans Affairs Medical Center, San Francisco, CA, USA.

M6
YOUNG INVESTIGATOR ABSTRACT AWARD
Transcriptional Activation of Vitamin D Receptor Mutants by Phosphorylation. Y. Liu*[1], P. Malloy*[2], D. Feldman*[2], S. Christakos[1]. [1]Dept. of Biochemistry, New Jersey Medical School, Newark, NJ, USA, [2]Dept. of Medicine, Stanford University School of Medicine, Stanford, CA, USA.

M7
YOUNG INVESTIGATOR ABSTRACT AWARD
Hypothalamic Neuropeptide Y (NPY) Y2 Receptors Protect Cancellous Bone From Leptin Induced Bone Loss. P. A. Baldock*[1], A. Sainsbury*[2], D. Lin*[3], M. Couzens*[2], R. F. Enriquez*[1], D. Matt*[3], H. Herzog*[2], E. M. Gardiner[1]. [1]Bone Program, Garvan Institute of Medical Research, Sydney, NSW, Australia, [2]Neurobiology Program, Garvan Institue of Medical Research, Sydney, NSW, Australia, [3]Dept of Molecular Medicine & Pathology, University of Auckland, Auckland, New Zealand.

M8
Prolonged Anabolic Steroid Therapy Promotes Bone Formation and Prevents Demineralization During Rehabilitation in Burned Children. K. D. J. Murphy*[1], S. Thomas*[1], D. L. Chinkes*[1], G. L. Klein[2], D. N. Herndon*[3]. [1]Department of Surgery, Shriners Hospitals for Children & The University of Texas Medical Branch, Galveston, TX, USA, [2]Department of Pediatrics, Shriners Hospitals for Children & The University of Texas Medical Branch, Galveston, TX, USA, [3]Departments of Surgery & Pediatrics, Shriners Hospitals for Children & The University of Texas Medical Branch, Galveston, TX, USA.

M9
Teriparatide Increases the Width of Modeling and Remodeling Osteons at the Trabecular and Endosteal Envelope. E. F. Eriksen, D. W. Donley*, Y. L. Ma. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

M10
The Risk of Developing Back Pain is Reduced in Postmenopausal Women with Osteoporosis following Teriparatide Compared with Alendronate Therapy. R. K. Dore[1], J. H. Krege[2], P. Chen*[2], E. V. Glass*[2], J. San Martin[2], P. D. Miller[3]. [1]UCLA, Anaheim, CA, USA, [2]Eli Lilly and Company, Indianapolis, IN, USA, [3]Colorado Center for Bone Research, Lakewood, CO, USA.

M11
Osteoformin Stimulates Differentiation of Human Chondrocytes. L. X. Bi[1], E. G. Mainous[2], W. L. Buford*[1]. [1]Depts of Surgery and Orthopaedics, University of Texas Medical Branch, Galveston, TX, USA, [2]Dept. of Surgery, University of Texas Medical Branch, Galveston, TX, USA.

M12
Clinical Observation Between Bone Mineral Density (BMD) and Testosterone (T) in Elderly Men. P. Li*. Elderly Ward, Beijing 304th Hospital of China Liberation Army, Beijing, China.

M13
PTH-dependent Osteocalcin Gene Expression Requires the Presence of an OSE1 Sequence in the Promoter and Multiple Signaling Pathways. G. Xiao, D. Jiang*, R. T. Franceschi, H. Boules*. Periodontics/Prevention/Geriatrics, The University of Michigan, Ann Arbor, MI, USA.

M14
Effects of 1,25-Dihydroxyvitamin D_3 and 25-Hydroxyvitamin D_3 on Osteoblast Differentiation in Human Marrow Stromal Cell Cultures. J. Glowacki[1], S. M. Mueller*[2], J. S. Greenberger[3], I. Bleiberg*[4], M. S. LeBoff[5]. [1]Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA, [2]University Hospital Zurich, Zurich, Switzerland, [3]Radiation Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [4]Sackler School of Medicine, Tel Aviv, Israel, [5]Medicine, Brigham and Women's Hospital, Boston, MA, USA.

M15
Parathyroid Hormone (1-14) Fragments Increase Bone Mass in OVX Rats. M. Shimizu*[1], H. Saito*[1], N. Shimizu*[1], N. Murao*[2], M. Kato*[2], J. T. Potts[3], T. J. Gardella[3], F. Makishima[1]. [1]Pharmaceutical Research Department II, Chugai Pharmaceutical Co., LTD., Shizuoka, Japan, [2]Pre-Clinical Research Department I, Chugai Pharmaceutical Co., LTD., Shizuoka, Japan, [3]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

M16
Clinical Experience with Serum Calcium and Vitamin D Levels in Patients Treated with Teriparatide [PTH(1-34)]. A. A. LICATA. Endocrinology, Cleveland Clinic Foundation, Cleveland, OH, USA.

M17
Circulating IGF-I is Essential for the Anabolic Effects of PTH on the Skeleton. S. Yakar[1], M. L. Bouxsein[2], H. Sun*[1], V. Glatt*[2], D. LeRoith[1], C. J. Rosen[3]. [1]Diabetes Branch, National Institutes of Health, Bethesda, MD, USA, [2]Orthopaedic Surgery, Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME, USA.

M18
Vitamin D3 and Ascorbic Acid 2-phosphate, a Long-acting Vitamin C Derivative Regulate Growth and Differentiation of Human Osteoblast-like Cells. R. Hata[1], Y. Maehata*[2], S. Takamizawa*[2], S. Ozawa*[2], S. Okada*[2], K. Izukuri*[2], Y. Kato*[2], S. Sato*[3], E. Kubota*[4], K. Imai*[5], H. Senoo*[5]. [1]Biochemistry and Molecular Biology, Research Center of Advanced Technology for Craniomandibular Func, Kanagawa Dental College, Yokosuka, Japan, [2]Biochemistry and Molecular Biology, Kanagawa Dental College, Yokosuka, Japan, [3]Department of Craniofacial Growth and Development Dentistry, Res Ctr Adv Techn Function, Kanagawa Dental College, Yokosuka, Japan, [4]Department of Oral Surgery, Res Ctr Adv Techn Function, Kanagawa Dental College, Yokosuka, Japan, [5]Department of Cell Biology and Histology, Akita University School of Medicine, Akita, Japan.

M19
Early Changes In Biochemical Markers Of Bone Formation Predict Improvements In Bone Structure During Teriparatide Therapy. A. Sipos*[1], H. Dobnig[2], A. Fahrleitner-Pammer[2], L. Ste-Marie[3], J. C. Gallagher[4], I. Pavo*[1], J. Wang*[1], E. F. Eriksen[1]. [1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [2]Internal Medicine, Medical University, Graz, Austria, [3]CHUM Hospital St-Luc, Montreal, PQ, Canada, [4]Bone Metabolism Unit, Creighton University, Omaha, NE, USA.

M20
A Single Intraosseous Injection of rhBMP-2/CPM Improves Femoral and Radial Structure in Ovariectomized Nonhuman Primates in 6 months. H. J. Seeherman, E. A. Smith-Adaline, J. D. Parkington*, H. Kim*, J. M. Wozney. Women's Health and Bone, Wyeth Research, Cambridge, MA, USA.

M21
Dkk2 Is Upregulated by Canonical Wnt and Stimulates Osteoblast Mineralization. X. Li*[1], P. Liu*[1], W. Liu*[1], Y. Zhang*[1], J. Zhang*[2], S. Harris*[2], D. Rowe*[1], D. Wu*[1]. [1]Genetics & Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Department of Oral Biology, University of Missouri at Kansas City, School of Dentistry, Kansas City, MO, USA.

M22
Preventive Effect of Zinc Acexamate Administration on Bone Loss in Diabetic Rats. M. Yamaguchi. Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, Shizuoka, Japan.

M23
Anabolic Effects of Nitric Oxide on Osteoblasts. S. J. Wimalawansa*. Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

M24
PTH Analogs Enhance Bone Formation at a Weight-Bearing Cement-Bone Interface. M. J. Allen*[1], J. E. Schoonmaker*[1], K. A. Mann*[1], V. Ross*[2], C. Allen*[2], G. E. Willick*[2], J. F. Whitfield[2]. [1]Orthopaedic Surgery, SUNY Upstate Medical University, Syracuse, NY, USA, [2]Biological Sciences, National Research Council, Ottawa, ON, Canada.

M25
Zoledronic Acid Increases Total Bone Volume in OP-1 Mediated Bone Formation in a Segmental Rat Femoral Defect Model. D. G. Little*, R. Bransford*, M. M. McDonald*, J. Briody*. Orthopaedic Research, The Children's Hospital Westmead, Sydney, Australia.

M26
A Novel Anabolic Peptide Activates Osteogenic Gene Expression in Rat Stromal Cells. G. Schneider*[1], K. J. Grecco*[2], D. McBurney*[2], W. E. Horton*[2]. [1]Weill Cornell Medical College in Qatar, New York, NY, USA, [2]Anatomy, NE Ohio Univers. Coll. of Med., Rootstown, OH, USA.

M27
PTHrP Down-Regulates Cyclin D1 Activity in Differentiating MC3T3 Cells. N. S. Datta, C. Chen*, L. K. McCauley. Perio/Prev/Geriatrics, University of Michigan, Ann Aror, MI, USA.

M28
A Mathematical Model for the Bone Remodeling Cycle Predicts the Effects of PTH(1-34) on Bone Turnover Markers in Rats. L. K. Potter*[1], G. B. Stroup[2], D. J. Rickard[2], Z. Wu*[3], B. J. Votta[2], S. M. Hwang*[2], F. L. Tobin*[4]. [1]Scientific Computing & Mathematical Modeling, GlaxoSmithKline, Research Triangle Park, NC, USA, [2]Musculoskeletal Diseases Biology, GlaxoSmithKline, Collegeville, PA, USA, [3]Assay Development and Compound Profiling, GlaxoSmithKline, Collegeville, PA, USA, [4]Scientific Computing & Mathematical Modeling, GlaxoSmithKline, King of Prussia, PA, USA.

M29
Revealing the Anabolic Effects of 1,25(OH)$_2$ Vitamin D3 by Co-Administration with Alendronate. A. A. Reszka, S. Pun*, L. P. Freedman, D. B. Kimmel. Molecular Endocrinology and Bone Biology, Merck Research Laboratories, West Point, PA, USA.

M30
PTH1R Edocytosis and G_q Signaling Independently Contribute to the Activation of the Mitogen-activated Protein Kinases ERK1 and ERK2. C. A. Syme*, A. Bisello. Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

M31
Teriparatide Mitigates the Cascade of Risk Associated With Increasing Osteoporosis Pathology. J. H. Krege*[1], H. K. Genant[2], G. G. Crans*[1], S. J. Vargas[3], J. C. Gallagher[4]. [1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [2]Osteoporosis and Arthritis Research Group, University of California - San Francisco, San Francisco, CA, USA, [3]Department of Endocrinology, William W. Backus Hospital, Norwich, CT, USA, [4]Bone Metabolism Section, Creighton University Medical Center, Omaha, NE, USA.

M32
Teriparatide Demonstrates Early Effects in Postmenopausal Women with Osteoporosis. W. J. Shergy*[1], M. Greenwald*[2], G. Woodson*[3], P. Chen*[4], D. A. Misurski*[4], R. B. Wagman[4]. [1]School of Medicine, University of Alabama at Birmingham, Huntsville, AL, USA, [2]Osteoporosis Medical Center, Palm Springs, CA, USA, [3]Department of Medicine, Emory School of Medicine, The Atlanta Research Center, Decatur, GA, USA, [4]US-Endocrinology, Eli Lilly and Company, Indianapolis, IN, USA.

M33
Mechanical Stimulation Prevents Osteocyte Apoptosis through an Integrin/Src/ERK Signalsome Localized in Caveolae: Involvement of a Ligand-Independent Function of the Estrogen Receptor. J. I. Aguirre, L. I. Plotkin, S. B. Berryhill*, R. S. Shelton*, S. A. Stewart*, R. S. Weinstein, A. M. Parfitt, S. C. Manolagas, T. Bellido. Endocrinology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

M34
Serum Protein Profiling by SELDI-TOF Mass Spectrometry for Biomarkers of PTH Response. A. K. Prahalad*[1], R. J. Hickey*[2,3], J. Huang*[4], S. Murthy*[1], T. Winata*[1], L. E. Dobrolecki*[2], J. M. Hock*[1,3]. [1]Anatomy & Cell Biology, Indiana Univ Sch. of Medicine, Indianapolis, IN, USA, [2]Dept of Medicine, Indiana Univ Sch. of Medicine, Indianapolis, IN, USA, [3]Indiana Cancer Research Institute, Indianapolis, IN, USA, [4]Dept of Computer Science, Indiana Univ, Indianapolis, IN, USA.

M35
Genetic and Activity Level Effects on Variation in BMD and BMC in a Human Genetic Isolate, the Schmiedeleut Hutterites. L. M. Havill[1], M. C. Mahaney[1], T. Binkley*[2], B. L. Specker*[2]. [1]Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA, [2]South Dakota State University, Brookings, SD, USA.

YOUNG INVESTIGATOR ORAL SESSION I

Moderators: Roland Baron and Joseph Zmuda

1:45 am – 2:45 pm

1:45 pm **11**
YOUNG INVESTIGATOR ABSTRACT AWARD
Hey1, a Direct Notch Target Gene, Is Up-regulated by BMP-2 and Reduces Osteoblast Matrix Mineralization and Cbfa1/Runx2 Transcriptional Activity. N. Zamurovic*, D. Cappellen*, D. Rohner*, M. Susa. Novartis Institutes for Biomedical Research, Basel, Switzerland.

2:00 pm **12**
YOUNG INVESTIGATOR ABSTRACT AWARD
Phosphophoryn, Encoded by Exon 5 of DMP-3, Regulates Osteoblast Differentiation via Integrin Signaling and MAP Kinase Pathway. J. A. Jadlowiec*[1], H. Koch*[2], P. Campbell[3], M. Seyedain*[4], C. Sfeir[5]. [1]Biological Sciences/Bone Tissue Engineering Center, Carnegie Mellon University, Pittsburgh, PA, USA, [2]Orthopaedic Surgery, University of Greifswald, Greifswald, Germany, [3]Institute for Complex Engineered Systems/Bone Tissue Engineering Center, Carnegie Mellon University, Pittsburgh, PA, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]Oral Medicine and Pathology, University of Pittsburgh, Pittsburgh, PA, USA.

2:15 pm **13**
YOUNG INVESTIGATOR ABSTRACT AWARD
Identification of LRP5 Sequences Responsible For and of Small Molecules Disrupting Dkk1-Mediated Antagonism. Y. Zhang*[1], X. Li*[1], J. Zhang*[2], S. E. Harries[2], J. Zheng*[3], D. Wu*[1]. [1]University of Connecticut Health Center, Farmington, CT, USA, [2]University of Missouri, Kansas City, MO, USA, [3]St. Jude Hospital, Memphis, TN, USA.

2:30 **14**
YOUNG INVESTIGATOR ABSTRACT AWARD
The Catechol-O-Methyltransferase val158met Polymorphism Is Associated with Bone Mineral Density in Young Adult Men. N. Andersson[1], A. Eriksson[1], M. Lorentzon[1], D. Mellström[2], C. Ohlsson[1]. [1]Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Department of Geriatric Medicine, Göteborg University, Göteborg, Sweden.

SESSION III

LRP5, Wnt and High Bone Mass

Moderators: Richard Bringhurst and Gerard Karsenty

2:45 pm – 4:15 pm

2:45 pm **15**
LRP5: Clinical Relevance
Karl L. Insogna, M.D.
Department of Internal Medicine and Endocrinology
Yale University School of Medicine
New Haven, Connecticut, USA

3:05 pm **16**
LRP5: Structural and Molecular Aspects
Mark L. Johnson, Ph.D.
Osteoporosis Research Center
Creighton University School of Medicine
Omaha, Nebraska, USA

3:25 pm **17**
LRP5 and the Wnt System
Peter V. N. Bodine, Ph.D.
Women's Health Research Institute
Wyeth Research
Collegeville, Pennsylvania, USA

3:45 pm **Questions**

BREAK
AND
POSTER VIEWING

4:00 pm – 4:15 pm

SESSION IV

Estrogens and SERMS

Moderators: Joel Finkelstein and Stavroula Kousteni

4:15 pm – 5:55 pm

4:15 pm **18**
The Classical ER Transcriptional Regulatory Pathway
Thomas C. Spelsberg, Ph.D.
Department of Biochemistry and Molecular Biology
Mayo Clinic College of Medicine
Rochester, Minnesota, USA

4:35 pm **19**
The Progesterone, Estrogen and Androgen Receptor Signaling Pathways Are Complex and Provide a Wealth of Opportunities for New Drug Discovery
Donald P. McDonnell, Ph.D.
Department of Pharmacology and Cancer Biology
Duke University Medical Center
Durham, North Carolina, USA

4:55 pm **20**
Activators of Non-Genotropic Estrogen-Like Signaling (ANGELS): A Novel Route to Bone Anabolism
Stavros C. Manolagas, M.D., Ph.D.
Department of Internal Medicine
Division of Endocrinology and Metabolism
Center for Osteoporosis and Metabolic Bone Diseases
University of Arkansas for Medical Sciences
Little Rock, Arkansas, USA

5:15 pm **21**
Can Estrogens and SERMs Be Anabolic?
Robert Lindsay, M.B.Ch.B., Ph.D., F.R.C.P.
Regional Bone Center
Helen Hayes Hospital
West Haverstraw, New York, USA
Columbia University
New York, New York, USA

5:35 pm **Questions**

POSTER VIEWING

5:55 pm – 7:00 pm

ADJOURN

7:00 pm

SESSION V

Growth Hormones and Growth Factors

Moderators: Roger Bouillion and Clifford J. Rosen

8:00 am – 10:20 am

8:00 am **22**
Genetic Strategies for Elucidating Insulin-like Growth Factor Action in Bone
Thomas L. Clemens, Ph.D.
Department of Pathology
University of Alabama at Birmingham
Birmingham, Alabama, USA

8:20 am **23**
Growth Hormone, IGFs and IGF-BPs: Clinical Aspects
Sundeep Khosla, M.D.
Endocrine Research Unit
Mayo Clinic College of Medicine
Rochester, Minnesota, USA

8:40 am **24**
BMP Biology Basics
Vicki Rosen, Ph.D.
Oral and Developmental Biology
Harvard University School of Dental Medicine and
The Forsyth Institute
Boston, Massachusetts, USA

9:00 am **25**
Sclerostin
Chris Paszty, Ph.D.
Department of Metabolic Disorders
Amgen Inc.
Thousand Oaks, California, USA

9:20 am **26**
FGF-2 in Bone Remodeling
Marja M. Hurley, M.D.
Division of Endocrinology
University of Connecticut Health Center
Farmington, Connecticut, USA

9:40 am **27**
Anabolic Factors for Fracture Healing
Thomas A. Einhorn, M.D.
Department of Orthopaedic Surgery
Boston University Medical Center
Boston, Massachusetts, USA

10:00 am **Questions**

BREAK AND POSTER VIEWING

10:20 am – 10:35 am

SESSION VI
PTH and PTHrP

Moderators: Elizabeth Shane and Dolores Shoback

10:35 am – 12:35 pm

10:35 am **28**
PTH: Basic Aspects
Henry M. Kronenberg, M.D.
Endocrine Unit
Massachusetts General Hospital and
Harvard Medical School
Boston, Massachusetts, USA

10:55 am **29**
Actions of PTH at the Tissue Level
David W. Dempster, Ph.D.
Department of Pathology
Columbia University
New York, New York, USA
Regional Bone Center
Helen Hayes Hospital
West Haverstraw, New York, USA

11:15 am **30**
PTH: Clinical Aspects
Susan L. Greenspan, M.D.
Department of Medicine
Osteoporosis Prevention and Treatment Center
University of Pittsburgh
Pittsburgh, Pennsylvania, USA

11:35 am **31**
Osteoblast-Derived PTHrP Is a Potent Endogenous Bone Anabolic Agent
Andrew C. Karaplis, M.D., Ph.D.
Department of Medicine
McGill University
Montreal, Quebec, Canada

11:55 am **32**
PTHrP: Clinical Aspects
Mara J. Horwitz, M.D.
Division of Endocrinology
Department of Medicine
University of Pittsburgh
Pittsburgh, Pennsylvania, USA

12:15 pm **Questions**

LUNCH
AND
POSTER VIEWING

12:35 pm – 1:45 pm

POSTER SESSION II
(T1 – T35)

1:00 pm – 1:45 pm

T1
YOUNG INVESTIGATOR ABSTRACT AWARD
Role of a Stretch-activated Potassium Channel in Mechanically-induced PTHrP Gene Expression in Osteoblasts. X. Chen, C. M. Macica*, B. E. Dreyer*, A. E. Broadus. Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA.

T2
YOUNG INVESTIGATOR ABSTRACT AWARD
CCAAT Enhancer Binding Proteins: Mediators of $1,25(OH)_2D_3$ and PTH Action that Affect Osteoblast Function. P. Dhawan*[1], X. Peng*[1], S. Williams*[2], S. Christakos[1]. [1]Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA, [2]Cell Biology and Biochemistry, Texas Tech University, Health Sciences Center, School of Medicine, Lubbock, TX, USA.

T3
YOUNG INVESTIGATOR ABSTRACT AWARD
Role of Crem Gene in the Anabolic Effect of PTH on Bone. F. Liu*[1], S. Lee[1], G. A. Gronowicz[2], D. J. Adams[2], B. E. Kream[1]. [1]Endocrionology, University of Connecticut Health Center, Farmington, CT, USA, [2]Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, USA.

T4
YOUNG INVESTIGATOR ABSTRACT AWARD
Individual and Combined Effects of Exercise and Alendronate on Material and Structural Properties of the Hip and Spine in Ovariectomized Rats. R. K. Fuchs[1,2], M. Shea[3], S. L. Durski*[2], B. Hanson*[3], B. K. Bay*[4], K. M. Winters[5], J. Widrick*[6], C. Snow[2]. [1]Anatomy and Cell Biology, Indiana University Medical School, Indianapolis, IN, USA, [2]Bone Researach Laboratory, Oregon State University, Corvallis, OR, USA, [3]Orthopedic Surgery, Oregon Health Science University, Portland, OR, USA, [4]Mechanical Engineering, Oregon State University, Corvallis, OR, USA, [5]Nursing, Oregon Health Science University, Portland, OR, USA, [6]Exercise and Sport Science, Oregon State University, Corvallis, OR, USA.

T5
YOUNG INVESTIGATOR ABSTRACT AWARD
A Mutation in the Osteoactivin/Gpnmb Gene Causes Osteopenia in Mice. M. C. Rico[1], M. G. Anderson*[2], A. Virgen*[1], S. W. M. John*[2], S. N. Popoff[1], F. F. Safadi[1]. [1]Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA, [2]HHMI and The Jackson Laboratory, Bar Harbor, ME, USA.

T6
YOUNG INVESTIGATOR ABSTRACT AWARD
Skeletal Disease Accompanying High Bone Mass and Novel LRP5 Mutation. M. R. Rickels*[1], X. Zhang*[2], S. Mumm[2,3], M. P. Whyte[2,3]. [1]Division of Endocrinology, Diabetes and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA, USA, [2]Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA, [3]Shriners Hospitals for Children, St. Louis, MO, USA.

T7
YOUNG INVESTIGATOR ABSTRACT AWARD
BMP6 Regulation of Human Marrow-derived Mesenchymal Stem Cell Differentiation. M. S. Friedman*[1], M. W. Long*[2], K. D. Hankenson[3]. [1]Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI, USA, [2]Pediatrics, University of Michigan, Ann Arbor, MI, USA, [3]Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA.

T8
Osteogenic Oxysterols Inhibit the Adverse Effects of Oxidative Stress on Osteogenic Differentiation of Marrow Stromal Cells. F. Parhami[1], C. M. Amantea*[1], J. A. Richardson*[1], T. J. Hahn[2], D. Shouhed*[1]. [1]Medicine, UCLA, Los Angeles, CA, USA, [2]Medicine, West L.A. VA Medical Center, Los Angeles, CA, USA.

T9
Decreased Estrogen May Contribute to Osteopenia in Unloaded Bones. J. C. Tou*, S. Arnaud*, R. Grindeland*, C. Wade*. Life Sciences Division, NASA Ames Research Center, Moffett Field, CA, USA.

T10
Anabolic Effects of Lactoferrin in Bone. J. Cornish, A. Grey, D. Naot, I. R. Reid. Medicine, University of Auckland, Auckland, New Zealand.

T11
Intermittent Parathyroid Hormone Treatment Enhances Guided Bone Regeneration in Rat Calvarial Bone Defects. T. T. Andreassen[1], V. Cacciafesta*[2]. [1]Department of Connective Tissue Biology, University of Aarhus, Aarhus C, Denmark, [2]Department of Orthodontics, The Royal Dental College, University of Aarhus, Aarhus C, Denmark.

T12
Cyclical Treatment with High Dose Calcitriol Increases Vertebral Bone Mass in Normal and Osteopenic Rats. R. G. Erben, K. Nägele*. Institute of Animal Physiology, University of Munich, Munich, Germany.

T13
The Acute Effects of a Novel Oral Formulation of Salmon Calcitonin on Bone Turnover in Healthy Postmenopausal Women. L. B. Tanko[1], Y. Z. Bagger[1], J. P. Devogelaer*[2], J. Y. Reginster*[3], L. Mindeholm*[4], M. Olson*[4], M. Azria*[4], C. Christiansen[1]. [1]Center for Clinical and Basic Research, Ballerup, Denmark, [2]Arthritis Unit, Université Catholique de Louvain, Brussels, Belgium, [3]3WHO Collaborating Center for Public Health Aspects of Osteoarticular Disease, Liege, Belgium, [4]Novartis, Basel, Switzerland.

T14
Sclerostin Is an Osteocyte-expressed Negative Regulator of Bone Formation, but not a Classical BMP Antagonist. C. W. Lowik*[1], P. ten Dijke*[2], R. L. van Bezooijen[1]. [1]Endocrinology, Leiden University Medical Center, Leiden, Netherlands, [2]Endocrinology, The Netherlands Cancer Institute, Division of Cellular Biochemistry, Netherlands.

T15
Increased Osteoblastic Differentiation in Cultured Marrow Cells After Blood Loss or Surgery. S. Odoi*, J. Burford*, R. Da Souza*, L. Parry*, T. Skerry. VBS, Royal Veterinary College, London, United Kingdom.

T16
Msx2 Regulates Mesenchymal Cell Lineage and Body Composition Via Paracrine Wnt-Dkk Signals. S. L. Cheng, N. Charlton-Kachigian, J. S. Shao, A. P. Loewy*, D. A. Towler. Dept of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA.

T17
Physical Activity Is Associated with the Size but not with the Volumetric Mineral Density of the Cortical Bone in Young Adult Men. M. Lorentzon[1], D. Mellström[2], C. Ohlsson[1]. [1]Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg, Sweden, [2]Department of Geriatric Medicine, Göteborg University, Göteborg, Sweden.

T18
Pathophysiology of Osteoporosis: Is the Major Defect (Bone Loss) Due to Metabolic Imbalance Between Bone Resorption and Formation, or to Insufficient Bone Collagen (Matrix) Formation? L. Klein. Biochemistry, Case Western Reserve Univ School of Medicine, Cleveland, OH, USA.

T19
Fluoroaluminate Stimulates and RGD Peptides Inhibit the Cellular Attachment and Spreading of Osteoblasts. C. J. C. Boersma*[1], R. J. Arends*[2], B. L. H. van Lith*[3], K. McGurk*[4]. [1]Target Discovery Unit Oss, NV Organon, Oss, Netherlands, [2]Pharmacology Unit Oss, NV Organon, Oss, Netherlands, [3]Target Discovery Unit Oss, NV Organon, Oss, Netherlands, [4]Lead Discovery Unit Oss, NV Organon, Oss, Netherlands.

T20
Bone Mass Has Reached its Peak in the Spine and Hip but Continues to Increase in the Cortices of the Long Bones in 18-20-Year-Old Men. M. Lorentzon[1], D. Mellström[2], C. Ohlsson[1]. [1]Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Department of Geriatric Medicine, Göteborg University, Göteborg, Sweden.

T21
Free Serum Estradiol Levels Correlate with both Trabecular and Cortical Volumetric Bone Mineral Density in Young Adult Swedish Men. M. Lorentzon, S. McGovern*, E. Svensson*, S. Heigis*, A. Eriksson, M. Svensson*, C. Ohlsson. Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden.

T22
Mice Deficient in ÃŸ-AR Signaling Have Increased Bone Mass Despite Increased Leptin Levels. M. L. Bouxsein, V. Glatt*, H. Dhillon*, E. Bachman*. Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, USA.

T23
Osteogenic Potential of Joint-Loading Modality. H. Yokota[1,2], S. M. Tanaka[1], H. B. Sun[1]. [1]Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Biomedical Engineering, Indiana University - Purdue University Indianapolis, Indianapolis, IN, USA.

T24
Loaded Bone Is the Target of the Anabolic Action of PTH. Y. Mikuni-Takagaki[1], K. Aoki[2], M. Takahashi*[2], K. Ohya[2]. [1]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan, [2]Department of Hard Tissue Engineering/Pharmacology, Tokyo Medical and Dental University, Graduate School, Tokyo, Japan.

T25
Exogenously Applied rhTGF-beta2 Enhances Bone Regeneration and Implant Fixation by Altering Gene Expression in a Rat Model. A. De Ranieri*[1], A. S. Virdi[1], S. Kuroda[1,2], D. R. Sumner[1]. [1]Anatomy & Cell Biology, Rush University Medical Center, Chicago, IL, USA, [2]Tokyo Dental and Medical University, Tokyo, Japan.

T26
The Phytoestrogen Genistein Enhances Osteoblastic Differentiation of Mouse Bone Marrow-derived Mesenchymal Stem Cells Through p38 MAPK Pathway. Q. C. Liao*[1], T. Liu*[1], L. D. Quarles[2], Y. F. Qin*[1], W. Pan*[1], H. H. Zhou*[1], Z. S. Xiao[1,2]. [1]Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, China, [2]Medicine, Duke University Medical Center, Durham, NC, USA.

T27
PTH-stimulated Cortical Bone Remodeling Is Differentially Regulated by Estrogens and Arrestins. D. Pierroz[1], S. L. Ferrari[1], V. Glatt*[2], R. Rizzoli[1], M. L. Bouxsein[2]. [1]Bone Diseases, Geneva University Hospital, Geneva, Switzerland, [2]Orthopedic Biomechanics Lab, Beth Israel Deaconess Medical Center, Boston, MA, USA.

T28
Use of a Simple Computerized Technique to Assess the Anabolic Effects of IGF-I in Mouse Bone Marrow Stromal Cells. T. L. Chen. Medicine/Endocrinology, V.A. Palo Alto Health Care System, Palo Alto, CA, USA.

T29
An Acceleration-based Anabolic Countermeasure to Bone Loss. R. Garman, C. Rubin, S. Judex. Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA.

T30
Connective Tissue Growth Factor (CTGF) Promotes Skeletogenesis. J. J. Song*, R. A. Kanaan*, F. F. Safadi, S. N. Popoff. Anatomy & Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA.

T31
Androstene Immune Regulating Hormones: A New Class of Potent Anabolic and Catabolic Regulators of Bone Resorption. N. H. Urban*[1], M. Holmes*[1], R. M. Loria*[2], M. J. Beckman[3]. [1]Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA, USA, [2]Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA, USA, [3]Biochemistry, Virginia Commonwealth University, Richmond, VA, USA.

T32
An Adynamic Osteodystrophy and Vascular Calcification Associated with the Metabolic Syndrome Is Worsened by CKD and Successfully Treated with Exogenous BMP-7. K. A. Hruska, R. J. Lund, M. R. Davies*, S. Mathew*. Pediatrics, Washington University, St. Louis, MO, USA.

T33
Dutasteride, a Potent 5 alpha Reductase Inhibitor, Does Not Effect Bone Density and Bone Metabolism in Healthy Men. R. V. Clark[1], A. M. Matsumoto*[2]. [1]Clinical Pharmacology, GlaxoSmithKline R & D, Research Triangle Park, NC, USA, [2]Internal Medicine, Univ of Washington School of Medicine, Seattle, WA, USA.

T34
Targeted Overexpression of Androgen Receptor in Osteoblasts Results in Complex Skeletal Phenotype. K. Wiren[1], M. Gentile*[2], S. Harada[2], K. Jepsen[3]. [1]VA Medical Center, Oregon Health & Science Univ, Portland, OR, USA, [2]Merck Research Laboratories, West Point, PA, USA, [3]Mt. Sinai School of Medicine, NY, NY, USA.

T35
Inorganic Phosphate Causes Rapid Changes in Gene Expression Through an ERK1/2 Dependent Pathway in MC3T3-E1 Osteoblasts. G. R. Beck, K. A. Simpson*. Center for Cancer Research, National Cancer Institute, Frederick, MD, USA.

YOUNG INVESTIGATOR ORAL SESSION II

Moderators: Jane Aubin and El-Hajj Fuleihan

1:45 pm – 2:45 pm

1:45 pm **33**
YOUNG INVESTIGATOR ABSTRACT AWARD
NF-E2 Megakaryocytes: A Novel Anabolic Pathway for Increased Bone Formation. M. A. Kacena[1], R. A. Shivdasani*[2,3], C. M. Gundberg[1], T. Nelson*[1], M. C. Horowitz[1]. [1]Orthopaedics, Yale University School of Medicine, New Haven, CT, USA, [2]Adult Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, [3]Medicine, Brigham and Women's Hospital, Boston, MA, USA.

2:00 pm **34**
YOUNG INVESTIGATOR ABSTRACT AWARD
Histone H4 Alternative Translation Stimulates Bone Mass Accrual. T. J. Noh*[1,2], E. Smith*[3,2], T. E. Myerrose*[4,5], T. Kohler*[6], M. Namdar-Attar*[7], N. Bab*[7], O. Lahat*[7], J. A. Nolta*[4,5], R. Müller[6], I. Bab[7], B. Frenkel[1,2]. [1]Biochemistry & Molecular Biology, Los Angeles, CA, USA, [2]Institute for Genetic Medicine, Los Angeles, CA, USA, [3]Orthopaedic Surgery, Los Angeles, CA, USA, [4]Pediatrics, Los Angeles, CA, USA, [5]and Children's Hospital Los Angeles, Keck School of Medicine at the University of Southern California, Los Angeles, CA, USA, [6]Institute for Biomedical Engineering, Swiss Federal Institute of Technology and University of Zurich, Zurich, Switzerland, [7]Bone Laboratory, The Hebrew University of Jerusalem, Jerusalem, Israel.

2:15 pm **35**
YOUNG INVESTIGATOR ABSTRACT AWARD
Irak-m Is a Negative Regulator of Osteoclast. H. Li*[1], E. Cuartas[1], W. Cui*[1], H. Lamallem*[1], Y. Choi*[2], H. Ke[3], R. Flavell*[4], K. Kobayashi*[4], A. Vignery[1]. [1]Orthopaedics Department, Yale University School of Medicine, New Haven, CT, USA, [2]University of Pennsylvania School of Medicine, Philadephia, PA, USA, [3]Pfizer Global Research and Development, Groton, CT, USA, [4]Section of Immunology, Yale University School of Medicine, New Haven, CT, USA.

2:30 pm **36**
YOUNG INVESTIGATOR ABSTRACT AWARD
Nonvertebral Fracture Risk Reduction During Treatment With Teriparatide Is Independent of Pretreatment Bone Turnover and Hip BMD. G. Crans*, B. Mitlak. Eli Lilly and Company, Indianapolis, IN, USA.

SESSION VII

Miscellaneous Anabolic Agents

Moderators: B. Lawrence Riggs and R. Graham Russell

2:45 pm – 4:35 pm

2:45 pm **37**
The Neuronal Control of Bone Formation
Gerard Karsenty, M.D., Ph.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas, USA

3:05 pm **38**
Prostaglandins: Basic and Clinical Studies
Lawrence G. Raisz, M.D.
Department of Endocrinology and Metabolism
University of Connecticut Health Center
Farmington, Connecticut, USA

3:25 pm **39**
Statins and Related Anabolic Agents
Gregory R. Mundy, M.D.
Department of Cellular and Structural Biology
University of Texas Health Science Center
at San Antonio
San Antonio, Texas, USA

3:45 pm **40**
Strontium
Pierre J. Meunier, M.D.
Faculty of Medicine
INSERM
Lyon, France

4:05 pm **Questions**

BREAK AND POSTER VIEWING

4:20 pm – 5:35 pm

CLOSING REMARKS

Moderator: Robert Nissenson

4:35 pm – 5:05 pm

4:35 pm **Future Directions in Skeletal Anabolic Research (Basic)**
Gerard Karsenty, M.D., Ph.D.
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas, USA

4:50 pm **Future Directions in Skeletal Anabolic Research (Clinical)**
Clifford J. Rosen, M.D.
Maine Center for Osteoporosis Research and Education
St. Joseph Hospital
Bangor, Maine, USA

POSTER VIEWING

5:05 pm – 6:00 pm

ADJOURN

6:00 pm

ABSTRACT KEY		
Monday Posters		**M**
Tuesday Posters		**T**
Denotes non-ASBMR membership		*** (asterisk)**
Session	**Presentation**	**Abstract**
Overview: Anabolic Therapy for Osteoporosis: The Urgent Need for a Consensus on Nomenclature	Oral Session	1
Session I: Exercise, Calcium and Vitamin D as Skeletal Anabolic Agents	Oral Session	2-5
Session II: Skeletal Anabolism: Transcriptional Regulation and Signaling	Oral Session	6-10
Poster Session I	Poster Session	M1-M35
Young Investigator Oral Session I	Oral Session	11-14
Session III: LRP5, Wnt and High Bone Mass	Oral Session	15-17
Session IV: Estrogens and SERMS	Oral Session	18-21
Session V: Growth Hormones and Growth Factors	Oral Session	22-27
Session VI: PTH and PTHrP	Oral Session	28-32
Poster Session II	Poster Session	T1-T35
Young Investigator Oral Session II	Oral Session	33-36
Session VII: Miscellaneous Anabolic Agents	Oral Session	37-40

1

Overview: Anabolic Therapy for Osteoporosis: The Urgent Need for a Consensus on Nomenclature. B. L. Riggs. Division of Endocrinology, Mayo Clinic, Rochester, MN, USA.

The convening of this workshop recognizes the development of a new class of drugs that promises to revolutionize osteoporosis therapy. However, as is made clear by the program of this meeting which groups diverse types of anti-osteoporotic drugs under the rubric of skeletal anabolic agents, a consensus on nomenclature is urgently needed. The new standard nomenclature should remove ambiguities, provide clarity of concept, and gain ready acceptance by the general medical and scientific community. The definition of terms should focus on effects on bone strength, describe cellular mechanisms, distinguish between the two major classes of anti-osteoporotic drugs, and incorporate evolving concepts about osteoporosis. One such concept is defining osteoporosis in terms of decreased bone strength rather than only decreased bone mineral density (BMD). Another concept is that the previously available class of drugs, which includes estrogen and the bisphosphonates, reduce fractures mainly by decreasing high bone turnover and its harmful effects on bone microstructure, rather than by increasing BMD. The final concept is that the new class of drugs, exemplified by its first member, PTH(1-34), reduce fractures not only by stimulating bone formation and producing large increases in BMD but also by improving bone quality. The earlier class of drugs reduces osteoclast activation, differentiation and function followed by a coupled decrease in bone formation. The new, emerging class enhances bone formation by amplifying mechanical signals, activating stem cells to differentiate along the osteoblast pathways, increasing work capacity or extending the lifespan of osteoblasts, or some combinations of these. The challenge will be to agree upon simple, widely acceptable names for each class - such as anti-catabolic and anabolic - and then to define them rigorously by incorporating as many of their unique characteristics as possible.

References:

1. Riggs, B.L. and Melton, L.J.: Bone turnover matters: the raloxifene treatment paradox of dramatic decreases in vertebral fractures without commensurate increases in bone density J Bone Miner Res 17:11-14, 2001.
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3. Parfitt, A.M.: Parathyroid hormone and periosteal bone expansion. J Bone Miner Res 17:1741-1743, 2002.
4. Dempster, D.W. et al. Effects of daily treatment with parathyroid hormone on bone microarchitecture and turnover in patients with osteoporosis: a paired biopsy study J Bone Miner Res 16:1846-1853, 2001.

Disclosures: **B.L. Riggs**, None.

2

Effects of Exercise on Bone. C. T. Rubin, Y. Qin, M. Hadjiargyrou, S. Judex. Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA.

Mechanical signals, in the guise of exercise, represent a key anabolic factor to the skeleton. The challenge remains in determining which specific components of mechanical loading represent anabolic signals, and then, if such signals can be applied to the skeleton in a manner which ultimately improves bone quantity and quality. Our own work has shown that extremely low magnitude (<<100 microstrain) mechanical signals can be strongly osteogenic if applied at a high frequency (10 to 100 Hz). Such high frequency low magnitude strains comprise a dominant component of a bone's strain history (Fritton et. al., J. Biomech, 2001), indicating that these mechanical events represent a significant determinant of bone morphology. Long term animal studies (one year) have shown that low level mechanical loading, inducing strains on the order of 5 microstrain, can increase cancellous bone volume fraction, thicken trabeculae, increase trabecular number and enhance bone stiffness and strength (Rubin et. al., Nature, 2001). Studies in the mouse have shown that these low level signals are not only anabolic, but extremely complex in terms of their molecular regulators (Judex et. al., FASEB, 2002). Preliminary work indicates that such signals can prevent bone loss in post-menopausal women (Rubin et. al., JBMR, 2004), and perhaps reverse osteopenia in children with disabling conditions (Ward et. al., JBMR, 2004). Such a biomechanical intervention is self-targeting, endogenous to bone tissue, and auto-regulated. In essence, these studies lay the groundwork for a unique, non-pharmacogenic and non-invasive intervention for osteoporosis, based purely on the premise of “form follows function” in the skeleton.

Disclosures: **C.T. Rubin**, Juvent, Inc. 4.

3

Calcium and Vitamin D: Basic Aspects. M. B. Demay. Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

The actions of 1,25-dihydroxyvitamin D are mediated by a nuclear receptor, the vitamin D receptor (VDR). In vitro analyses have demonstrated a key role for 1,25-dihydroxyvitamin D in the regulation of genes encoding bone matrix proteins and RANK ligand. In vivo studies demonstrate that deficiency of vitamin D or the VDR results in hypocalcemia, hyperparathyroidism, hypophosphatemia, rickets, and osteomalacia. Early institution of a diet high in calcium, phosphorus and lactose prevents these abnormalities (Amling M et al Endocrinology 140:4982), suggesting that 1,25-dihydroxyvitamin D and its receptor have a non-essential or redundant role in the skeleton. Notable in this respect is the observation that, although 1,25-dihydroxyvitamin D induces RANK ligand production, VDR null osteoblasts can support osteoclastogenesis when co-cultured with normal spleen cells, PTH and interleukin 1 alpha (Takeda S et al Endocrinology 140:1005). These data support the hypothesis that in the absence of the VDR, other regulatory molecules act to maintain skeletal homeostasis.

Other models reveal that both the receptor and ligand have skeletal effects in vivo. Mice overexpressing the VDR in osteoblasts have increased bone volume, demonstrating significant anabolic effects of the VDR (Gardiner E et al FASEB J 14:1908). In contrast, studies in 24-hydroxylase null mice suggest that high levels of 1,25-dihydroxyvitamin D impair mineralization during development by a VDR - dependent mechanism (St-Arnaud R et al Endocrinology 141:2658).

In spite of numerous elegant studies, questions regarding the role of the VDR and its ligand on the osteoblast remain unanswered. To address this, studies were performed in primary calvarial osteoblasts isolated from VDR null mice. These studies demonstrate that the VDR attenuates osteoblast differentiation and may play a role in lineage determination.

Disclosures: **M.B. Demay**, None.

4

Calcium and Vitamin D: Clinical Aspects. B. Dawson-Hughes. Calcium and Bone Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center at Tufts University, Boston, MA, USA.

Inadequate intakes of calcium and vitamin D have been associated with higher bone-remodeling rates, increased bone loss, and reduced secondary bone mineralization. Total body retention of calcium increases as calcium intake increases up to a plateau intake of about 1,200 mg/d in adult men and women. A mean serum 25(OH)D level of at least 75 to 80 nmol/L is needed for optimal bone health and reduced risk of falling (Dawson-Hughes B, Heaney RP, Holick M, Lips P, Meunier PJ, Vieth R. Vitamin D Roundtable. In: Nutritional Aspects of Osteoporosis, Burckhardt, Dawson-Hughes, Heaney (eds), Academic Press, San Diego, 2004). Randomized, controlled trials link increased calcium and vitamin D intakes to suppressed parathyroid hormone levels, reduced rates of bone turnover, and reduced bone loss but effects of calcium and vitamin D, individually, on fracture rates have been mixed. The combination of calcium and vitamin D supplements however lowers hip and all non-vertebral fracture rates in older adults (Chapuy MC, et al. Brit Med J 1994;308:1081-2 and Dawson-Hughes et al. New Engl J Med 1997;337:670-6). The National Academy of Sciences recommends 1,200 mg/d of calcium for men and women age 51 and older and 400 IU/d of vitamin D for those age 51-70 and 600 IU/d for men and women over age 70. There is increasing recognition however that these intakes of vitamin D will not raise mean 25(OH)D levels to the desired 80 nmol/L (32ng/ml) and that higher intakes are needed.

Disclosures: **B. Dawson-Hughes**, GlaxoSmithKline 5; Dairy Managment Inc. 2.

5

Mechanisms and Novel Anabolic Actions of Vitamin D and Its Analogs in Bone. J. W. Pike. Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.

The 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) hormone regulates mineral homeostasis in vertebrate organisms through its ability to control the expression of gene networks in kidney, intestine and bone. Its actions are mediated by the vitamin D receptor (VDR), a nuclear protein that is produced in vitamin D target tissues and which interacts at the level of DNA to modulate the transcriptional output of selected genes. Recent studies have indicated that the association of the VDR with DNA requires participation of a partner protein termed retinoid X receptor and the subsequent recruitment of numerous multi-protein complexes essential for the trans-regulation process. In studies to be discussed, we first explore the molecular dynamics of $1,25(OH)_2D_3$ activation using endogenous 25-hydroxyvitamin D_3-24-hydroxylase and osteopontin gene promoters as molecular targets. These studies together with experiments employing an unusual vitamin D analog lead us to propose specific principles that may underlie the molecular mechanisms responsible for gene selectivity. We also investigate the molecular actions of the novel $1,25(OH)_2D_3$ analog 2-methylene-19-nor-(20S)-$1,25(OH)_2D_3$ (2MD) synthesized by scientists at Deltanoid Pharmaceuticals, Inc. This highly potent compound exhibits a unique mineralizing activity on osteoblasts in vitro that is not seen with $1,25(OH)_2D_3$. These and other studies prompted an evaluation of the anabolic properties of 2MD in the aged, ovariectomized rat model. The results of this study suggest that low levels of 2MD potently increase overall bone mineral density at trabecular, vertebral and cortical bone surfaces via new bone formation. These and additional data indicate that 2MD shows significant promise as a potential anabolic therapeutic for bone loss associated with age, glucocorticoid therapy and menopause.

References:

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Disclosures: **J.W. Pike**, None.

6

Anabolic Actions of PTH in Bone: Role of AP-1 and Osteoclastogenesis. A. J. Koh*[1], J. Berry*[1], E. L. Ealba*[1], A. Mattos*[1], L. McCabe[2], L. K. McCauley[3]. [1]Perio/Prev/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [2]Physiology, Michigan State University, East Lansing, MI, USA, [3]Perio/Prev/Geriatrics and Dept. Pathology, University of Michigan, Ann Arbor, MI, USA.

The AP-1 family of transcription factors includes the major members, Fos (c-Fos, Fra-1, Fra-2, FosB) and Jun (c-Jun, JunB, JunD) in addition to ATF (activating transcription factor) and MAF (musculoaponeurotic fibrosarcoma) families (1). These pleiotropic transcriptional regulators form heterodimers (Fos/Jun) or homodimers (Jun/Jun) to interact with AP-1 sites on a large number of genes important in bone formation. Gene targeting models have revealed critical roles for AP-1 in skeletal development with osteosclerotic and osteopetrotic phenotypes emphasizing the role of specific family members (1). Skeletal hormones and growth factors such as PTH and PTHrP have been found to regulate AP-1 family members and suggest these transcriptional regulators may be instrumental in effects of anabolic agents in bone (2). In osteoblastic cells, PTHrP rapidly increases nuclear levels of c-Fos, Fra-1, Fra-2, FosB, and JunB proteins. The mRNA levels of these genes also increases but c-Jun and JunD are not altered. In vivo studies have also implicated these mediators (3). Since c-Fos is significantly upregulated in response to PTH, the anabolic actions of PTH in c-*fos* knockout mice were evaluated and PTH was ineffective at increasing bone volume during growth (4). The c-*fos* knockout mice are osteopetrotic and lack osteoclasts, making it difficult to discriminate specific dependence on c-*fos,* osteoclasts, or other hematopoietic components. Primary osteoblasts from c-*fos* genotypes, wildtype (WT), heterozygote (HET) or null, were found to have similar responses to PTH relative to proliferation, apoptosis, gene expression, and differentiation *in vitro* suggesting lack of osteoblast-associated c-*fos* dependence for PTH anabolic effects. A novel osseous transplant model where vertebral bodies (vossicles) were isolated from c-*fos* null, HET and WT mice, and implanted into athymic mice, was used to rescue the hematopoietic complement (e.g. osteoclasts) and test this dependence. hPTH (1-34) was administered daily for 3wks and histomorphometry revealed increased bone mass per area with PTH in vossicles from all genotypes. BRdU staining revealed that PTH increased numbers of proliferating cells in vossicles regardless of genotype, while vehicle-treated mice had proliferation limited to the bone lining cells. Taken together, these results indicate that the presence of c-*fos* positive osteoclasts or other cells in the bone marrow may be more important than c-*fos* expression in osteoblasts for PTH to exert its anabolic actions. In summary, AP-1 transcription factors are modified by anabolic agents and have important roles in the specific expression of bone associated proteins, and hence the process of bone formation. Targeted manipulation of these factors could facilitate the development of novel strategies to more directly treat bone loss than do current therapies.

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3) Stanislaus D, Devanarayan V, Hock JM. In vivo comparison of activated protein-1 gene activation in response to human parathyroid hormone (hPTH) (1-34) and hPTH(1-84) in the distal femur metaphyses of young mice. Bone 27:819-826, 2000.

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Disclosures: **L.K. McCauley**, None.

7

Δ FosB, a Truncated Isoform of FosB Lacking Transactivation Domains Induces Osteosclerosis and Inhibits Adipogenesis in Transgenic Mice. M. Wu*[1], G. Rowe*[1], M. Kveiborg[2], W. C. Horne[1], R. Baron[1]. [1]Orthopaedics, Yale University, School of Medicine, New Haven, CT, USA, [2]Institute of Molecular Pathology, Copenhagen, Denmark.

The AP-1 family of transcription factors consists of Fos- and Jun-related proteins, several of which play important roles in bone cell differentiation. ΔFosB and Δ2ΔFosB are naturally occurring splicing isoforms of the AP-1 transcription factor, FosB. Although both maintain the ability to heterodimerize with Jun proteins and bind consensus AP-1 sites, they lack the major C-terminal transactivation domain of FosB. In addition, a potential N-terminal transactivation domain is also absent in Δ2ΔFosB. Transgenic mice overexpressing ΔFosB under the control of the non-restricted NSE promoter develop an osteosclerotic phenotype as well as a dramatic decrease in adipose tissue. Furthermore, both in vitro studies and transgenic mice overexpressing ΔFosB specifically targeted to osteoblasts demonstrated that these effects are mediated by cell autonomous and independent mechanisms. Transgenic mice overexpressing only Δ2ΔFosB under the control of the NSE-promoter also develop a severe osteosclerosis, and significantly increased dynamic bone formation parameters. NSE-Δ2ΔFosB, as ΔFosB transgenic mice, exhibited a decrease in adipose tissue, with a reduced ability of the cells to differentiate into adipocytes. Thus, overexpression of the Δ2ΔFosB isoform, which conserves the DNA-binding and heterodimerization capacity, but is lacking any known transactivation domain, can induce both the osteoblast and the adipocyte phenotypes. Thus, ΔFosB affects osteoblast and adipocyte differentiation by mechanisms that do not require its own transcriptional activity. Since ΔFosB and Δ2ΔFosB interact with other AP1 family members and with Smads, Runx2 and CEBP-β, they induce osteoblast and inhibit adipocyte differentiation by interfering with the activity of other transcription factors or co-factors.

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proteins induces bone formation and inhibits adipogenesis *in vivo*. Nature Medicine 6:985-990, 2000.
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Disclosures: **R. Baron**, ProSkelia Pharmaceuticals 2, 3, 4, 7.

8

Runx/Cbfa1 Factors: Multifunctional Regulators of Skeletal Development. J. B. Lian, G. S. Stein, J. L. Stein*, A. J. van Wijnen, A. Javed. Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

All known mammalian *runt*-related transcription factors are expressed in osteogenic lineage cells. While Runx2 is obligatory for endochondral and intramembranous bone formation, Runx1 and Runx3 are also expressed in the skeleton. Runx factors share several unique properties that facilitate their functions as “master regulators” for maturation of cell phenotypes from stem cells. Detailed studies demonstrate that Runx2 (i) activates or represses target gene transcription in a promoter context dependent manner, a requirement for cell maturation; (ii) integrates signal transduction cascades induced by growth factors, cytokines and hormones, a requirement for physiologic responses (Zaidi et al., EMBO J., Epub ahead of print, 2004); (iii) maintains commitment to the osteogenic phenotype during proliferative expansion of osteoprogenitor populations (Pratap et al., Cancer Res., 63:5357, 2003; Zaidi et al, Proc. Natl. Acad. Sci. USA 100:14852, 2003); and (iv) contributes to developmental signaling (e.g., TGFβ/BMP smads, Src, Wnt factors, homeodomain proteins) for the control of bone formation (Zaidi et al, Proc. Natl. Acad. Sci. USA 99:8048, 2002). The tissue- specific formation of Runx multifunctional complexes on gene promoters facilitated by a unique nuclear matrix targeting signal that directs Runx factors to specific subnuclear domains for recruitment of co-regulatory proteins in a gene selective manner (Stein et al, Trends Cell Biology 13:584, 2003). Examples of the biological significance for this targeting function of Runx factors to support regulation of skeletal cell differentiation and tissue formation will be presented. The implication of these findings for potential applications to support Runx anabolic properties of bone will be discussed.

Disclosures: **J.B. Lian**, None.

9

PTH, Apoptosis and Bone Anabolism. T. Bellido, S. C. Manolagas, R. L. Jilka. Endocrinology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

The mechanism underlying the anabolic effect of intermittent PTH administration has remained elusive for over 60 years. Recent studies of ours in mice have revealed that daily injections of PTH attenuate osteoblast apoptosis, thereby increasing osteoblast number, bone formation rate, and bone mass, without affecting osteoclast number. In sharp contrast, sustained elevation of PTH by continuous infusion or by raising endogenous hormone secretion with a calcium-deficient diet, does not affect osteoblast apoptosis; as expected, however, it causes an increase in RANKL-mediated osteoclastogenesis and thereby osteoclast number and bone resorption. The anti-apoptotic effect of PTH is mediated by a cAMP/PKA signaling cascade leading to phosphorylation and inactivation of the pro-apoptotic protein Bad as well as CREB- and Runx2-mediated gene transcription. Consistent with its dependence on transient elevations of the hormone, the anti-apoptotic effect of PTH on osteoblasts is short-lived because PTH also increases proteasomal proteolysis of Runx2. In fact, the duration of the anti-apoptotic effect of PTH on osteoblasts can be prolonged by overexpressing Runx2, preventing the proteolytic degradation of Runx2, or blocking the interaction of Runx2 with Smurf1 E3 ligase which targets it for proteasomal degradation. The self-limiting nature of PTH-induced survival signaling on osteoblastic cells offers a mechanistic explanation for the requirement of repeated administration of the hormone for bone anabolism. Moreover, along with the recent discoveries that prostaglandin administration, ANGELS, sclerosteosis, or activating mutations of the Wnt receptor LRP5 all attenuate osteoblast apoptosis, our findings provide proof of principle that prevention of osteoblast apoptosis is a fundamental control mechanism for augmenting bone mass and a rational target for bone anabolic therapies at large.
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Disclosures: **T. Bellido**, NuVios 1.

10

PTH Responsive Genes and Novel Anabolic Targets. N. C. Partridge. Department of Physiology and Biophysics, University of Medicine and Dentistry at New Jersey and Robert Wood Johnson Medical School, Piscataway, NJ, USA.

Parathyroid hormone (PTH) is the major mediator of calcium homeostasis and bone remodeling, but has both bone resorption and bone formation actions. In fact, intermittent injections of PTH significantly increase bone apposition rate (Tam et al., Endocrinology 110, 506, 1982) and PTH (1-34, teriparatide) is approved for use as a treatment for osteoporosis. Yet the mechanisms responsible for the anabolic effects are still open to speculation (Qin et al., Trends Endo. Metab. 2004, in press). Our recent studies identified about 150 genes regulated by PTH (1-34) in a rat osteoblastic osteosarcoma cell line, UMR 106-01 (Qin et al., J. Biol. Chem. 278, 19723, 2003). We have investigated several of those genes further. Amphiregulin, a member of the epidermal growth factor family, and the cytokine, interleukin-18 are two of the genes highly regulated by PTH. The hormone stimulates expression of both genes in osteoblastic cells via the PKA signaling pathway and by primary events. In vivo studies suggest that both factors may be involved in the anabolic actions of PTH and/or be required for normal bone development. Overall, our work indicates that a network of genes is responsible for PTH’s effects in bone. Support: NIH grant DK48109.

Disclosures: **N.C. Partridge**, Orthofix 5.

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Hey1, a Direct Notch Target Gene, Is Up-regulated by BMP-2 and Reduces Osteoblast Matrix Mineralization and Cbfa1/Runx2 Transcriptional Activity. N. Zamurovic*, D. Cappellen*, D. Rohner*, M. Susa. Novartis Institutes for Biomedical Research, Basel, Switzerland.

To examine early events in osteoblast differentiation, we analyzed the expression of about 9,400 genes in the murine MC3T3 cell line, whose robust differentiation was documented cytochemically and molecularly. The cells were stimulated for 1 and 3 days with the osteogenic stimulus containing BMP-2. Total RNA was extracted and analyzed by Affymetrix GeneChip oligonucleotide arrays. A regulated expression of 394 known genes and 295 expressed sequence tags (EST) was detected. The sensitivity and reliability of detection by microarrays was shown by confirming the expression pattern for 20 genes by radioactive quantitative RT-PCR. Functional classification of regulated genes was performed, defining the groups of regulated Growth Factors, Receptors and Transcription Factors. The most interesting finding was concomitant activation of TGF-beta, Wnt and Notch signaling pathways, confirmed by strong up-regulation of their target genes by PCR. TGF-beta pathway is activated by stimulated production of the growth factor itself, while mechanism of Wnt and Notch activation remains elusive. We showed BMP-2 stimulated expression of Hey1, a direct Notch target gene, in mouse C2C12 cells, human mesenchymal cells and mouse calvaria. SiRNA-mediated inhibition of Hey1 induction led to an increase in osteoblast matrix mineralization, suggesting that Hey1 is a negative regulator of osteoblast maturation. This negative regulation is apparently achieved via interaction with Cbfa1/Runx2: Hey1 completely abrogated Cbfa1/Runx2 transcriptional activity. These findings identify Notch-Hey1 pathway as a negative regulator of osteoblast differentiation / maturation, which is a completely novel aspect of osteogenesis and could point to possible new targets for bone anabolic agents.

Disclosures: **N. Zamurovic**, Novartis Pharma AG 3.

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Phosphophoryn, Encoded by Exon 5 of DMP-3, Regulates Osteoblast Differentiation via Integrin Signaling and MAP Kinase Pathway. J. A. Jadlowiec*[1], H. Koch*[2], P. Campbell[3], M. Seyedain*[4], C. Sfeir[5]. [1]Biological Sciences/Bone Tissue Engineering Center, Carnegie Mellon University, Pittsburgh, PA, USA, [2]Orthopaedic Surgery, University of Greifswald, Greifswald, Germany, [3]Institute for Complex Engineered Systems/Bone Tissue Engineering Center, Carnegie Mellon University, Pittsburgh, PA, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]Oral Medicine and Pathology, University of Pittsburgh, Pittsburgh, PA, USA.

Extracellular matrix proteins (ECM) serve as both a structural support for cells and also as a dynamic bionetwork that directs cellular activities. ECM proteins such as those of the SIBLING family (Small Integrin-Binding LIgand Glycoprotein) could possess inherent growth factor activity. Phosphophoryn (exon 5 of dentin matrix protein 3) is a member of the SIBLING family and has been implicated in biomineralization as a nucleator/modulator of crystal formation. In this study, we demonstrate that PP may also have a signaling role. Quantitative real-time PCR technology was used to demonstrate up-regulation of *Runx2*, *Osx* and *Ocn* in primary human adult mesenchymal stem cells (hMSC), a mouse osteoblastic cell line (MC3T3-E1), and a mouse fibroblastic cell line (NIH3T3). Further, PP increased OCN protein production in hMSC and MC3T3-E1. ALP activity and calcium deposition were enhanced in hMSC. An $\alpha_v\beta_3$ integrin-blocking antibody significantly inhibited rPP-induced expression of *Runx2* in hMSC, suggesting that signaling by PP is mediated through the integrin pathway. Our data further shows that PP signals via the MAP kinase pathway; treatment of hMSC with rPP caused activation of the p38 component. These data demonstrate a novel signaling function for PP in regulating the expression of bone gene markers in addition to its hypothesized role in biomineralization. The implication is that PP could be combined with growth factors in novel tissue-engineered approaches for an enhanced therapeutic effect.

Disclosures: **J.A. Jadlowiec**, None.

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Identification of LRP5 Sequences Responsible for and of Small Molecules Disrupting Dkk1-Mediated Antagonism. Y. Zhang*[1], X. Li*[1], J. Zhang*[2], S. E. Harries[2], J. Zheng*[3], D. Wu*[1]. [1]University of Connecticut Health Center, Farmington, CT, USA, [2]University of Missouri, Kansas City, MO, USA, [3]St. Jude Hospital, Memphis, TN, USA.

The mechanism underlying the high bone mass mutation (G171V) has been investigated. This mutation reduces Dkk-1-mediated antagonism, suggesting that the first YWTD-EGF repeat domain (YED) where G171 is located may be responsible for Dkk-mediated antagonism. However, we found that the third YED, but not the first YED, is required for DKK1-mediated antagonism. Instead, the G171V mutation disrupts the interaction of LRP5 with Mesd, a LRP5/6 chaperon protein required for coreceptors' transport to cell surfaces and results in less LRP5 molecules on the cell surface. Although the reduction in the level of cell surface LRP5 molecules led to a reduction in Wnt signaling in a paracrine paradigm, the mutation did not appear to affect the activity of coexpressed Wnt in an autocrine paradigm. Together with the observation that osteoblast cells produce an autocrine canonical Wnt and that osteocytes produce paracrine Dkk1, we believe that the G171V mutation may cause an increase in Wnt activity in osteoblasts by reducing the number of targets for paracrine Dkk1 to antagonize without affecting the activity of autocrine Wnt. Moreover, identification of the third YTD as the Dkk1-binding domain led us to map the interaction surface by mutagenesis. This information, together with the deduced tertiary structure of the third YTD, allowed us to identify, via "virtual" computer-aided screening, small molecule compounds that may in theory disrupt the Dkk and LRP5 interaction. Many of the compounds were tested and showed potent inhibition of the interaction. Their *in vivo* and *in vitro* effects on osteogenesis are being investigated..

Disclosures: **Y. Zhang**, None.

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The Catechol-O-Methyltransferase val158met Polymorphism Is Associated with Bone Mineral Density in Young Adult Men. N. Andersson[1], A. Eriksson[1], M. Lorentzon[1], D. Mellström[2], C. Ohlsson[1]. [1]Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Department of Geriatric Medicine, Göteborg University, Göteborg, Sweden.

Peak bone mineral density (peak BMD) is an important predictor of future risk of osteoporosis. Estrogens influence the accretion of bone mass during puberty. Catechol-O-Methyltransferase (COMT) is involved in the degradation of estrogens. There is a functional polymorphism in the COMT gene (val158met), resulting in a 60-75% difference in enzyme activity between the val (high activity = H) and met (low activity = L) variants. The aim of the present study was to investigate the associations between this polymorphism and peak BMD in young men. 458 healthy men (age 19, SD 0.6) were genotyped and classified as $COMT^{LL}$, $COMT^{HL}$ or $COMT^{HH}$. Bone parameters were measured using both DXA and pQCT. Regression models using physical activity, height, weight, age and COMT genotype as covariates showed that COMT genotype was an independent predictor of areal BMD in the total body, total femur and trochanter ($p<0.01$) but not in the spine. Areal BMD of the femur was 3.7% lower in $COMT^{LL}$ than in $COMT^{HL}$, while the values for $COMT^{HL}$ and $COMT^{HH}$ were very similar. pQCT analyses demonstrated that COMT genotype was an independent predictor of trabecular vBMD in the tibia, radius and fibula ($p<0.05$). Trabecular vBMD of the radius in $COMT^{LL}$ was 5.4% and 5.1% lower than that of $COMT^{HL}$ and $COMT^{HH}$ respectively. COMT genotype was associated with cortical volumetric BMD ($p<0.05$) but not with cortical cross sectional area in the tibia. These findings demonstrate that the COMT polymorphism is associated with BMD in young adult men.

Disclosures: **N. Andersson**, None.

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LRP5: Clinical Relevance. K. L. Insogna. Yale University School of Medicine, New Haven, CT, USA.

Gain of function mutations in LRP-5 cause inherited syndromes of high bone mass that appear to result from alterations in osteoblast function. Conversely, loss of function mutations cause low bone mass and low bone formation rates. Two kindreds with high bone mass and a G171V mutation in LRP-5 have been identified. Skeletal anatomy was largely normal except that one kindred (Boyden et al, New Engl J. Med. 346:1513, 2002) showed a widened mandible and torus palatinus. Interestingly, in a study of 452 women, the presence and size of torus palatinus was significantly associated with higher bone mass (Belsky et al, J. Clin Endocrinol Metab. 88:2081, 2003). Six other LRP-5 mutations have been identified that cause dominantly-inherited high bone mass, all occurring in a restricted region of the extracellular domain (Van Wesenbeeck et al, Am. J. Hum. Genet. 72: 763, 2003). The G171V mutation confers resistance to Dkk1, an endogenous inhibitor of Wnt/LRP5 signaling. Bone cells isolated from a patient with the G171V mutation evidence accelerated mineralization compared to normal cells, suggesting that Wnt/LRP-5 signaling may play a role in mineralization (Yao et al, J Bone Min Res 17 (Suppl 1): S196, 2002). A population-based study of five LRP-5 polymorphisms with allele frequencies >2% found that missense substitutions V667M and A1330V and their haplotypes were associated with vertebral BMC, projected area and stature, accounting for up to 15% of population variance in adult males (Ferrari S et al, Am J Hu Genet, in press). Moreover, the A1330V substitution was associated with increased risk for idiopathic male osteoporosis. In summary, mutations in LRP-5 cause syndromes of low and high bone mass, and allelic variants in LRP-5 may contribute to the heritable component of bone mass. Exploring the Wnt/LRP-5 pathway may provide targets for discovery of skeletal anabolics.

Disclosures: **K.L. Insogna**, None.

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LRP5: Structural and Molecular Aspects. M. L. Johnson. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

New insights into the regulation of bone formation have recently emerged from studies of mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) {Little et al., Am J. Hum. Genetics, 70:11-19, 2002; Gong et al., Cell 107:513-523, 2001; Boyden et al., N. Engl. J. Med. 346:1513-1521, 2002; Van Wesenbeck et al. Am. J. Hum. Genet. 72: 763-771, 2003} that have identified this receptor and the Wnt signaling pathway as critical in the control of bone formation. The LDL receptors are a family of multifunctional cell surface proteins. LRP5, along with its close relative LRP6, and the *Drosophila* homologue, *Arrow*, have been identified as co-receptors with the seven-transmembrane receptor, *frizzled*, in the canonical Wnt signaling pathway {Wehri et al. Nature 407: 527-530, 2000}. Binding of Wnt to the LRP5-*frizzled* co-receptor complex leads to increases in the cytoplasmic concentration of β -catenin and changes in transcription of several target genes. A secreted inhibitor to Wnt signaling called Dickkopf-1 (Dkk1) has recently been shown to bind LRP5 to a transmembrane protein, kremen, and this leads to its internalization and ultimate degradation {Mao et al., Nature 417: 664-667, 2002; Rothbacher et al., Nature Cell Biology 4: 172-173, 2002}. The G171V mutation, that causes high bone mass in humans and transgenic mice (HBM), has been shown to result in reduced inhibition of the canonical Wnt signaling pathway by Dkk1, and increased activation of the pathway in response to mechanical loading. The mutation also lowers the threshold for response to mechanical loading. Furthermore, the G171V mutation results in increased production of OPG mRNA in response to loading, which could result in a reduction in osteoclastogenesis. These data suggest that the Lrp5-Wnt signaling pathway is an integral part of the cellular machinery that mediates responsiveness to mechanical loads.

Disclosures: **M.L. Johnson**, Genome Therapeutics Corporation and Wyeth Research 2.

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LRP5 and the Wnt System. P. V. N. Bodine. Women's Health Research Institute, Wyeth Research, Collegeville, PA, USA.

Regulation of canonical Wnt signaling in osteoblasts has been shown to play an important role in bone formation. Loss-of-function mutations in the Wnt co-receptor, low-density lipoprotein receptor-related protein (LRP) 5, cause osteoporosis pseudoglioma syndrome in humans and mice, while gain-of-function mutations (e.g., G171V) lead to high bone mass phenotypes. Additionally, deletion of LRP6 and ablation of the Wnt antagonist secreted frizzled-related protein (sFRP)-1 from mice has extended our understanding of Wnt pathways in osteoblast function. LRP5-/- mice exhibit decreased trabecular bone volume (TBV) at 2 weeks of age due to reduced osteoblast proliferation and activity (Kato et al. 2002 J. Cell Biol. 157: 303-314). LRP6-/+ mice also display diminished TBV indicating that LRP5 and 6 are both required for optimal osteoblast function (Kharode et al. 2003 J. Bone Miner. Res. 18: S60). In contrast, transgenic LRP5G171V/+ mice demonstrate increased TBV at 5 weeks of age due to reduced osteoblast/osteocyte apoptosis and elevated osteoblast number (Babij et al. 2003 J. Bone Miner. Res. 18: 960-974). Additionally, sFRP-1-/- mice show heightened TBV, but not until ~27 weeks of age when enhanced osteoblast proliferation, differentiation and activity, as well as diminished osteoblast/osteocyte apoptosis are observed (Bodine et al. 2004 Molec. Endocrinol. 18: in press). Thus, regulation of the canonical Wnt pathway via modulation of LRP5/6 affects early postnatal bone accrual by alteration of osteoblast proliferation, activity and/or apoptosis, while control of Wnt signaling by sFRP-1 does not affect bone formation until adulthood when it restrains many aspects of osteoblast physiology. Taken together, these data suggest a role for non-canonical Wnt pathways in osteoblast function. Moreover, differential expression of LRP5 and sFRP-1 during osteoblast development and the ability of sFRP-1 to act as a paracrine/autocrine regulator may contribute to these phenotypes.

Disclosures: **P.V.N. Bodine**, Wyeth Research 3.

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The Classical ER Transcriptional Regulatory Pathway. T. C. Spelsberg[1], D. G. Monroe[1], F. J. Secreto[1], D. C. Muddiman*[1], B. L. Riggs[2], S. Khosla[2]. [1]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, USA, [2]Department of Internal Medicine, Division of Endocrinology, Mayo Clinic College of Medicine, Rochester, MN, USA.

Estrogens (E) and SERMs serve as major regulators of skeletal homeostasis in males and females and primary therapies for prevention of bone loss. E directly regulates both osteoblasts (OB) and osteoclast (OC) gene expression and activity, and indirectly OB-OC coupling via E-regulated paracrine factors (reviewed in 1). The current classical ER transcription (genomic) pathway also occurs in human OB cells. The pathway (reviewed in Figure) involves E or SERM binding to the estrogen receptor isoforms (ERα and ERβ), the subsequent binding of the complex to target gene promoters, followed by the association of specific nuclear co-activators or co-repressors, which, in turn, activate or inhibit gene transcription, respectively (reviewed in 2,3). This laboratory has recently described some of the actions of ER isoforms and selected co-regulators on human OB gene expression and cell functions when exposed to E and SERM (4,5). In OB cell lines, containing doxycycline regulated ERα, ERβ, or both ERα and ERβ isoforms, specific patterns of gene expression are observed (4,5). Recent studies have revealed gene specific antagonisms as well as unique patterns of gene expression in OB cells containing both isoforms. Studies on ER-co-activator interactions in OB cells demonstrate a selectivity of ERα for SRC-2 and ERβ for SRC-1 (5). The SRC-3 co-activator is not present in these cells. This laboratory has recently identified a ligand specific binding of OB cell co-activators (SRC-1 and SRC-2), as well as the co-repressor (REA), to each of the ER isoforms using GST pull-downs, western blotting, and mass spectrometry.

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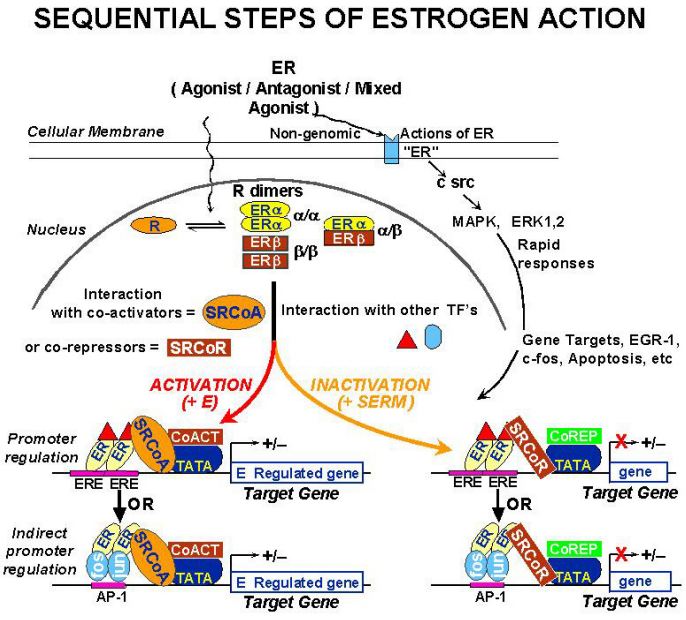

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Disclosures: **T.C. Spelsberg**, None.

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The Progesterone, Estrogen and Androgen Receptor Signaling Pathways Are Complex and Provide a Wealth of Opportunities for New Drug Discovery. D. P. McDonnell*. Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA.

The classical models of steroid receptor pharmacology held that agonists functioned by binding to their cognate receptors facilitating their conversion from an inactive form to one that was capable of activating transcription. By extrapolation, it was believed that antagonists functioned by competitively inhibiting agonist binding, freezing the receptor in an inactive state. However, as early as 1967 when the biological actions of the "antiestrogen" tamoxifen were first described it was clear that this simple model did not adequately describe estrogen receptor (ER) pharmacology. Tamoxifen is more appropriately classified as a Selective Estrogen Receptor Modulator (SERM), one of a group of compounds whose agonist or antagonist activity can differ between cells. Similarly, tissue selective progesterone, androgen and glucococorticoid receptor modulators have also been identified indicating that the observed complexity of ER action extends to other steroid receptors. Significant progress has been made in defining the molecular mechanism(s) by which cells distinguish between agonists and antagonists and how some receptor modulators can manifest their actions in a cell-selective manner. The most important of these are (1) differences in the relative expression level of receptor isoforms or subtypes, (2) the impact which the bound ligand has on the structure of its cognate receptor, and (3) the complement of coactivators and corepressors in a target cell which can interact with the activated receptor. This presentation will focus on the role of coactivators and corepressors in nuclear receptor pharmacology and how these proteins regulate cellular responses to agonists and antagonists and how perturbations in these regulatory mechanisms can have pathological consequences.

Disclosures: **D.P. McDonnell**, Ligand Pharmaceuticals 5; Wyeth Pharmaceuticals 8; GlaxoSmithKline 2.

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Activators of Non-Genotropic Estrogen-Like Signaling (ANGELS): A Novel Route to Bone Anabolism. S. C. Manolagas, S. Kousteni, T. Bellido, R. S. Weinstein, C. O'Brien, R. L. Jilka. Department of Internal Medicine, Division of Endocrinology and Metabolism, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Estrogens and androgens slow the rate of bone remodeling by attenuating the birth rate of osteoclasts and osteoblasts from their respective progenitors. They also act to maintain a focal balance within each remodeling cycle by shortening the lifespan of osteoclasts and prolonging the lifespan of osteoblasts. At least part of these effects stem from nongenotropic actions of the ligand-activated receptors resulting in kinase (Src/ERK, PI3K and JNK)-mediated regulation of common transcription factors. Such nongenotropic actions can be functionally dissociated from classical genotropic transcriptional activity of the receptors with synthetic ligands, dubbed ANGELS. This function-selective class of ER/AR ligands is capable of increasing bone mineral density and bone strength in both female and male mice, significantly more than estrogens or androgens without affecting reproductive organs by up- or down-regulating a pool of genes that is distinct from that regulated by the classical ligands. Hence, classical genotropic actions of sex steroid receptors are essential for their effects on reproductive tissues, but dispensable for their bone protective effects; and ANGELS have the potential to cause positive focal balance between formation and resorption and continuous gain in bone mass. Consistent with unique bone anabolic effects of ANGELS, as distinguished from the anti-remodeling/anti-catabolic effects of estrogens, ANGELS (but not estradiol, androgens or their metabolites) induce the commitment of pluripotent mesenchymal stem cell progenitors and also promote the differentiation of committed osteoblastic cells toward the osteoblastic lineage. These actions are mediated by Src-, PI3K- and JNK-mediated potentiation of BMP-2 and Wnt signaling cascades and β-catenin mediated transcription.

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Disclosures: **S.C. Manolagas**, NuVios 1, 2, 4, 5.

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Can Estrogens and SERMs Be Anabolic? R. Lindsay[1,2]. [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Columbia University, New York, NY, USA.

Estrogen traditionally has been thought of as an anti-resorptive agent, whose primary effect is inhibition of bone remodeling. However, estrogen deficiency at the time of menopause often results in prolonged loss of bone mass, with architectural disruption that in combination lead to increased fracture risk. Thus, in addition to increased of activation of remodeling, there must also be a deficit in new bone formation within each remodeling cycle to produce progressive bone loss. This could occur because of impaired formation, or a failure of the osteoblast population to respond to increased avidity of the osteoclasts. While Albright considered the former the likely culprit, it has become a common assumption that it is the latter. In animal models estrogen has been shown by some groups to increase osteoblast number, and active formation surface, and clearly stimulates medullary bone formation during the egg laying cycle. Evidence that estrogen is anabolic in human bone has been harder to obtain. Mostly, studies with estrogen in humans suggest reduction in remodeling and activation frequency as the major effect; some have suggested that there is positive bone balance within each remodeling site; others have suggested a more definitive anabolic effect at high doses. It has also been suggested that there may be some anabolic activity related to the progestin, especially the 19-nortestosterone derivatives. Anabolic activity of the SERMs has not been demonstrated in humans.

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Disclosures: **R. Lindsay**, Wyeth 2, 8; Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and Aventis) 2, 8; Ilex 2; Novartis, Berlex 5; Eli Lilly and Company 5, 8.

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Genetic Strategies for Elucidating Insulin-like Growth Factor Action in Bone. T. L. Clemens. Department of Pathology, University of Alabama at Birmingham, Birmingham, OH, USA.

Insulin like growth factor-I (IGF-I) exerts profound anabolic effects on bone and has been postulated to mediate the anabolic actions of PTH. The recent development of genetically engineered mice offers an appropriate bridge between cell culture models and studies in humans. Transgenic mice overexpressing IGF-I in osteoblasts have an increased rate of bone formation and a decrease in the mineralization lag time (Zhao et al, *Endocrinology* 2674-82, 2000). Therefore, locally produced IGF-I not only accelerates new bone formation but also increases the pace at which matrix is mineralized. Remarkably, these changes occur without any change in the total number of osteoblasts, implying that IGF-I functions primarily to increase the performance of resident osteoblasts. By contrast, bones from mice lacking the IGF-IR generated by Cre-mediated recombination methods are normal in length but display a striking reduction in cancellous bone. Surprisingly however, the amount of unmineralized osteoid is increased in the mutants and is accompanied by an increase in osteoclast erosion surface. Primary osteoblasts from the mutant mice fail to mineralize in culture and exhibit a marked reduction in several genes associated with mineral deposition (Zhang et al*, J Biol Chem* 44005-12, 2002). Thus despite defective IGF-I signaling, osteoblasts are able to mature and deposit osteoid normally, but are unable to perform their final function, namely to mineralize bone matrix. Current studies are underway to manipulate additional components of the IGF axis in a time and tissue-specific manner (Zhang et al, *J Bone Miner Res* 836-43, 2003). Results from these studies should lead to new hypotheses concerning the roles of the IGF system in maintenance of bone health and hopefully will identify new therapeutic opportunities.

Disclosures: **T.L. Clemens**, None.

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Growth Hormone, IGFs and IGF-BPs: Clinical Aspects. S. Khosla. Endocrine Research Unit, Mayo Clinic College of Medicine, Rochester, MN, USA.

Puberty is associated with a marked increase in bone and muscle mass, driven in large part by activation of the growth hormone (GH)-insulin-like growth factor (IGF) axis. By contrast, senescence is characterized by significant loss of bone and muscle mass (osteopenia and sarcopenia, respectively) associated with declining GH and IGF-I production. This has led to the longstanding, plausible, and yet unproven hypothesis that treatment of aging individuals with GH or IGFs could reverse osteopenia and sarcopenia without significant adverse side-effects. A number of small, randomized trials of GH therapy of aging individuals have been conducted over the past decade and the results have been equivocal, at best (1). However, a recent randomized, placebo controlled trial of 80 postmenopausal women on estrogen therapy found remarkable increases in bone mineral content (BMC) and bone mineral density (BMD) at several skeletal sites after 48 months, with GH having been administered for 36 months, consistent with a delayed and extended effect of GH on bone (2). Lean mass also increased significantly. There is even more limited data on the use of IGF-I as an anabolic agent, in large part due to significant, dose-dependent side effects (3). Since IGF binding proteins (IGFBPs) can both modulate IGF action as well as serve to potentially transport/target IGFs to particular tissues, combinations of IGF-1 and IGFBP-3 have been used in animal and in a small human study (4). Finally, based on findings in the rare syndrome of hepatitis C-associated osteosclerosis, we have suggested that a combination of IGF-II (or its precursor, IGF-IIE) and IGFBP-2 may be effective in targeting IGFs to bone, with subsequent anabolic effects (5). In summary, the GH-IGF axis remains a promising, but as yet unproven, target for novel anabolic approaches to treat age-related osteopenia and sarcopenia.

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Disclosures: **S. Khosla**, None.

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BMP Biology Basics. V. Rosen. Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, MA, USA.

Bone morphogenetic proteins (BMPs) are secreted signaling molecules used by all multicellular organisms including those without skeletons. They act locally on target cells to affect cell survival, proliferation and differentiation (1). BMPs have clinical utility as bone regeneration agents in adults, inducing de novo bone formation when implanted into bone defects, and accelerating the rate of fracture healing when applied at the fracture site (2). The potent osteogenic activity of exogenously applied BMPs suggests that BMPs may both regulate formation of skeletal tissue during embryogenesis and help maintain bone mass in adults. Data from studies of embryonic skeletal development support the idea that BMP activity is a central effector of bone formation and also highlight the many levels of control that exist to modulate BMP action (3). Studies of statins, estrogens and other bone anabolic agents suggest that regulation of BMP synthesis and deposition into bone matrix may be key to maintaining bone mass in adults (4). This idea is also supported by experiments in which transgenic mice that have been engineered to over express BMP antagonists display osteopenia and spontaneous fractures in postnatal life (5). As a whole, our current understanding of BMP biology highlights the potential of BMPs as anabolic agents in bone and points to the need for future studies focusing on the regulation of BMP activity in the adult skeleton.

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Disclosures: **V. Rosen**, None.

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Sclerostin. N. Sun*[1], Y. Gao*[2], J. Pretorius*[3], S. Morony[2], K. Warmington*[2], Z. Geng[2], P. J. Kostenuik[2], S. Simonet[2], D. L. Lacey[2], I. Sarosi*[3], C. Kurahara*[1], C. Paszty[2]. [1]Functional Genomics, Amgen Inc., Thousand Oaks, CA, USA, [2]Metabolic Disorders, Amgen Inc., Thousand Oaks, CA, USA, [3]Pathology, Amgen Inc., Thousand Oaks, CA, USA.

In humans, complete lack of the protein sclerostin due to homozygosity for null mutations in the SOST gene is responsible for causing sclerosteosis, a rare genetic disease characterized by increased bone mineral density (BMD) throughout the skeleton (Brunkow ME *et al.* 2001. Am. J. Hum. Genet. 68: 577-589). Sclerostin, together with Sclerostin-like, forms a two member gene family distantly related to the DAN family of bone morphogenetic protein (BMP) antagonists. Sclerostin is expressed in bone by osteocytes, binds to BMPs and has an inhibitory effect on osteoblast differentiation/function in cell culture and in transgenic mice (Winkler DG *et al.* 2003. EMBO J 22: 6267-6276). Wise, the Xenopus ortholog of Sclerostin-like, has been shown to bind LRP6 and to be both an inhibitor and activator of Wnt signaling in a context-dependent manner (Itasaki N *et al.* 2003. Development 130: 4295-4305). At present, the details of Sclerostin's mechanism(s) of action *in vivo* remain somewhat obscure, however, based on the genetic and expression data, a novel bone homeostatic pathway in humans has been discovered, in which osteocytes, by secreting sclerostin, negatively modulate osteoblast mediated bone anabolic activity.

Similar to humans with sclerosteosis, knock-out mice homozygous for a deletion of the SOST gene have increased BMD throughout their skeleton but are otherwise essentially normal. The conservation of sclerostin biology from human to mouse, coupled with further *in vitro* and *in vivo* studies should serve as a means towards gaining a deeper understanding of the basic biology surrounding this novel pathway, as well as it's therapeutic potential in the anabolic treatment of osteoporosis.

Disclosures: **C. Paszty**, Amgen Inc 1, 3.

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FGF-2 in Bone Remodeling. M. M. Hurley. Endocrinology & Metabolism, University of Connecticut Health Center, Farmington, CT, USA.

Fibroblast growth factor (FGFs) ligands and receptors (FGFRs) are important in bone development and remodeling. FGFs signal via activation of receptor tyrosine kinases and recruitment of intracellular signaling proteins. FGF2 is produced by osteoblasts/stromal cells, stored in bone matrix, and could function in an intracrine, paracrine or autocrine manner to modulate bone cell function. FGF2 has dual effects on osteoblast in vitro with stimulatory effects on proliferation of precursors and inhibitory effects on type 1 collagen synthesis by differentiated osteoblasts. FGF2 also potentiates the survival effects of IGF-1. Similar to PTH, FGF2 increases osteoclast formation and bone resorption. In addition, continuous FGF2 treatment inhibits, while intermittent FGF-2 stimulates bone formation in vitro and in vivo. In rodents, FGF2 induces new bone formation on endosteal and trabecular bone surfaces. FGFs also play an important role in fracture repair. Further evidence for the importance of FGF2 in bone is derived from the finding that Fgf2-/- mice develop low bone mass and decreased bone formation with age. Anabolic factors such as prostaglandins, TGFb, BMP2 and PTH regulates Fgf2 mRNA and protein levels in osteoblasts. Interestingly, the osteoclastogenic and anabolic effects of PTH are impaired in the Fgf2-/- mice suggesting a role for endogenous FGF2 in PTH responses. Finally transgenic mice with targeted over-expression of one isoform of FGF2 in osteoblasts have increased bone mass and formation. Thus FGF2 may play a role in the pathogenesis of bone disorders while isoforms of FGF2 may be therapeutic targets for the management of bone loss.

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Disclosures: **M.M. Hurley**, None.

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Anabolic Factors for Fracture Healing. T. A. Einhorn. Orthopaedics, Boston University School of Medicine, Boston, MA, USA.

Local and systemic therapies for the enhancement of fracture healing may greatly impact the field of orthopaedic surgery. Historically, biophysical modalities such as electrical field stimulation[1] and ultrasound[2] have been shown to enhance the healing of fresh fractures as well as delayed unions and nonunions. However, the use of biological therapies carry the promise of providing more robust bone formation. Currently, two bone morphogenetic proteins (BMPs), BMP-2 and BMP-7 (OP-1) have been approved by the FDA for single-level intervertebral body lumbar spine fusion[3] and the treatment of recalcitrant nonunions of long bones, respectively.[4] Clinical trials to expand these indications to the treatment of compound fractures of long bones and other spinal applications are currently underway. Improvements in growth factor delivery and the use of gene therapy are of substantial interest for their ability to enhance the bone formed for these indications. Other therapies currently under investigation include the use of platelet derived growth factor, vascular endothelial growth factor, and prostaglandin receptor agonists. Systemic therapies for the enhancement of fracture healing have focused on the potential use of parathyroid hormone (1-34) and growth hormone. Recent studies have shown that PTH (1-34) at low doses comparable to those used in humans may be effective.[5]

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Disclosures: **T.A. Einhorn**, Eli Lilly 2, 5; Stryker Biotech 2; Novo-Nordisk 2, 5.

28

PTH: Basic Aspects. H. M. Kronenberg. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

Parathyroid hormone (PTH) is the major peptide regulator of calcium homeostasis. The 84 residue protein works primarily by activating the PTH/PTHrP receptor (PTHR1), a G protein-coupled receptor. Carboxy-terminal fragments of PTH cannot active this receptor but circulate at higher levels than does the intact hormone. These fragments bind to receptors and have biologic actions that are still poorly understood. Only the first 34 residues of PTH interact with the PTHR1. Most of the binding of PTH to its receptor involves interactions between the amino-terminal extracellular domain of the receptor and the carboxy-terminal portion of the 1-34 region of PTH. This interaction then positions the first 14 residues of the ligand to interact effectively with 7 membrane-spanning domains of the receptor and their associated extracellular loops. Mutated versions of this 14-residue region can fully activate the receptor and have anabolic actions on bone analogous to those of PTH 1-34. Binding of PTH to its receptor leads to activation of Gs, Gi and the Gq family of G proteins. Which G proteins are activated is determined in a cell-specific fashion. Scaffolding proteins such as NHERF 1 and 2 assemble the PTHR1 with other signaling molecules and strongly influence the choice of G proteins activated by the receptor. Some actions of PTH on cells of the osteoblast lineage are cell autonomous and depend only on activation of the PTHR1 on these cells. Other actions require, as well, the activity of osteoclasts in ways that are not yet well understood.

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Disclosures: **H.M. Kronenberg**, Chugai Pharmaceuticals 2.

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Actions of PTH at the Tissue Level. D. W. Dempster. Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

Antiresorptive drugs, as their name implies, inhibit bone resorption. However, the secondary consequence is a rapid decrease in bone formation. By contrast, PTH stimulates bone formation through an increase in the bone remodeling rate. Under the influence of PTH(1-34), the amount of bone laid down in each remodeling unit is increased. This distinguishes the effects of PTH treatment from other high remodeling states, such as estrogen deficiency, in which the net balance favors resorption. In addition to stimulation of bone formation through this mechanism, termed remodeling-based formation, there is also biochemical and histomorphometric evidence that PTH(1-34) is initially able to uncouple formation from resorption to stimulate formation directly. This is termed modeling-based formation. This may occur by activation of lining cells on previously quiescent surfaces, as well as by osteoblasts engaged in remodeling-based formation migrating outside the borders of the resorption cavity (1). PTH (1-34) not only increases trabecular thickness but also may improve trabecular connectivity (2). The mechanism is uncertain but could involve the initial thickening of trabeculae followed by intra-trabecular tunneling. Beneficial effects of PTH treatment are not, as was once thought, restricted to cancellous bone. Recent histomorphometric and absorptiometric studies demonstrate increases in both cortical thickness and bone diameter under the influence of PTH. The fundamentally different actions of antiresorptive and anabolic agents and PTH at both the cellular and tissue levels provide rationale for continuing to explore their combined or sequential use in the treatment of severe osteoporosis.

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Disclosures: **D.W. Dempster**, Eli Lilly 2,5,8; Proctor and Gamble 2,5,8; Merck 2, 5, 8.

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PTH: Clinical Aspects. S. L. Greenspan. Osteoporosis Prevention and Treatment Center, University of Pittsburgh, Pittsburgh, PA, USA.

Parathyroid hormone (1-34) [teriparatide] is the first anabolic agent that is FDA-approved for the treatment of osteoporosis. In a randomized, double-blind, placebo-controlled trial in 1600 postmenopausal women with osteoporosis, teriparatide 20 F g as a daily subcutaneous injection resulted in an increased bone density of 9.7% at the spine and 2.6% at the hip after approximately 21 months (1). In addition, vertebral fractures decreased 65% and nonvertebral fractures decreased 53% (1). This study was terminated early due to the development of osteosarcoma in rats, although osteosarcoma has not been associated with hyperparathyroidism in humans. Side effects of teriparatide were minor and included rare hypercalcemia, dizziness, and leg cramps. A 9-month study in older, osteoporotic men revealed similar increases in spine bone mineral density and trends for fracture reduction (2). Antiresorptive agents have been associated with a decline in markers of bone turnover, whereas teriparatide has been associated with increases in markers of bone formation followed by increases in markers of bone resorption. Upon discontinuation of teriparatide, the improvement in bone mineral density may be maintained if followed with an antiresorptive agent (3). Combination therapy with parathyroid hormone plus alendronate has not been found to be more beneficial than monotherapy with parathyroid hormone (4). Although combination therapy with hormone replacement and parathyroid hormone appears to significantly improve bone mass more than hormone replacement alone, it is not known whether parathyroid hormone monotherapy is preferential to the combination of the two. New forms of parathyroid hormone, such as parathyroid hormone (1-84) (5), and alternative schedules for administration are currently under investigation.

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Disclosures: **S.L. Greenspan**, Eli Lilly & Co. 2, 5, 8; NPS 3,5; Allelix 3, 5.

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Osteoblast-Derived PTHrP Is a Potent Endogenous Bone Anabolic Agent. A. C. Karaplis, D. Miao, D. Goltzman. Medicine, McGill University, Montreal, PQ, Canada.

Although mice homozygous for targeted disruption of the *Pthrp* locus (*Pthrp*$^{-/-}$) die at birth with severe skeletal deformities (Karaplis *et al*. Genes & Dev 1994;8:277), *Pthrp*$^{+/-}$ animals survive to develop, by three months of age, decreased bone volume and skeletal microarchitectural changes indicative of premature and advanced osteoporosis (Amizuka *et al*. Dev Biol 1996;175:166). Defective bone formation was identified as the underlying etiology for the low bone mass in these mice, as determined by histomorphometric studies and *ex vivo* bone marrow cultures. Daily PTH administration had a more profound bone anabolic effect in *Pthrp*$^{+/-}$ mice than in wild type litter mates. Moreover, PTHrP haploinsufficiency also reduced trabecular bone of *Pth*$^{-/-}$ mice to levels below wild-type by decreasing osteoprogenitor cell recruitment, enhancing osteoblast apoptosis and diminishing bone formation, suggesting that the increased trabecular bone volume in *Pth*$^{-/-}$ mice is due to diminished PTH-induced osteoclastic bone resorption and persistent PTHrP-stimulated osteoblastic bone formation (Miao *et al*. Endocrinology 2004). To substantiate the pivotal anabolic action of osteoblast-derived PTHrP, mice were generated with selective disruption of *Pthrp* exclusively in cells of the osteogenic lineage (*Pthrp*$^{flox/flox}$;*cre*$^{col\ I}$). The osteoporotic phenotype was again recapitulated in this setting, as PTHrP-null osteogenic cells displayed diminished precursor cell recruitment and increased apoptotic death, leading to an overall impairment in bone formation. These findings establish a central role for osteoblast-derived PTHrP in bone formation and provide insight into the profound anabolic action of PTH/PTHrP peptides in patients with osteoporosis.

Disclosures: **A.C. Karaplis**, None.

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PTHrP: Clinical Aspects. M. J. Horwitz, M. Tedesco*, A. F. Stewart. Division of Endocrinology, University of Pittsburgh, Pittsburgh, PA, USA.

Parathyroid hormone-related protein (PTHrP) binds to, and signals, through the common PTH/PTHrP receptor identically to PTH. Human (h) PTHrP(1-36) and hPTH(1-34) also display identical pharmacokinetics following intravenous (IV) administration (2). In contrast, and serendipitously, following subcutaneous (SQ) injection, PTHrP appears to be absorbed (peak <15 min) more rapidly than PTH (peak 30-45 min) (1). This leads to a requirement for larger doses of PTHrP(~400 ug/day) than PTH (20 ug/day) (1-4).

To study the efficacy of PTHrP in osteoporosis, we treated 16 postmenopausal osteoporotic (T < -2.5) women on stable, long-term estrogen and calcium/vitamin D with either a daily SQ injection of PTHrP (6.56 ug/kg/day ~ or 400 ug) or vehicle for three months (4). Lumbar spine BMD, the primary outcome measure, increased by 4.7% in the PTHrP group ($p < 0.025$). Surprisingly, despite the large dose of PTHrP, serum calcium did not increase above 10.1 mg/dl in any subject over the 3 months, and no adverse effects were observed. Moreover, bone formation (serum osteocalcin) increased by 60% over baseline, whereas bone resorption (N-telopeptide and deoxypyridinoline) did not change. These results confirm a prior two week SQ PTHrP study in non-estrogenized women (2). PTHrP appears to be a pure skeletal anabolic agent in human osteoporosis, and thus far appears to be devoid of adverse effects, including hypercalcemia. Current studies are focused on determining the maximum tolerable dose of PTHrP, and confirming the efficacy of PTHrP in a larger cohort of postmenopausal women receiving the previously described 400 ug/day and higher doses.

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Disclosures: **M.J. Horwitz**, Merck 8; **A. F. Stewart**, Osteotrophin LLC 4; Eli Lilly and Company 5.

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NF-E2 Megakaryocytes: A Novel Anabolic Pathway for Increased Bone Formation. M. A. Kacena[1], R. A. Shivdasani*[2,3], C. M. Gundberg[1], T. Nelson*[1], M. C. Horowitz[1]. [1]Orthopaedics, Yale University School of Medicine, New Haven, CT, USA, [2]Adult Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, [3]Medicine, Brigham and Women's Hospital, Boston, MA, USA.

Normal bone homeostasis requires an exquisite balance between bone formation and resorption. Increases in formation or decreases in resorption result in increased bone mass. The identification of new models of anabolic bone growth is critical for the development of new approaches to treat osteoporosis, fracture repair, and tumor induced bone loss. NF-E2 is a transcription factor required for megakaryocyte differentiation. Mice that are deficient in NF-E2 have a developmental arrest of megakaryocyte differentiation, resulting in the accumulation of immature megakaryocytes in the spleen and bone marrow, with essentially no platelets (<5%). Interestingly these mice also exhibit a high bone mass phenotype with up to a 6-fold increase in trabecular bone volume. The increased bone mass phenotype in these animals was not due to osteoclast defects because osteoclast number and function were not compromised in vitro or in vivo. In contrast, in vivo osteoblast number and bone formation parameters were significantly elevated. When wild-type or NF-E2 osteoblasts were cultured with megakaryocytes from NF-E2 deficient mice, osteoblast proliferation increased 3-6-fold by a mechanism that required cell-to-cell contact. We demonstrated that the increased bone phenotype could be adoptively transferred into irradiated wild-type mice using NF-E2 spleen cells. The wealth of evidence points to a cell-to-cell contact mediated response that enhances osteoblast proliferation and in turn results in an increased bone phenotype in vivo, through a novel anabolic pathway.

Disclosures: **M.A. Kacena**, None.

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Histone H4 Alternative Translation Stimulates Bone Mass Accrual. T. J. Noh*[1,2], E. Smith*[3,2], T. E. Myerrose*[4,5], T. Kohler*[6], M. Namdar-Attar*[7], N. Bab*[7], O. Lahat*[7], J. A. Nolta*[4,5], R. Müller[6], I. Bab[7], B. Frenkel[1,2]. [1]Biochemistry & Molecular Biology, Los Angeles, CA, USA, [2]Institute for Genetic Medicine, Los Angeles, CA, USA, [3]Orthopaedic Surgery, Los Angeles, CA, USA, [4]Pediatrics, Los Angeles, CA, USA, [5]and Children's Hospital Los Angeles, Keck School of Medicine at the University of Southern California, Los Angeles, CA, USA, [6]Institute for Biomedical Engineering, Swiss Federal Institute of Technology and University of Zurich, Zurich, Switzerland, [7]Bone Laboratory, The Hebrew University of Jerusalem, Jerusalem, Israel.

The evolutionary conserved *histone H4* genes encode at least two peptides: the 103 amino acid H4 protein and a circulating mitogen, Osteogenic Growth Peptide (OGP). The latter is synthesized *de novo* from H4 mRNA following leaky ribosomal scanning through the imperfect H4 AUG initiator and alternative translation starting at codon 85, a perfect AUG initiator. To test the function of *H4* alternative translation *in vivo*, we engineered transgenic mice ubiquitously and constitutively expressing a mutant H4 mRNA, *H4tTG1*, which does not encode H4 protein. Quantitative micro-computed tomographic analysis of femora from 8, 17 and 34 week-old mice revealed a marked increase in trabecular, but not cortical, bone volume density at all ages. This effect was particularly strong in females, which exhibited a significant 2-fold increase in trabecular bone density compared to wild-type controls. The enhancement of trabecular bone density was accompanied by increased trabecular number and connectivity, parameters that contribute to bone strength. Dynamic histomorphometric analysis demonstrated a significant 35% increase in the percentage of trabecular surface engaged in bone formation and a significant 23% increase in the mineral appositional rate in females. Osteclast number was not significantly altered. No adverse effect of OGP over-expression was noticeable in transgenic mice up to 18 months of age. Thus, continuous OGP over-expression throughout life results in a specific augmentation of trabecular bone without noticeable effects on cortical bone or extra-skeletal tissues. In summary, transgenic expression of H4 mRNA lacking the upstream initiation codon in post-mitotic cells results in increased trabecular bone accrual.

Disclosures: **T.J. Noh**, None.

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Irak-m Is a Negative Regulator of Osteoclast. H. Li*[1], E. Cuartas[1], W. Cui*[1], H. Lamallem*[1], Y. Choi*[2], H. Ke[3], R. Flavell*[4], K. Kobayashi*[4], A. Vignery[1]. [1]Orthopaedics Department, Yale University School of Medicine, New Haven, CT, USA, [2]University of Pennsylvania School of Medicine, Philadephia, PA, USA, [3]Pfizer Global Research and Development, Groton, CT, USA, [4]Section of Immunology, Yale University School of Medicine, New Haven, CT, USA.

Toll-like receptors (TLRs), like IL-1R, modulate osteoclast differentiation and activation via IRAK (IL-1 associated kinase). Phosphorylated IRAK binds TRAF6 which activates NF-kB and MAPKs. IRAKM, unlike IRAK, IRAK-2 and IRAK-4 is expressed only in monocytes/macrophages, lacks kinase activity and is a negative regulator of IL-1R/TLR signaling. We therefore hypothesized that IRAK-M down-regulates osteoclasts and positively regulates bone mass. To test that hypothesis, we used mice with a homozygous deletion in IRAKM. 4 month old IRAKM -/- mice weighed less than +/+ and developed a hunchback that was confirmed by X-ray analysis. In male -/-, total body bone mineral density (PIXIMUS) was lower at 2, 4 and 6 month of age, while in female it was only lower at 4 months. PQCT analysis of femurs from 4 month old -/- mice demonstrated a 35% decrease in cortical and trabecular areas when compared with +/+ mice. MicroCT analysis of distal femurs from IRAKM -/- mice confirmed a 60% reduction in trabecular bone volume. Histomorphometry analysis revealed a 3.8-fold increase in osteoclast number associated with a 2-fold increase in bone turnover in IRAKM -/- mice when compared with +/+ mice. IRAKM -/- bone marrow cells treated with M-CSF and RANKL demonstrated an accelerated rate of multinucleation and differentiation into TRAP+ cells. Such cells had an extended half-life and hyper-phosphorylated IκB, JNK and ERK1/2 upon stimulation with IL-1α and β. IRAK-M appears to negatively regulate osteoclast differentiation and activation via NF-κB and MAPK signaling pathways, and positively regulate bone mass.

Disclosures: **H. Li**, None.

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Nonvertebral Fracture Risk Reduction During Treatment With Teriparatide Is Independent of Pretreatment Bone Turnover and Hip BMD. G. Crans*, B. Mitlak. Eli Lilly and Company, Indianapolis, IN, USA.

Teriparatide [rhPTH(1-34)] is a bone-forming agent that increases BMD and reduces the risk of fracture. Currently the relationship between the nonvertebral antifracture efficacy of teriparatide and pretreatment bone turnover, or hip BMD is unclear.

To quantify this relationship, data from the Fracture Prevention Trial, in which 1637 subjects were assigned to treatment with 20 or 40 mcg of teriparatide or placebo were examined in two logistic regression analyses. The first analysis evaluated the relationship between baseline bone turnover and the risk of nonvertebral fracture; the second evaluated the impact of baseline femoral neck BMD on nonvertebral fracture risk. Nonvertebral fracture risk was modeled as a function of therapy, baseline covariate, and the therapy-by-baseline covariate interaction. To determine the final model, a stepwise selection procedure was used, whereby only those terms that were statistically significant at the 0.05 level were included.

Lower BMD and higher bone turnover were associated with an increased risk of fracture. Importantly, while the main effect of therapy was statistically significant in each model ($p<0.01$), the therapy-by-baseline covariate interaction was not, suggesting that the effect of teriparatide therapy to decrease the risk of nonvertebral fractures is independent of both baseline hip BMD and baseline bone turnover. While the nonvertebral fracture risk was related to bone turnover and hip BMD, the efficacy of teriparatide was independent of these baseline characteristics.

Disclosures: **G. Crans**, Eli Lilly and Company 1, 3.

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The Neuronal Control of Bone Formation. F. Elefteriou[1], S. Takeda[2], G. Karsenty[1]. [1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA, [2]Tokyo Medical and Dental University, Saginuma, Miyamae-ku, Kanagawa, Japan.

The hypothesis that bone mass, body weight and reproduction could share common endocrine regulators has been the basis of our study of the endocrine control of bone mass. This hypothesis had one implication since the control of body weight and reproduction is largely of hypothalamic nature it implies that bone mass could also be controlled by the hypothalamus. Testing this hypothesis in vivo led us to uncover that leptin is a powerful physiological inhibitor of bone formation. This appears to be true in mice and in humans. In agreement with our working hypothesis leptin inhibits bone formation by acting on a subpopulation of hypothalamic neurons that mediates its antiosteogenic but not its anorexigenic function. This anatomical distinction between anorexigenic and antiosteogenic neurons can be established genetically and chemically, it has important histological and therapeutic implications. The mediator of the function of this antiosteogenic neurons is the sympathetic nervous system through B2 adrenergic receptors that are present on osteoblasts. Leptin controls this regulatory loop via its serum concentration and even high serum leptin level do decrease bone mass suggesting leptin resistance may not occur in the control of bone formation. Recent studies of wildtype and B2 adrenergic receptor deficient mice have revealed an unanticipated role for the sympathetic nervous system in the control of bone mass that will be presented at the meeting.

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Disclosures: **G. Karsenty**, None.

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Prostaglandins: Basic and Clinical Studies. L. G. Raisz, C.C., Pilbeam. Department of Medicine, University of Connecticut Health Center, Farmington, CT, USA.

Prostaglandins, particularly PGE_2, are potent multifunctional regulators of bone formation and resorption[1]. In humans a role for PGE_2 as a stimulator of bone resorption was first recognized in rare cases of humoral hypercalcemia of malignancy [2]. Subsequently PGE infusion was shown to stimulate bone formation in infants with congenital heart disease [3]. In cell and organ culture and in animal models the predominant effects of PGE_2 are to stimulate bone resorption and formation. However inhibition of the function of differentiated osteoblasts and osteoclasts has also been observed. Endogenous prostaglandin production, which is largely dependent on stimulation of inducible cyclooxygenase (COX-2), has been shown to mediate the anabolic response to mechanical forces, as well as to enhance the osteoclastogenic response to most stimulators of bone resorption. . Prostaglandins may also mediate resorptive responses in inflammation. Both phospholipase A2, which releases arachidonic acid, and COX-2 have been implicated in this process [4]. There are two receptors for PGE_2 that stimulate cyclic AMP production, EP2R and EP4R, and these receptors have been shown to mediate stimulation of bone resorption and formation in vivo and in vitro. Selective EP2 and EP4 receptor agonists can enhance bone formation and fracture healing [5]. In all of these responses initial prostaglandin effects production may be “auto-amplified” by the ability of prostanoids themselves to induce COX-2. Knockouts of EP2R and EP4R and COX-2 do not show a marked skeletal phenotype, but do show altered responses to a variety of perturbations. Thus the role of prostaglandins in bone metabolism may be largely to facilitate or enhance the responses to physiologic or pathologic stimuli, probably acting in concert with other regulatory factors.

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Disclosures: **L.G. Raisz**, None.

39

Statins and Related Anabolic Agents. G. R. Mundy. Cellular & Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Multiple molecular targets have been identified that could be used as tools for drug discovery as anabolic agents, including transcription factors, signal transduction molecules and the bone growth factor promoters. Among the latter is the Bone Morphogenetic Protein-2 (BMP-2) gene, which is regulated by a complex promoter. This promoter has proven a useful molecular target for the stimulation of osteoblast differentiation and bone formation. We have identified a number of different classes of small molecules that stimulate this promoter and cause increased bone formation, including statins and inhibitors of the ubiquitin-proteasome pathway. In both cases, the role of the BMP family has been demonstrated by inhibition with noggin. The Gli family of transcription factors that mediate hedgehog signaling control expression of the BMPs in vertebrates, and their ortholog Ci similarly regulates decapentaplegic (dpp) expression in drosophila, an effect that is proteasome- and E3 ligase-dependent. The BMP-2 promoter in cells in the osteoblast lineage is regulated by nitric oxide, and BMP-2 expression in osteoblast lineage cells is enhanced by increased expression of eNOS mRNA. The statins stimulate BMP-2 transcription, which ultimately leads to osteoblast proliferation and differentiation by enhancing eNOS mRNA stability, which in turn leads to enhanced NO generation and BMP-2 transcription. The effects of statins to increase mRNA stability is mediated by their capacity to inhibit HMG CoA reductase, which in turn leads to impaired generation of small GTPases that require prenylation for activity. This latter step is responsible for increasing expression of eNOS mRNA. A similar process occurs in endothelial cells and is responsible for NO generation and beneficial effects on cerebral blood flow and protection against ischemic cerebral infarcts. These observations suggest novel ways in which effects of statins unrelated to their capacity to lower serum cholesterol may have important effects in target cells which in turn may lead to therapeutic benefit. These mechanisms will be discussed during this presentation.

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Disclosures: **G.R. Mundy**, None.

40

Strontium. P. J. Meunier. Faculty Laennec, INSERM Unit 403, Lyon, France.

Strontium (Sr) is a natural bone-seeking element. In the 50's preliminary open trials of Sr salts suggested that Sr may have potential benefits for osteoporotic patients (Bull Hosp Joint Dis 1952;13:59-66). This has incited Servier chemists to synthesize a new salt of Sr,strontium ranelate (SR),composed of an organic moiety (ranelic acid)and of two atoms of stable Sr. In vitro and in vivo in several animal models SR appeared capable to both stimulate osteoblastic bone formation and reduce osteoclastic bone resorption (Calcif Tissue Int 2001;69:121-9). Results from a 2 year controlled phase II dose-response study have shown that ingestion of 2 g. a day of SR increased bone mineral density (BMD) and may reduce the incidence of new vertebral fractures (VF) in ostoporotic women (J Clin Endocrinol Metab 2002;87:2060-6). Two large phase III trials were then designed in 6740 osteoporotic women receiving 2 g.a day of SR : one assessing the effects on the risk of new VF (SOTI),and one evaluating the effects on the risk of non-vertebral fractures (TROPOS).In SOTI new VF occurred in fewer patients in the SR group than in the placebo group ,with a risk reduction of 49 % in the first year and 41% during the 3 year study period. SR increased lumbar spine BMD at month 36 by 14.4% and was well tolerated (N Engl J Med 2004;350:459-68). TROPOS study showed a significant 16 % reduction in the risk of a first non-vertebral fracture in the group treated for 3 years with SR (Osteoporos Int 2002;13:Suppl 3 :S 14). Although further studies are needed to elucidate the cellular mechanisms of action of SR, this compound already appears as an effective and safe novel therapy of postmenopausal osteoporosis.

Disclosures: **P.J. Meunier**, Servier 5.

M1

Amphiregulin Is a Novel Growth Factor in Bone Stimulated by Parathyroid Hormone and Required for Normal Bone Development. L. Qin[1], J. Tamasi[2], L. Raggatt[1], X. Li[1], J. H. M. Feyen[2], D. Lee*[3], E. DiCicco-Bloom*[4], N. C. Partridge[1]. [1]Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [2]Bristol-Myers Squibb Pharmaceutical Research Institute, Pennington, NJ, USA, [3]Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC, USA, [4]Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.

Parathyroid hormone (PTH) is the major mediator of calcium homeostasis and bone remodeling, having both bone resorption and bone formation actions. Intermittent injection of PTH increases bone formation and has been approved as a treatment for osteoporosis. Our recent studies identified more than 100 genes regulated by rPTH(1-34) in a rat osteoblastic cell line, UMR 106-01. Amphiregulin, a member of the EGF family, is one of those genes. Real-time RT-PCR demonstrated that amphiregulin is rapidly and highly up-regulated by PTH in several osteoblastic cell lines. Moreover, hPTH(1-38) injection into rats dramatically elevated the amphiregulin level in the femoral metaphyses. The up-regulation of amphregulin by PTH is PKA-dependent and is a primary response. Study of the expression patterns of all the EGF-like ligands (EGF, TGF-α, amphiregulin, epiregulin, HB-EGF and betacellulin) and their receptors (EGFR and ErbB2) in UMR 106-01 cells shows that all of these proteins are expressed but amphiregulin is the only member that is highly regulated by PTH. Functional studies using cell numbers, [^{3}H]thymidine incorporation into DNA and cell cycle analysis indicated amphiregulin is a potent growth factor for osteoblastic cells. Amphiregulin also strongly and quickly stimulated Akt and ERK phosphorylation, c-fos and c-jun expression in osteoblastic cells. All of these functions require the EGFR. Finally, microCT analysis of tibiae from amphiregulin knockout mice revealed that those mice have significantly less trabecular bone than wild-type. In summary, our data demonstrated that amphiregulin is a novel growth factor in bone stimulated by parathyroid hormone and required for normal bone development.

Disclosures: **L. Qin**, None.

M2

Endogenous PKIgamma Regulates Immediate-early Gene Expression Induced by PTH in Osteoblasts. X. Chen[1], J. Dai[1], S. A. Orellana*[2], E. M. Greenfield[1]. [1]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA, [2]Pediatrics, Case Western Reserve University, Cleveland, OH, USA.

Immediate-early genes, such as c-fos and IL-6, mediate the anabolic and catabolic effects of PTH. Thus, termination of immediate-early gene expression may regulate the anabolic/catabolic balance after exposure to PTH. We have shown that the primary mechanism responsible for termination of immediate-early gene expression following stimulation by PTH acts downstream of receptor desensitization, adenylyl cyclase activation, and cAMP degradation (Am J Physiol Cell Physiol 283:1432-40, 2002). We therefore hypothesized that inhibition of PKA activity by the protein kinase inhibitor (PKI) family terminates transcription factor phosphorylation and gene expression. We found that PKIgamma mRNA and protein are constitutively expressed in osteoblasts and fibroblasts at high levels, while PKIalpha and PKIbeta are weakly expressed. PKIgamma knock down by siRNA or antisense transfection substantially extends PTH-induced nuclear PKA activity, CREB phosphorylation, and expression of c-fos and IL-6. These findings are the first in any cell type showing that endogenous PKIgamma regulates PKA signaling. PKIgamma likely transports PKA out of the nucleus since (1) PKIgamma contains a potent nuclear export signal (NES) and is therefore rapidly re-exported to the cytoplasm following PTH-induced nuclear translocation, (2) PKIgamma knock down blocks nuclear export of all three isoforms of the catalytic subunit of PKA, and (3) although PKA catalytic subunits lack a NES, the NES inhibitor, Leptomycin B, blocks nuclear export of both PKIgamma and PKA. However, since Leptomycin B has no effect on nuclear PKA activity, CREB phosphorylation, or immediate-early gene expression, nuclear export is not required and PKIgamma binding is sufficient to terminate PKA signaling.

Disclosures: **X. Chen**, None.

M3

Anabolic Actions of PTH: Temporal Effects on Tissue-engineered Bone. G. J. Pettway*[1], A. J. Koh*[2], E. Widjaja*[3], M. Morris*[3], L. K. McCauley[2]. [1]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA, [2]Perio/Prev/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [3]Chemistry, University of Michigan, Ann Arbor, MI, USA.

Although in clinical use, the mechanisms for PTH anabolic actions are still unclear. A novel tissue-engineering model using bone marrow stromal cell (BMSC) implants was used to analyze actions of PTH. Mice with 1wk old BMSC implants received PTH (40μg/kg/d) or vehicle injections for 1wk (group 1), 3wks (group 2), or 7wks (group 3), or 3wks initiated 12wks after implantation (group 4). Increased cellularity was noted in group 1 PTH-treated ossicles, increased bone in group 2 PTH-treated ossicles (54.6% vs. 28.6%), but similar amounts of bone in group 3&4 ossicles regardless of treatment. Ossicles from group 1, PTH-treated mice, showed reduced mineralization via microradiography. Interestingly, endogenous vertebral bone was not significantly affected in the PTH groups, suggesting that the anabolic effects of PTH are more pronounced in growing bone. Phosphate mineral in group 1 ossicles was determined using raman spectroscopy and gene expression of ossicles and calvaria evaluated by northern blot analysis. PTH inhibited mineralization in ossicles, as indicated by low osteocalcin (OCN) mRNA expression. PTH increased matrix γ-carboxyglutamic acid protein (MGP) slightly and PTH1R significantly in calvaria but not in ossicles. BrdU labeling performed on vertebral implants in athymic mice revealed more widespread BrdU labeling observed in the bone marrow from PTH-treated mice versus a focused BrdU positivity along the trabecular bone in controls. These results indicate that tissue-engineered bone is particularly responsive to PTH during the modeling phase and suggest PTH inhibits mineralization in early ossicle development, augmenting bone formation later.

Disclosures: **G.J. Pettway**, None.

M4

The Role of IGF-I in Regulating the Skeletal Response to PTH. Y. Wang[1], S. Nishida[1], H. Z. ElAlieh*[1], S. Majumdar[2], A. Burghardt*[2], T. L. Clemens[3], B. P. Halloran[1], D. D. Bikle[1]. [1]Endocrine Unit, Veterans Affairs Medical Center,University of California, San Francisco, San Francisco, CA, USA, [2]Radiology, University of California, San Francisco, San Francisco, CA, USA, [3]Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA.

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Although the effects of parathyroid hormone (PTH) on bone metabolism are well established, the mechanisms are unclear. To examine the role of insulin-like growth factor I (IGF-I) signaling in mediating the actions of PTH on bone, we investigated the bone response to PTH in 3 month old control mice and mice with a bone specific IGF-I receptor null mutation (bIGF-IR$^{-/-}$), (floxed IGF-IR x osteocalcin promoter driven Cre recombination), treated with vehicle or PTH (80 μg/kg bw/day for 2 weeks). In vehicle treated mice, fat free/body weight of the tibia (FFW/BW), bone volume (BV), and cortical thickness (C.Th) were significantly less in bIGF-IR$^{-/-}$ mice than in control mice. PTH treatment significantly decreased FFW/BW, decreased BV and increased C.Th in both control and bIGF-IR$^{-/-}$mice. Furthermore, PTH increased mRNA levels of RANKL, alkaline phosphatase (ALP) and osteocalcin in the bone from both control and bIGF-IR$^{-/-}$ mice. However, administration of PTH increased the number of alkaline phosphatase positive colonies and mineralization only in bone marrow stromal cells (BMSCs) from control mice, without any effects in BMSCs from bIGF-IR$^{-/-}$ mice. Our results suggest that the IGF-IR in mature osteoblasts is required for PTH to stimulate osteoprogenitor cell proliferation and/or differentiation, perhaps similar to the mechanism by which PTH stimulates hematopoietic cell proliferation and/or differentiation.

Disclosures: **Y. Wang**, None.

M5

The Mechanism for IGF-I Resistance Induced by Skeletal Unloading Is Not Shared by Other Growth Factors. S. Nishida, Y. Wang, H. Z., E. I. Alieh*, B. P. Halloran, D. D. Bikle. Endocrine Unit, University of Calfornia, Veterans Affairs Medical Center, San Francisco, CA, USA.

Skeletal unloading leads to decreased bone formation and decreased bone mass. These results can be explained in part by a failure of IGF-I to activate its signaling pathways in unloaded bone. To determine whether this resistance is specific for IGF-I or common to all skeletal growth factors acting through receptor tyrosine kinase mechanisms we compared the effect of IGF-1 and PDGF in a rat model using hindlimb suspension. IGF-I (10ng/ml)did not increase bone marrow osteoprogenitor (BMOp) cell proliferation in bone marrow stromal cells (BMSC) taken from unloaded bone, whereas PDGF was fully effective. The ability of IGF-I to stimulate IGF-I receptor phosphorylation was blocked in BMSC from unloaded bone but not the ability of PDGF to stimulate PDGF receptor phosphorylation. Integrins are likely to serve as mechanical sensors in bone, and integrin activation is known to augment growth factor signaling. In recent studies we found that unloading resulted in decreased integrin expression. Echistatin,an inhibitor of integrin signaling, blocked IGF-I stimulated BMOp cell proliferation and IGF-I receptor phosphorylation but was much less effective in blocking these actions of PDGF. These results indicate that the mechanism by which skeletal unloading leads to IGF-I resistance has little impact on the anabolic response to PDGF, suggesting that PDGF and possibly other growth factors may be of clinical use in preventing and /or treating bone loss during immobilization and other forms of skeletal unloading.

Disclosures: **S. Nishida**, None.

M6

Transcriptional Activation of Vitamin D Receptor Mutants by Phosphorylation. Y. Liu*[1], P. Malloy*[2], D. Feldman*[2], S. Christakos[1]. [1]Dept. of Biochemistry, New Jersey Medical School, Newark, NJ, USA, [2]Dept. of Medicine, Stanford University School of Medicine, Stanford, CA, USA.

Studies were done using mutant vitamin D receptors containing inactivating mutations in the ligand binding domain observed in patients with human $1,25(OH)_2D_3$ resistant rickets (F251C, I268T, H305Q, E420K). Using 24(OH)ase and osteopontin promoter constructs, we found okadaic acid (OA), an inhibitor of phosphatase, can enhance the transcriptional activity of mutant VDRs (H305Q, F251C, I268T) 3-7 fold. The enhancement was found to be correlated to increased interaction between DRIP205 and mutant VDRs. Hexafluoro $1,25(OH)_2D_3$ analogs, that are more potent than $1,25(OH)_2D_3$, resulted in at least partial rescue of the transcriptional responsiveness of these mutant VDRs and increased interaction between the mutant VDRs and DRIP205. The E420K mutant, which prevents coactivator binding, was unresponsive to both OA and analogs. We found that VDR was not phosphorylated in the presence of 50 nM OA. To address the possibility that OA may be acting by enhancing the phosphorylation of another protein required for the transcriptional activation of VDR, we examined CREB binding protein (CBP). We found that treatment of cells with OA consistently resulted in the appearance of a slower migrating form of CBP as visualized by SDS-PAGE and Western blotting. The slower migrating form was no longer detected after subsequent incubation with phosphatase, providing evidence that OA induces phosphorylation of CBP. The phosphorylation of CBP was not induced by $1,25(OH)_2D_3$ and analogs. These findings suggest that transcriptional activity of mutant VDRs can be enhanced by phosphorylation and this may be mediated by increased coactivator binding and phosphorylation of specific VDR associated cofactors.

Disclosures: **Y. Liu**, None.

M7

Hypothalamic Neuropeptide Y (NPY) Y2 Receptors Protect Cancellous Bone From Leptin Induced Bone Loss. P. A. Baldock*[1], A. Sainsbury*[2], D. Lin*[3], M. Couzens*[2], R. F. Enriquez*[1], D. Matt*[3], H. Herzog*[2], E. M. Gardiner[1]. [1]Bone Program, Garvan Institue of Medical Research, Sydney, NSW, Australia, [2]Neurobiology Program, Garvan Institue of Medical Research, Sydney, NSW, Australia, [3]Dept of Molecular Medicine & Pathology, University of Auckland, Auckland, New Zealand.

Leptin and NPY Y2 receptors are strongly co-expressed in the hypothalamus. Both leptin deficiency and Y2 deletion increase hypothalamic NPY and bone formation. In contrast, chronic leptin excess in obesity is not associated with bone loss or reduced hypothalamic NPY, suggesting a role for NPY signalling in the protection of bone from elevated leptin levels, possibly involving the Y2 receptor. The effect of the Y2 receptor on the bone response to elevated leptin levels was investigated.

Bone was assessed in the chronic leptin deficient (ob/ob) and (Y2/ob) double knockout mice was compared to normoleptinaemic Y2 knockout ($Y2^{-/-}$) mice and in wildtype and $Y2^{-/-}$ mice 3 weeks after hyperleptinaemia produced by viral hypothalamic over-expression of NPY.

Cancellous bone volume was similarly elevated in $Y2^{-/-}$, ob/ob and Y2/ob compared to wildtype, despite the difference in leptin between these 3 anabolic models. NPY overexpression increased body weight by 60% in wildtype and $Y2^{-/-}$, elevating circulating leptin levels. This was associated with a 45% decrease of cancellous bone volume in $Y2^{-/-}$ with no change in wildtype. Osteoid width was significantly reduced 3.5 fold in obese $Y2^{-/-}$ and 2 fold in obese wildtype, suggesting a loss of protective effect in $Y2^{-/-}$.

In chronic leptin deficiency the Y2 receptor does not appear to mediate a pathway distinct from that involving the leptin receptor. In the 3 week model, the osteopenic effect of increased leptin is attenuated by Y2. This finding suggests that the Y2 receptor may protect against bone loss resulting from increases in leptin signalling.

Disclosures: **P.A. Baldock**, None.

M8

Prolonged Anabolic Steroid Therapy Promotes Bone Formation and Prevents Demineralization during Rehabilitation in Burned Children. K. D. J. Murphy*[1], S. Thomas*[1], D. L. Chinkes*[1], G. L. Klein[2], D. N. Herndon*[3]. [1]Department of Surgery, Shriners Hospitals for Children & The University of Texas Medical Branch, Galveston, TX, USA, [2]Department of Pediatrics, Shriners Hospitals for Children & The University of Texas Medical Branch, Galveston, TX, USA, [3]Departments of Surgery & Pediatrics, Shriners Hospitals for Children & The University of Texas Medical Branch, Galveston, TX, USA.

Introduction: Post-burn catabolism prevents bone formation and accelerates bony demineralization after severe pediatric burns. Anabolic agents such as growth hormone and oxandrolone have been used successfully for constitutional delays of growth.

Methods: Fifty-five burned children with burns greater than 40% total body surface area were enrolled in a randomized controlled trial to investigate the effect of the anabolic agent, oxandrolone, on bone growth and mineralization. Oxandrolone (0.1mg/kg PO BID) or placebo was administered from discharge from the intensive care unit until 12 months after injury. Dual-Energy X-ray Absorptiometry (DEXA) measured whole body bone mineral content (BMC), whole body bone mineral density (BMD), Spine BMC and Spine BMD, at discharge, 6, 9 and 12 months post-burn. DEXA software calculated age and sex matched Spine BMD Z-scores. Liver function tests and including alkaline phosphatase were serially measured.

Results: Oxandrolone subjects had significantly greater BMC twelve months after burn ($p<0.016$), with differences at time points becoming more disparate over time. Although Spine BMC & BMD did not differ between groups, BMD z-scores were significantly better with oxandrolone than controls ($p<0.016$) especially twelve months after injury. This was associated with significantly greater alkaline phosphatase levels in treated patients ($p<0.001$) and low normal levels in controls even 12 months after injury. Liver transaminases remained normal for both groups. There were no significant side-effects.

Conclusions: Low-dose oxandrolone, administered during rehabilitation until one year after burns, successfully and safely promotes bone formation and prevents osteopenia induced by severe burns in children.

Disclosures: **K.D.J. Murphy**, None.

M9

Teriparatide Increases the Width of Modeling and Remodeling Osteons at the Trabecular and Endosteal Envelope. E. F. Eriksen, D. W. Donley*, Y. L. Ma. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

Teripararatide [rhPTH(1-34), TPTD], a new bone formation agent for osteoporosis, reverses osteoporotic changes in bone structure and decreases vertebral and nonvertebral fracture rates. A significant proportion of new bone formed during teriparatide treatment seems to be formed via modeling, i.e. formation of new bone on quiescent bone surfaces without previous resorption. We analyzed the occurrence and dimensions of modeling and remodeling osteons in iliac crest biopsies obtained from patients treated 12-24 months with placebo (n=20) or teriparatide 20 (TPTD20, n=19) or 40 (TPTD40, n=13) µg/day s.c. in a large randomized trial. Active bone forming, tetracycline-labeled osteons on trabecular and endocortical surfaces were studied. Trabecular and endosteal osteons were classified according to the presence of smooth or scalloped cement lines and collagen orientation (i.e. modeling and remodeling osteons, respectively). Remodeling wall width was also quantified. A dose-dependent increase in modeling osteons was seen for TPTD20 (0.4%) and TPTD40 (3.8%) ($P<0.001$). Mixed remodeling/modeling trabecular osteons showed a dose dependent increase (TPTD20 (2.4%) and TPTD40 (3.9%) ($P<0.001$). Significant increases in the remodeling wall width of trabecular and endosteal packets were noted in both teriparatide groups compared with placebo ($P<0.05$). In conclusion, this study suggests that teriparatide induces pure modeling bone formation at quiescent surfaces, and increases bone formation at remodeling sites. This leads to increased thickness of completed bone structural units at both the trabecular and endosteal envelope. These mechanisms may contribute to the improvement of trabecular and cortical architecture demonstrated after teriparatide treatment.

Disclosures: **E.F. Eriksen**, Eli Lilly and Company 3.

M10

The Risk of Developing Back Pain Is Reduced in Postmenopausal Women with Osteoporosis following Teriparatide Compared with Alendronate Therapy. R. K. Dore[1], J. H. Krege[2], P. Chen*[2], E. V. Glass*[2], J. San Martin[2], P. D. Miller[3]. [1]UCLA, Anaheim, CA, USA, [2]Eli Lilly and Company, Indianapolis, IN, USA, [3]Colorado Center for Bone Research, Lakewood, CO, USA.

The relative risk of back pain, moderate or severe back pain and severe back pain was significantly reduced following teriparatide 20 and 40 mcg/d (TPTD20 and TPTD40) treatment of postmenopausal women with osteoporosis compared with placebo (Neer, NEJM 2001, Genant, ASBMR 2003). We compared back pain incidence in postmenopausal women with osteoporosis given oral alendronate 10 mg/d (ALN10) plus placebo injection with teriparatide injection plus oral placebo. Back pain data was collected during adverse event monitoring. In study A, women were randomized to TPTD20 or ALN10 for 18 months. In study B, women were randomized to TPTD40 or ALN10 for 14 months and most women completing this trial were enrolled in a follow-up study. In each trial, baseline differences in patient demographics between treatment groups were not statistically significant. TPTD20-treated women had reduced risk of back pain (P=0.051), moderate or severe back pain (P=0.003), and severe back pain (P=0.04), with relative risk reductions of 27%, 44%, and 52%, respectively, versus ALN10-treated women (Table, I). TPTD40-treated women had reduced risk of back pain (P=0.012) and moderate or severe back pain (P=0.016), with relative risk reductions of 71% and 80%, respectively versus ALN10-treated women (Table, II). During the trial plus 18 months of follow-up, TPTD40-treated women had reduced risk of back pain (P=0.015), and moderate or severe back pain (P=0.016), with relative risk reductions of 66% and 80%, respectively, versus the ALN10 group. (Table, III). In conclusion, women randomized to teriparatide had reduced risk of back pain compared to women randomized to alendronate.

I. Study A

	ALN10 N=101		TPTD20 N=102		Relative Risk (P-value)
	n	%	N	%	
Back Pain	39	38.6	26	25.5	.73 (0.051)
Moderate or Severe Back Pain	33	32.6	15	14.7	.56 (0.003)
Severe Back Pain	12	11.9	4	3.9	.48 (0.04)

II. Study B

	ALN10 N=73		TPTD40 N=73		
	n	%	N	%	
Back Pain	14	19.2	4	5.5	.29 (0.012)
Moderate or Severe Back Pain	10	13.7	2	2.7	.20 (0.016)
Severe Back Pain	2	2.7	1	1.4	.52 (NS)

III. Study B + 18 Months Follow-up

	ALN10 N=53		TPTD40 N=52		
	n	%	N	%	
Back Pain	15	28.3	5	9.6	.34 (0.015)
Moderate or Severe Back Pain	10	18.9	2	3.9	.20 (0.016)
Severe Back Pain	3	5.7	1	1.9	.33 (NS)

Disclosures: **J. San Martin**, Eli Lilly and Company 3.

M11

Osteoformin Stimulates Differentiation of Human Chondrocytes. L. X. Bi[1], E. G. Mainous[2], W. L. Buford*[1]. [1]Depts of Surgery and Orthopaedics, University of Texas Medical Branch, Galveston, TX, USA, [2]Dept. of Surgery, University of Texas Medical Branch, Galveston, TX, USA.

Our previous studies have shown that negatively charged resins increase bone formation and accelerate bone defect healing in vivo. Now, we are seeking a synthetic negatively charged peptide, which demonstrates low antigenicity and is biodegradable, injectible, and low-cost, to stimulate bone formation. In order to investigate potential effects of osteoformin, negatively charged peptide (polyaspartate), on human chondrocytes, we examined expression of bone morphogenetic protein -2 [BMP-2], alkaline phosphatase activity (ALP) and mineralization after treatment of cells with osteoformin. Human chondrocytes were cultured in a-minimum essential medium [α-MEM] and 10% fetal bovine serum with or without osteoformin (5ug/ml) for 7, 10, 14 and 18 days, respectively. To determine mineralization, the cells were cultured in mineralizing-growth medium. The levels of ALP were assayed using a commercial kit (Sigma Chemical Co., St. Luis, MO). Expression of BMP-2 (anti-rhBMP-2 monoclonal antibody, Genetics Institute, Cambridge, MA) was examined using immunoquantitative assay. The mineralization was assessed by Von Kossa staining. ALP activities were significantly elevated (55-67%, $P<0.001$) in osteoformin treated group, compared to control group. BMP-2 expression was increased (23-34%, $P<0.01$) after osteoformin treatment compared to control. There were significant increases in the levels of mineralization (2-3 fold, $P<0.001$) within 18 days of culture. We conclude that osteoformin significantly stimulated chondrocyte differentiation in vitro. It might be an important regulator of bone formation by accelerating fracture, bone defect healing, repairing traumatic articular cartilage and controlling bone diseases.

Disclosures: **L.X. Bi**, None.

M12

Clinical Observation Between Bone Mineral Density (BMD) and Testosterone (T) in Elderly Men. P. Li*. Elderly Ward, Beijing 304th Hospital of China Liberation Army, Beijing, China.

Objective: To search the correlation between bone mineral density (BMD) and testosterone(T) in elderly men who are ≥60 year, and analyze the effect of testostrone in osteoporosis of aged males.

Method: 26 elderly men in hospital, (average 68±6.17y). Liver disease, kidney disease endocrinopathy and second osteoporosis are eliminated. No one used cordisone and testostrone. To check up sex hormones, include testosterone (T), estrogen (E), follicle-stimulation hormone(FSH), luteinizing hormone(LH), prolactin(PLT).on an empty stomach in the morning. BMD were measured by NOLAND dual energy X-ray absorptionmetry, it made in USA. T-score<2.5SD was diagnosed osteoporosis. Sex hormones were measured by ELISA.

Results:

1. The levels of sex hormones: T 3.99±2.25ng/ml; E 25.25±21.31pg/ml; FSH 9.08±8.01miU/ml; LH 5.43±3.17miU/ml; PLT 302.94±156.39uiU/ml.
The mean BMD L2-41.0043±0.24g/cm2; Ward's triangle 0.5381±0.15g/cm2.
2. 6 cases were diagnosed osteoporosis; it is 23.08% of total patients.
3. The levels of LH correlated with BMD at L2-4 ($r=0.46$, $p<0.01$); the levers of T correlated with BMD at Ward's triangle ($r=0.41$ $p<0.025$).
The relation between BMD and sex hormones: ① ②

r	T	E	FSH	LH	PLT
L2-4	0.0261	-0.0768	0.0081	0.4649①	-0.0429
Ward's triangle	0.4075②	-0.2090	-0.0888	0.1947	-0.0277

① $p<0.01$; ② $p<0.025$

Conclusion: Our study revealed the level of T correlated with BMD at Ward's triangle, the lower T level is an important factor in osteoporosis in old men.

Disclosures: **P. Li**, None.

M13

PTH-dependent Osteocalcin Gene Expression Requires the Presence of an OSE1 Sequence in the Promoter and Multiple Signaling Pathways. G. Xiao, D. Jiang*, R. T. Franceschi, H. Boules*. Periodontics/Prevention/Geriatrics, The University of Michigan, Ann Arbor, MI, USA.

Parathyroid hormone (PTH) is an important peptide hormone regulator of bone formation and osteoblast activity. PTH has both catabolic and anabolic effects on osteoblasts and bone, which depend on the temporal pattern of administration; continuous administration decreases bone mass whereas intermittent administration increases bone mass. However, the mechanism is largely unknown. This study examined the effect of PTH on mouse osteocalcin gene expression in MC3T3-E1 preosteoblastic cells and primary cultures of bone marrow stromal cells. PTH increased the levels of osteocalcin mRNA 4- to 5-fold in both cell types. PTH also stimulated transcriptional activity of a 1.3 kb fragment of the mouse osteocalcin gene 2 (mOG2) promoter. Inhibitor studies revealed a requirement for protein kinase A, protein kinase C and mitogen-activated protein kinase pathways in the PTH response. Deletion of the mOG2 promoter sequence from -1316 to -116 caused no loss in PTH responsiveness while deletion from -116 to -34 completely prevented PTH stimulation. Interestingly, this promoter region does not contain the Runx2 binding site shown to be necessary for PTH responsiveness in other systems. Nuclear extracts from PTH treated MC3T3-E1 cells exhibited increased binding to OSE1, a previously described osteoblast-specific enhancer in the mOG2 promoter. Furthermore, mutation of OSE1 in DNA transfection assays established the requirement for this element in the PTH response. Collectively, these studies establish that actions of PTH on the OCN gene are mediated by multiple signaling pathways and require OSE1 and associated nuclear proteins.

Disclosures: **G. Xiao**, None.

M14

Effects of 1,25-Dihydroxyvitamin D_3 and 25-Hydroxyvitamin D_3 on Osteoblast Differentiation in Human Marrow Stromal Cell Cultures. J. Glowacki[1], S. M. Mueller*[2], J. S. Greenberger[3], I. Bleiberg*[4], M. S. LeBoff[5]. [1]Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA, [2]University Hospital Zurich, Zurich, Switzerland, [3]Radiation Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [4]Sackler School of Medicine, Tel Aviv, Israel, [5]Medicine, Brigham and Women's Hospital, Boston, MA, USA.

Vitamin D deficiency is common and is associated with reduced bone strength and fractures. A sub-population of marrow stromal cells has osteoblastogenic potential. We had reported an age-related decrease in osteoblastogenesis with marrow from men 38 to 80 years old. We now test the hypothesis that both 1,25-dihydroxyvitamin D_3 and $25OHD_3$ stimulate osteoblastogenesis in marrow from elderly men and women undergoing total hip replacement for osteoarthritis. Adherent cells were cultured in osteogenic supplements (10 nM dexamethasone, 5 mM ß-glycerophosphate, 170 µM ascorbic phosphate). AlkP activity was measured colorimetrically after 6d.

First, in 13 samples (men, age 27-79 years), 10 nM $1,25(OH)_2D_3$ stimulated AlkP in all, but with a significant decrease with age ($r=-0.803$, $p=0.0009$). Second, dose-response studies showed that $1,25(OH)_2D_3$ stimulated 16/17 (94%) samples (men and women, 64-83y), with peak stimulation between 1 and 10 nM $1,25(OH)_2D_3$. Third, 7/9 (78%) were stimulated by $1,25(OH)_2D_3$ and $25OHD_3$, with equivalent stimulation in cells from 2 men and 1 woman.

Thus, although there are differences in magnitude of stimulation, peak dose, and relative effects of D metabolites, marrow from elders shows osteoanabolic response to $1,25(OH)_2D_3$ and $25OHD_3$. Although *in vivo* vitamin D levels were not studied herein, we speculate that vitamin D status of the subject may account for *in vitro* differences in behavior of marrow stromal cells. We recently reported vitamin D-deficiency in 22% of women admitted for hip replacement from the same pool as those whose discarded marrow was used in this work [J Bone Joint Surg 85A: 2371, 2003].

Disclosures: **J. Glowacki**, None.

M15

Parathyroid Hormone (1-14) Fragments Increase Bone Mass in OVX Rats. M. Shimizu*[1], H. Saito*[1], N. Shimizu*[1], N. Murao*[2], M. Kato*[2], J. T. Potts[3], T. J. Gardella[3], F. Makishima[1]. [1]Pharmaceutical Research Department II, Chugai Pharmaceutical Co., LTD., Shizuoka, Japan, [2]Pre-Clinical Research Department I, Chugai Pharmaceutical Co., LTD., Shizuoka, Japan, [3]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

We have previously shown that modified PTH(1-14) analogs stimulate cAMP and PLC signaling in PTH-1 (P1R) receptor-expressing cells with potencies comparable to that of PTH(1-34). Here we used such N-terminal PTH analogs to further define the minimum PTH pharmacophore that can mediate the in vivo bone-anabolic response. We utilized [M]-PTH(1-21) (M = $Aib^{1,3}$,Gln^{10},Har^{11},Ala^{12},Trp^{14},Arg^{19}), [M]-PTH(1-14), [Ac_5c^1,M]-PTH(1-14), [Ac_5c^1,M]-PTH(1-11) and PTH(1-34), which, in cell-based assays, exhibited apparent binding affinities (IC_{50}s measured with ^{125}I-[M]-PTH(1-15) or ^{125}I-[M]-PTH(1-21) radioligand) of 0.8, 180, 24, 260 and 3.4 nM, respectively; the cAMP-stimulating potencies (EC_{50}s) of these analogs were similar to each other (~2 nM). We administered the peptides intravenously (i.v.) and daily for six weeks to seven-week-old ovariectomized rats. Relative to vehicle, PTH(1-34), [M]-PTH(1-21) and the two PTH(1-14) analogs each significantly and dose (31 to 1,000 nmol/kg)-dependently increased spine BMD, and each achieved approximately the same maximum effect (~20% increase), albeit, compared to PTH(1-34), 10-, 300-, and 100-fold higher concentrations of [M]-PTH(1-21), [M]-PTH(1-14) and [Ac_5c^1,M]-PTH(1-14) were required. [Ac_5c^1,M]-PTH(1-11) (1,000 nmol/kg) did not increase BMD. We developed an LC-MS/MS-based method to assess the concentrations over time of the [M]-PTH(1-14) analogs in plasma of injected rats. The disappearance time ($T_{1/2}$) of [M]-PTH(1-14) was three-fold shorter than that of PTH(1-34) (3 min. versus 10 min.). The overall data show that the essential bone-anabolic pharmacophore of PTH is contained within residues (1-14). Further development of the PTH(1-14) scaffold to improve P1R-binding affinity and/or the pharmacokinetic profile could permit further minimization of the peptide chain length needed to build bone in vivo.

Disclosures: **M. Shimizu**, Chugai Pharmaceutical Co., LTD 3.

M16

Clinical Experience with Serum Calcium and Vitamin D Levels in Patients Treated with Teriparatide [PTH(1-34)]. A. A. LICATA. Endocrinology, Cleveland Clinic Foundation, Cleveland, OH, USA.

This study evaluated changes in mineral metabolism in patients(n=12) using teriparatide for 3-6 months. Serum calcium, PTH, 25 vitamin D, 1,25 vitamin D, bone turnover markers (serum osteocalcin [OSC], and urinary (NTX)] were measured using standard clinical procedures. Patients were using supplemental calcium and vitamin D per standard published guidelines. During treatment, serum calcium increased 5-6% and PTH decreased 16-17% ($p<0.05$). All changes, however, were within the normal reference interval. Seventy-five percent of calcium values were between 10 and 10.5 mg/dL. OSC and NTX increased 3.5 ($p<0.05$) and 4.2($p<0.1$) -fold, respectively. 25 vitamin D did not significantly change. 1,25 vitamin D increased 2-3 fold ($p<0.05$) from baseline. Seventy- five percent of values were greater than the upper reference cutoff (66 pg/mL). the average was. 98.8 pg/ml.. Patients experienced no symptoms during this period of treatment. Adjustment of the daily mineral supplement corrected the serum calcium changes when seen. Summary: teriparatide increases serum 1,25 vitamin D and calcium, the later change reflecting the amount of daily mineral supplement used. Conclusion: for patients using teriparatide, clinicians may need to adjust the mineral and vitamin D doses recommended by published guidelines.

Disclosures: **A.A. Licata**, Eli Lilly 8.

M17

Circulating IGF-I is Essential for the Anabolic Effects of PTH on the Skeleton. S. Yakar[1], M. L. Bouxsein[2], H. Sun*[1], V. Glatt*[2], D. LeRoith[1], C. J. Rosen[3]. [1]Diabetes Branch, National Institutes of Health, Bethesda, MD, USA, [2]Orthopaedic Surgery, Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME, USA.

Using three mouse strains with null mutations of the hepatic IGF-I gene(*LID)*, the ALS gene*(ALSKO)* or both(*LA)*, but normal expression of skeletal IGF-I, we established that circulating IGF-I is essential for peak bone acquisition. To test the importance of circulating IGF-I in mediating PTH's anabolic action, we injected 12wk old male LID, ALSKO, LA and wildtype(WT) mice with PTH(1-34)(50ug/kg/d) or vehicle(VEH) for 4 weeks. At baseline, there was a significant decrease in total cross-sectional area ($p<0.0001$) at the femoral mid-shaft (-8-to -41%) proportional to serum IGF-I. BV/TV and trabecular number in both the vertebrae and distal femur were lower in WT than LID, ALSKO, and LA. After PTH, vertebral BV/TV increased in WT and LID(18%-29%), but unchanged in ALSKO or LA mice. Similarly, in the distal femur, BV/TV increased 20-40% with PTH in WT and LID, but PTH-treated ALSKO and LA declined (22% and 29%, respectively). Trabecular number dropped in ALSKO and LA in response to PTH. Mid-femoral cortical bone area increased 9-16% in PTH-treated WT, LID, and ALSKO but was unchanged in LA. We found that LID, ALSKO and LA mice have reduced femoral cross-sectional area, yet LID and ALSKO have markedly increased BV/TV compared to WT. LA and ALSKO mice do not exhibit a net trabecular anabolic response to intermittent PTH. In comparison, the anabolic cortical bone response to PTH is similar in WT, LID and ALSKO, but nonexistent in LA. We conclude that circulating IGF-I is critical for the optimal skeletal anabolic response to PTH.

Disclosures: **C.J. Rosen**, NIHAR45433 R.

M18

Vitamin D3 and Ascorbic Acid 2-phosphate, a Long-acting Vitamin C Derivative Regulate Growth and Differentiation of Human Osteoblast-like Cells. R. Hata[1], Y. Maehata*[2], S. Takamizawa*[2], S. Ozawa*[2], S. Okada*[2], K. Izukuri*[2], Y. Kato*[2], S. Sato*[3], E. Kubota*[4], K. Imai*[5], H. Senoo*[5]. [1]Biochemistry and Molecular Biology, Research Center of Advanced Technology for Craniomandibular Func, Kanagawa Dental College, Yokosuka, Japan, [2]Biochemistry and Molecular Biology, Kanagawa Dental College, Yokosuka, Japan, [3]Department of Craniofacial Growth and Development Dentistry, Res Ctr Adv Techn Func, Kanagawa Dental College, Yokosuka, Japan, [4]Department of Oral Surgery, Res Ctr Adv Techn Func, Kanagawa Dental College, Yokosuka, Japan, [5]Department of Cell Biology and Histology, Akita University School of Medicine, Akita, Japan.

In order to investigate regulation mechanisms of growth and differentiation of human osteoblasts by dihydroxy vitamin D3 (VD3), and L-ascorbic acid 2-phosphate (Asc 2-P), a long-acting vitamin C derivative, we cultured MG-63 in the presence of VD3 and/or Asc 2-P. Cell growth as observed by cell number was decreased by the presence of VD3 in the culture medium, but it was increased by the presence of Asc 2-P. Type I collagen synthesis and alkaline phosphatase (ALP) activity, early osteoblast differentiation markers, were stimulated by the presence of VD3 as well as by that of Asc 2-P. The co-presence of Asc 2-P and VD3 had an synergistic effect on the collagen synthesis and ALP activity of the cells. Inhibition of collagen synthesis by the presence of inhibitors of collagen synthesis attenuated the stimulative effect of VD3 and Asc 2-P on ALP activity. On the other hand, ALP activity was significantly increased and the growth rate was decreased when the cells were cultured on type I collagen-coated dishes. These results indicate that collagen mediates effects of Asc 2-P and VD3 on the differentiation stimulation of human osteoblast-like cells. VD3 also increased the levels of mRNA for Cbfa1/Runx2 and Osterix, transcription factors critical for osteoblast differentiation as well as those of differentiation markers such as ALP, type I collagen and osteocalcin. These results also suggest that VD3 control growth and differentiation of human osteoblast-like cells by regulating gene expression of osteoblast-related transcription factors as well as type I collagen.

Disclosures: **R. Hata**, None.

M19

Early Changes in Biochemical Markers of Bone Formation Predict Improvements in Bone Structure during Teriparatide Therapy. A. Sipos*[1], H. Dobnig[2], A. Fahrleitner-Pammer[2], L. Ste-Marie[3], J. C. Gallagher[4], I. Pavo*[1], J. Wang*[1], E. F. Eriksen[1]. [1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [2]Internal Medicine, Medical University, Graz, Austria, [3]CHUM Hospital St-Luc, Montreal, PQ, Canada, [4]Bone Metabolism Unit, Creighton University, Omaha, NE, USA.

A significant proportion of new bone formed during teriparatide treatment seems to be formed via modeling, i.e. formation of new bone without previous resorption. To evaluate biochemical markers of bone metabolism as early predictors of the effect of teriparatide therapy on new bone formation at the tissue level, we correlated the change in markers of bone formation at 1 and 3 months with change in bone structure parameters, assessed by 2-dimensional (2D) histomorphometry and 3-dimensional (3D) structural μCT analysis. Thirty-one paired iliac crest biopsies were obtained from patients treated with teriparatide for 12-24 months. At 1 month, increases in serum bone specific alkaline phosphatase (BAP) and serum C-terminal propeptide of type I procollagen (PICP) were significantly correlated with the increase in 2D mean wall thickness (BAP, $r=0.61$, $p<0.001$; PICP $r=0.43$, $p=0.02$) and 3D trabecular bone volume (BAP $r=0.46$, $p=0.009$; PICP $r=0.39$, $p=0.03$). Increases in BAP also correlated with increases in 2D trabecular bone volume ($r=0.51$, $p=0.01$), 3D trabecular bone volume ($r=0.41$, $p=0.02$) and 3D trabecular thickness ($r=0.44$, $p=0.01$). At 3 months, S-PICP showed an inverse correlation with marrow star volume ($r=-0.44$, $p<0.05$), an index inversely related to trabecular connectivity. Early changes in markers of bone resorption were not correlated to changes in structural parameters. In conclusion, our data suggest that early increases in biochemical markers of bone formation predict improvements in bone structure during teriparatide therapy as reflected in increased mean wall thickness, trabecular thickness, trabecular connectivity and trabecular bone volume.

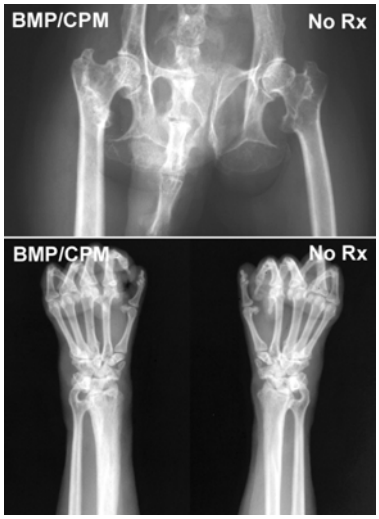
Disclosures: **A. Sipos**, Eli Lilly and Company 3.

M20

A Single Intraosseous Injection of rhBMP-2/CPM Improves Femoral and Radial Structure in Ovariectomized Nonhuman Primates in 6 months. H. J. Seeherman, E. A. Smith-Adaline, J. D. Parkington*, H. Kim*, J. M. Wozney. Women's Health and Bone, Wyeth Research, Cambridge, MA, USA.

Recombinant BMP-2 (rhBMP-2) is a potent osteoinductive factor with the potential to lower the risk of hip and wrist fractures in osteopenic patients by inducing dramatic bone formation following local injection. This study evaluated the efficacy of intraosseous (IO) delivery of rhBMP-2 in a calcium phosphate matrix (CPM) to increase bone mass. One distal radius of six adult female cynomolgus monkeys at least 5 years post-ovariectomy received a single 0.25ml IO injection of 1.5mg/ml rhBMP-2 delivered in CPM (Etex Corporation, Cambridge, MA). Three monkeys also received a single 0.7ml injection of 1.5mg/ml rhBMP-2/CPM (BMP/CPM) in one proximal femur. The contralateral radii and femora remained untreated. Six months post-injection, ex vivo pQCT analysis indicated that BMP/CPM substantially increased total, cortical, and trabecular bone areas, and cortical thickness in both the femoral neck (~36%) and the subtrochanteric region (~27%) (see table and figure). In the distal radius, BMP/CPM significantly increased total (30%), cortical (35%) and trabecular (19%) bone areas and cortical thickness (23%) in the metaphysis ($p<0.05$, paired t-test). Similarly, cortical area of the radial shaft increased by 25% ($p=0.078$) leading to a 52% increase in polar moment of inertia ($p=0.073$). In summary, local administration of BMP/CPM resulted in a rapid increase in cortical and trabecular bone in the proximal femur and distal radius. This change in geometry is expected to substantially enhance the structural integrity of bones at these sites. Mechanical testing is currently ongoing. BMP/CPM represents a promising anabolic therapy for prevention of osteoporosis-related hip and wrist fractures.

pQCT results from the proximal femur. All values represented as mean (standard deviation)		
	BMP/CPM	No Treatment
Fem. neck cortical area (mm^2)	41.9 (9.4)	30.7 (8.4)
Fem. neck cortical thickness (mm)	1.55 (0.38)	1.11 (0.26)
Fem. neck total density (mg/cm^3)	697.3 (126.8)	557.1 (82.5)
Subtrochanteric cortical area (mm^2)	71.7 (7.3)	58.8 (10.5)
Subtrochanteric cortical thickness (mm)	1.78 (0.11)	1.40 (0.23)
Subtrochanteric total density (mg/cm^3)	649.8 (8.7)	550.5 (47.2)



Disclosures: **H.J. Seeherman**, Wyeth 3.

M21

Dkk2 Is Upregulated by Canonical Wnt and Stimulates Osteoblast Mineralization. X. Li*[1], P. Liu*[1], W. Liu*[1], Y. Zhang*[1], J. Zhang*[2], S. Harris*[2], D. Rowe*[1], D. Wu*[1]. [1]Genetics & Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Department of Oral Biology, University of Missouri at Kansas City, School of Dentistry, Kansas City, MO, USA.

The role of a Wnt antagonist DKK2 in the regulation of osteogenesis is investigated. The expression of DKK2 was significantly (about 20 folds) upregulated during mouse primary BMS osteoblast differentiation and that this upregulation immediately followed that of canonical Wnt7b expression, suggesting that canonical Wnts may regulate Dkk2 expression. In fact, Wnt1 indeed increases Dkk2 expression in MC3T3 cells. In addition, Dkk2 expression coincides with GFP expression in BMS cultures derived from the 2.3 Col1A1-GFP transgenic mice, in which GFP is expressed only in osteoblasts and osteocytes. The finding that differentiation of GFP-negative osteoblasts into GFP positive ones requires canonical Wnt stimulation suggests that canonical Wnt signaling may upregulate DKK2 expression in primary osteoblasts. Moreover, we found that overexpression of DKK2 at a late stage of BSM osteoblast differentiation stimulated mineralization. Based on all these results, we propose a model for the involvement of Wnt and Dkk2 in the regulation of osteogenesis; the expression of Wnt7b is upregulated at an early stage of osteoblast differentiation, which stimulates further differentiation of the osteoblasts into 2.3 Col1A1 GFP positive cells and upregulates the expression of Dkk2. Dkk2 in turn stimulates terminal osteoblast differentiation. To investigate the role of Dkk2 *in vivo*, a mouse line that lacks Dkk2 was generated. The Dkk2-null mice are viable, but preliminary examination of bone mineral density (BMD) suggests that these mice have significantly lower whole body or femoral BMDs. Comprehensive characterization of these mice is currently under the way.

Disclosures: **X. Li**, None.

M22

Preventive Effect of Zinc Acexamate Administration on Bone Loss in Diabetic Rats. M. Yamaguchi. Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, Shizuoka, Japan.

Zinc is essential for growth in human and many animals. Bone growth retardation is a common finding in various condition associated with zinc deficiency. Zinh has been shown to stimulate bone formation and to inhibit bone resorption. Zinc has a potent stimulatory effect on protein synthesis in osteoblastic cells. Zinc can directly activate aminoacylt-RNA synthetase in the cells.
Zinc acexamate has a potent stimulatory effect on bone formation in femoral tissue culture in vitro as compared with zinc sulfate, estrogen, IGF-I, and fluoride. Zinc acexamate has a significant stimulatory effect on femoral fracture healing in rats. Furthermore, zinc acexamate was found to have a preventive effect on bone loss in streptozotocin (STZ)-induced diabetic rats in vivo. Rats received a single subcutaneous administration of STZ, and the animals were orally administered once daily for 14 days with zinc acexamate (2.5, 5 or 10 mg/l00 g body weight). STZ administration caused a significant increase in serum glucose, triglyceride and calcium levels and a significant decrease in body weight, serum zinc and inorganic phosphorus levels. Moreover, calcium content, alkaline phosphatase activity and DNA content in the femoral-diaphyseal and -metaphyseal tissues were significantly reduced in STZ-diabetic rats. The change in these serum and bone components of STZ-diabetic rats was significantly restored by the oral administration of zinc acexamate (2.5, 5 or 10 mg/l00 g body weight).
Thus, zinc acexamate has been demonstrated to have a preventive and restorative effect on STZ-induced diabetic condition and bone loss in rats in vivo.

Disclosures: **M. Yamaguchi**, None.

M23

Anabolic Effects of Nitric Oxide on Osteoblasts. S. J. Wimalawansa*. Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

We have previously demonstrated that treatment with nitroglycerine (NG), a nitric oxide (NO) donor is equivalent to estrogen replacement therapy in postmenopausal women on prevention of bone loss; administration of NG prevents ovariectomy as well as glucocorticoid-induced bone loss. Furthermore, endothelial NO synthase enzyme (NOS) deficient mice have lower bone densities; use of NOS inhibitor completely abolished the beneficial effects of estrogen on bone. Paradoxically, generation of NO via i-NOS as with inflammation, or administration of high doses of NO (cardiac patients) enhances bone resorption, while lower levels of NO (c/e-NOS) maintains bone homeostasis.
Accumulating data suggests that NO has direct stimulating effects on osteoblasts, and this leads to an increase or stabilization of serum bone-formation markers, bone-specific alkaline phosphatase and osteocalcin, in contrast to decrease of bone-formation marker seen with anti-resorptive therapies, estrogen and bisphosphonates. Our studies revealed a dose-dependent increase of osteoblastic functions, in response to treatment with NO. In addition to its known anti-osteoclastic effects, histomorphometric data suggests that the administration of NO donor compounds *in vivo* has an anabolic effect on osteoblasts.
NO produced in bone cells via NOS enzyme (and administration of NO compounds into osteoblast cultures, enhanced mineralization) in response to cytokines, mechanical stress, and sex-steroid hormones (by osteoblasts, osteocytes, and endothelial cells) may represent an important regulatory mechanism in both osteoblasts and osteoclasts. This may be especially important under pathological conditions characterized by local increases in the release of inflammatory cytokines, and withdrawal of estrogen as in postmenopausal women and hypogonadism in men.

Disclosures: **S.J. Wimalawansa**, None.

M24

PTH Analogs Enhance Bone Formation at a Weight-Bearing Cement-Bone Interface. M. J. Allen*[1], J. E. Schoonmaker*[1], K. A. Mann*[1], V. Ross*[2], C. Allen*[2], G. E. Willick*[2], J. F. Whitfield[2]. [1]Orthopaedic Surgery, SUNY Upstate Medical University, Syracuse, NY, USA, [2]Biological Sciences, National Research Council, Ottawa, ON, Canada.

This study tests tactical post-operative dosing with parathyroid hormone (PTH) analogs as a means of reducing the risk of implant osteolysis. Aseptic loosening of cemented total joint replacements occurs when the integrity of the cement-bone or stem-cement is compromised as a result of adverse biological or mechanical factors. Promising candidate therapies for this application include anabolic growth factors such as (PTH). PTH (1-34) has been shown previously to increase the interfacial tensile strength of the PMMA-bone interface [1].
An established rat model of implant osteolysis was selected as the ideal model for studying this [2]. The current study is a proof of concept experiment to determine whether a short-course of therapy with either human PTH (1-34)NH_2 or a novel cyclic derivative, [Leu^{27}]cyclo(Glu^{22}-Lys^{26})PTH(1-31)NH_2 (Ostabolin-C™) [3], was sufficient to enhance bone apposition around a PMMA mantle in this rat model. The histomorphometric data showed that a short (4-week) course of treatment with human PTH analogs is sufficient to enhance new bone formation around the PMMA interface and also a possible increased efficacy of the cyclic hPTH(1-31) in comparison to hPTH(1-34).
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2. Allen, M *et al J. Bone. Joint. Surg. Br.* **1996,** *78-B*, 32.
3. Whitfield, J. F. *et al J. Bone Miner. Res.* **1997,** *12*, 1246.

Disclosures: **G.E. Willick**, None.

M25

Zoledronic Acid Increases Total Bone Volume in OP-1 Mediated Bone Formation in a Segmental Rat Femoral Defect Model. D. G. Little*, R. Bransford*, M. M. McDonald*, J. Briody*. Orthopaedic Research, The Children's Hospital Westmead, Sydney, Australia.

Recombinant bone morphogenetic proteins (BMPs) are often used to treat fracture non-union. BMPs enhance osteoblastogenesis, however they also up-regulate osteoclasts. Clinically, this could limit the volume/strength of the callus produced. We hypothesised that the anabolic response to OP-1 (rhBMP-7), may be optimised through reduction of osteoclast activity using zoledronic acid (ZA), thereby increasing callus volume/strength over BMP alone.
A rat femoral fracture non-union model was taken to 8 weeks. 15mg of bovine collagen carrier or carrier containing 0.05mg OP-1 was placed in each defect. Treatment groups were: carrier ± ZA and carrier plus OP-1 ± ZA. ZA (0.1mg/kg) was administered systemically either at surgery or 2 weeks post-operation. Callus formation was assessed by plain radiograph, QCT and undecalcified histomorphometry. Callus strength was assessed by 3-point bending.
Radiological union was absent in carrier ± ZA, whereas all OP-1 groups united. QCT revealed that BMC was increased 45% and 96% over OP-1 alone in OP-1+ZA at 0 and 2 weeks, respectively ($p<0.01$). Similarly, callus volume was increased 45% and 86% over OP-1 alone in OP-1+ZA at 0 and 2 weeks, respectively ($p<0.01$).
BV/TV was increased 72% and 82% over OP-1 alone in the OP-1+ZA at 0 and 2 weeks, respectively ($p<0.05$). Callus strength was increased 107% ($p<0.05$) and stiffness increased 148% ($p<0.05$) in OP-1+ZA 2 weeks over OP-1 alone.
In conclusion, ZA significantly increased OP-1 induced callus formation and strength in our rat fracture non-union model. Hence, combined anabolic and anti-catabolic therapies may significantly improve outcome in treatment of fracture non-union.

Disclosures: **M.M. McDonald**, None.

M26

A Novel Anabolic Peptide Activates Osteogenic Gene Expression in Rat Stromal Cells. G. Schneider*[1], K. J. Grecco*[2], D. McBurney*[2], W. E. Horton*[2]. [1]Weill Cornell Medical College in Qatar, New York, NY, USA, [2]Anatomy, NE Ohio Univers. Coll. of Med., Rootstown, OH, USA.

Two weeks of intermittent subcutaneous injections of short peptides from the third domain of the human serum vitamin D binding protein (DBP) to intact, adult rats results in significant increases in total density and strength of long bones. We tested whether these peptides would act directly on differentiated osteoblasts or less differentiatied stromal cells derived from tibias of post-natal rats. The cells were treated with PTH (5 nMolar) or 5 ng/ml of either peptide 10 or peptide 12 (10 and 12 amino acid fragments of DBP) for time periods ranging from 24 to 72 hours. Total RNA was extracted, reverse transcribed into cDNA, and the relative expression level of a group of marker genes was determined by quantitative real time pcr (QtRT-PCR) normalized to 18S RNA. Peptide 10 induced the up-regulation of mRNA coding for alkaline phosphatase (2.3-fold), collagen I (3-fold), osteocalcin (17-fold), and osteopontin (4.7-fold) by 72 hours of treatment. An independent experiment showed a similar pattern of induction at the 48 hour time point and indicated an upregulation of PCNA mRNA suggesting a proliferative response. PTH and peptide 12 induced a more variable and less robust pattern of osteogenic gene expression. Peptide 10 also induced the expression of these genes in osteoblast cells but the response was slower and less dramatic. These results suggest that the relatively undifferentiated stromal cells present in the marrow may be the target for the anabolic effects of the DBP peptides.

Disclosures: **G. Schneider**, None.

M27

PTHrP Down-Regulates Cyclin D1 Activity in Differentiating MC3T3 Cells. N. S. Datta, C. Chen*, L. K. McCauley. Perio/Prev/Geriatrics, University of Michigan, Ann Aror, MI, USA.

Bone turnover is controlled by the coordination of osteoblast proliferation and differentiation, and PTH and PTHrP have been implicated in these events; however their specific targets are still unclear. The mechanisms by which PTHrP impacts cell cycle proteins and the role of signaling pathways in differentiating MC3T3 osteoblastic cells were investigated. Western blot analysis revealed that PTHrP inhibited cyclin D1 protein expression (7 fold) in a dose and time dependent manner. PTHrP did not alter levels of its kinase partners CDK4, CDK6 or CDK inhibitors p21 and p27, but increased the level of p16 protein. However, PTHrP did decrease the association of cyclin D1 with CDK4/CDK6 protein (3-5 fold) as determined by cyclin D1 immunoprecipitation and western blot for CDK4/CDK6. Forskolin, a PKA agonist, mimicked the action of PTHrP and the PKC inhibitor, GF109203X, slightly blocked PTHrP inhibition of cyclin D1 implying involvement of both PKA and PKC pathways. U0126, a MAPK inhibitor, alone decreased cyclin D1 protein expression suggesting basal cyclin D1 protein is MAPK dependent. Interestingly, in proliferating MC3T3 cells, cyclin D1 remained unchanged with PTHrP. In conclusion, PTHrP down-regulates the G1 phase specific cyclin D1 protein in differentiating but not proliferating osteoblastic cells. These data suggest cell cycle control may be a mechanism through which PTHrP allows osteoblasts to progress while in a proliferating state, but when differentiating, PTHrP may induce G1 cell cycle arrest. Such regulation in cell cycle could be an important determinant of the life span and bone forming activity of osteoblasts.

Disclosures: **N.S. Datta**, None.

M28

A Mathematical Model for the Bone Remodeling Cycle Predicts the Effects of PTH(1-34) on Bone Turnover Markers in Rats. L. K. Potter*[1], G. B. Stroup[2], D. J. Rickard[2], Z. Wu*[3], B. J. Votta[2], S. M. Hwang*[2], F. L. Tobin*[4]. [1]Scientific Computing & Mathematical Modeling, GlaxoSmithKline, Research Triangle Park, NC, USA, [2]Musculoskeletal Diseases Biology, GlaxoSmithKline, Collegeville, PA, USA, [3]Assay Development and Compound Profiling, GlaxoSmithKline, Collegeville, PA, USA, [4]Scientific Computing & Mathematical Modeling, GlaxoSmithKline, King of Prussia, PA, USA.

We are developing a mathematical model for the bone remodeling cycle to predict the effects of different PTH(1-34) dosing regimens on bone formation and resorption in rats. The current approach incorporates the kinetics of osteoblast and osteoclast proliferation, differentiation and apoptosis. While not incorporating all biology into the model, the most salient features necessary for PTH interactions have been introduced. Osteoblast-osteoclast interactions are represented in the model by RANK/RANKL/OPG dynamics. The action of PTH includes: (1) parathyroid hormone receptor kinetics, (2) effects on RANKL and OPG, and (3) effects on osteoblast recruitment and apoptosis. The model hypothesizes that a key difference between the effects of continuous and intermittent PTH dosing is that continuous dosing increases osteoclasts through the RANK/RANKL pathway significantly more than intermittent dosing (Locklin et al., J. Cell. Biochem. 89: 180-190, 2003). Model-simulated levels of active osteoblasts and osteoclasts are correlated with the bone turnover markers plasma osteocalcin and urinary deoxypyridinoline, respectively. The model is able to capture the differential effects of intermittent and continuous PTH dosing on osteoblasts and osteoclasts, including the substantial increase in the RANKL:OPG ratio that is seen with continuous dosing (Locklin et al.). Moreover, in comparing the effects of different PTH(1-34) dosing frequencies and dose levels in rats, model simulations predict that a small dose given daily can result in greater bone formation than a significantly larger dose given weekly. This mathematical model is a useful tool that can help in the design of optimal PTH dosing regimens.

Disclosures: **L.K. Potter**, None.

M29

Revealing the Anabolic Effects of 1,25(OH)$_2$ Vitamin D3 by Co-Administration with Alendronate. A. A. Reszka, S. Pun*, L. P. Freedman, D. B. Kimmel. Molecular Endocrinology and Bone Biology, Merck Research Laboratories, West Point, PA, USA.

The accepted paradigm for bone remodeling in adult animals holds that bone formation is coupled to resorption. Pure antiresorptives, such as alendronate (ALN), reduce bone formation via coupling. 1,25(OH)$_2$ Vitamin D3 (1,25(OH)$_2$D3) displays antiresorptive effects that appear to be uncoupled from anabolism. We hypothesized that anabolic effects of 1,25(OH)$_2$D3 could be better revealed in the presence of a pure anti-resorptive. Three month-old rats were ovariectomized (OVX), and, three months later, treated with 1,25(OH)$_2$D3 (0.1 µg/kg/d, p.o.) alone or in combination with ALN (10 µg/kg, thrice weekly, s.c.) or ALN alone. Despite equivalent reductions of bone resorption by ALN (64%) and 1,25(OH)$_2$D3 (61%), lumbar vertebral cancellous bone formation rate (BFR) was reduced by 98% and 58%, respectively, suggesting an uncoupled bone formation response to 1,25(OH)$_2$D3. Interestingly BFR with 1,25(OH)$_2$D3 was essentially identical in the absence or presence of ALN. Dose-dependent effects were seen with two lower doses of 1,25(OH)$_2$D3 (0.01 and 0.03 µg/kg) combined with ALN, suggesting authentic anabolic action. At the central femur, endocortical BFR was reduced 76% by ALN, but increased with 1,25(OH)$_2$D3 with or without ALN. Periosteal bone formation was unchanged by ALN and doubled by 1,25(OH)$_2$D3. ALN/1,25(OH)$_2$D3 increased periosteal BFR by 3-fold vs. ALN alone. These data demonstrate that 1,25(OH)$_2$D3 increases bone formation both when administered alone and in the presence of ALN. These anabolic effects of 1,25(OH)$_2$D3 occurred despite significant decreases in osteoclast activity, suggesting an independent effect of 1,25(OH)$_2$D3 on bone formation.

Disclosures: **A.A. Reszka**, Merck and Co., Inc. 1, 3.

M30

PTH1R Edocytosis and G_q Signaling Independently Contribute to the Activation of the Mitogen-activated Protein Kinases ERK1 and ERK2. C. A. Syme*, A. Bisello. Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

Agonist-mediated activation of the type I parathyroid hormone receptor (PTH1R) results in several signaling events, and receptor endocytosis. While it is known that activation of the G_s/cAMP/PKA and ERK1/2 pathways contribute in different ways to the anabolic effect of PTH on bone, whether PTH1R trafficking contributes to signaling by PTH remains unclear. To begin addressing this, we investigated the role of PTH1R trafficking in cAMP signaling and ERK1/2 activation in HEK-293T cells. Dominant-negative forms of dynamin (K44A) and β-arrestin2 (319-418) abrogated PTH1R internalization but had no effect on cAMP signaling (desensitization and resensitization) in either the absence or presence of cyclohexamide. Therefore, PTH1R endocytosis is not necessary for regulation of cAMP signaling. ERK1/2 activation by PTH(1-34) peaked at 5 min (average 2.6-fold ± 0.2, n=12) and subsided by 30 min. A PTHrP-based analog (Bpa^1-PTHrP-(1-36)) that activates the G_s/cAMP/PKA without inducing PTH1R endocytosis, failed to activate ERK1/2, indicating that G_q-signaling and/or PTH1R internalization are required for ERK1/2 activation. Inhibition of PTH1R internalization by K44A-dynamin dampened ERK1/2 activation in response to PTH(1-34) by 45%. Conversely, a PTH1R mutant (T410P), which is constitutively associated with β-arrestin2 and does not require occupancy by agonist for internalization, was able to activate ERK1/2 in response to either PTH agonist or antagonist. Therefore, PTH1R trafficking and G_q (but not G_s) signaling independently contribute to ERK1/2 activation. Together with previous reports, these findings underlie the complexity of the molecular mechanisms leading to PTH-stimulated ERK1/2 activity, which may involve G_q-mediated ERK1/2 activation, transactivation of EGF receptors and PTH1R trafficking.

Disclosures: **C.A. Syme**, None.

M31

Teriparatide Mitigates the Cascade of Risk Associated with Increasing Osteoporosis Pathology. J. H. Krege*[1], H. K. Genant[2], G. G. Crans*[1], S. J. Vargas[3], J. C. Gallagher[4]. [1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [2]Osteoporosis and Arthritis Research Group, University of California - San Francisco, San Francisco, CA, USA, [3]Department of Endocrinology, William W. Backus Hospital, Norwich, CT, USA, [4]Bone Metabolism Section, Creighton University Medical Center, Omaha, NE, USA.

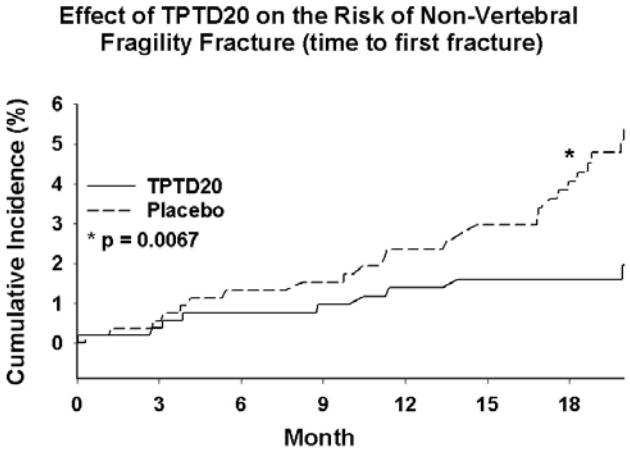
The relationship between increasing number and severity of prior fractures and the corresponding increased risk of developing new fractures was evaluated in women who participated in the teriparatide Fracture Prevention Trial. Data from women with postmenopausal osteoporosis who received placebo or treatment with teriparatide 20 µg once-daily for a median 19 months duration were analyzed to determine the risk of fracture during the trial. Among women in the placebo group with mild, moderate, or severe prevalent vertebral fractures, 9.6%, 12.9%, and 28.4%, respectively, developed new vertebral fractures (Armitage trend test $P<0.001$), whereas 4.0%, 7.9%, and 23.2%, respectively, suffered moderate or severe fractures ($P<0.001$). Among placebo patients with 1, 2, or ≥3 prevalent vertebral fractures, 6.8%, 15.7%, and 22.6%, respectively, developed new vertebral fractures ($P<0.001$), whereas 3.0%, 8.8%, and 17.1%, respectively, developed new moderate or severe vertebral fractures ($P<0.001$). Among placebo patients with 0, 1, or ≥2 prior nonvertebral fractures, 3.4%, 9.4%, and 20.9%, respectively, developed new nonvertebral fractures ($P<0.001$). In contrast to the placebo group, women in the teriparatide 20 µg group showed no significant increase in fracture risk with increasing severity of baseline pathology as determined by number and severity of prevalent vertebral fractures and number of prior nonvertebral fractures. Increasing severity and number of fractures contribute to an increasing cascade of risk for future fractures among untreated patients. Treatment with teriparatide 20 µg once-daily mitigated or eliminated this cascade of risk in women with osteoporosis.

Disclosures: **J.H. Krege**, Eli Lilly and Company 3.

M32

Teriparatide Demonstrates Early Effects in Postmenopausal Women with Osteoporosis. W. J. Shergy*[1], M. Greenwald*[2], G. Woodson*[3], P. Chen*[4], D. A. Misurski*[4], R. B. Wagman[4]. [1]School of Medicine, University of Alabama at Birmingham, Huntsville, AL, USA, [2]Osteoporosis Medical Center, Palm Springs, CA, USA, [3]Department of Medicine, Emory School of Medicine, The Atlanta Research Center, Decatur, GA, USA, [4]US-Endocrinology, Eli Lilly and Company, Indianapolis, IN, USA.

Treatment with rhPTH (1-34) (teriparatide, TPTD) in the Fracture Prevention Trial for a median of 19 months decreased the risk of vertebral and non-vertebral fractures in postmenopausal women with osteoporosis (Neer, 2001). Statistically significant increases in serum levels of procollagen type I of carboxy-terminal propeptide (PICP) at one month and lumbar spine BMD at three months provide early evidence of bone formation after TPTD initiation (Heathman, 2000; Marcus, 2003). These were the earliest time points at which these two parameters were measured. We performed an analysis on time to first fracture at six non-vertebral sites (hip, distal forearm, humerus, clavicle, pelvis, leg). Radiographs for evaluation of vertebral fractures were performed at baseline and study endpoint so only non-vertebral fractures were available for earlier analysis. Those who received TPTD20 demonstrated a reduced incidence of non-vertebral, fragility fracture compared with placebo at 18 months ($p = 0.0067$). After four months, the cumulative percentage of events between the treated and non-treated groups began to diverge. This divergence continued to increase across the duration of the trial (Figure). These results represent continued evidence demonstrating the early effects of TPTD therapy.



Disclosures: **R.B. Wagman**, Eli Lilly and Company 3.

M33

Mechanical Stimulation Prevents Osteocyte Apoptosis through an Integrin/Src/ERK Signalsome Localized in Caveolae: Involvement of a Ligand-Independent Function of the Estrogen Receptor. J. I. Aguirre, L. I. Plotkin, S. B. Berryhill*, R. S. Shelton*, S. A. Stewart*, R. S. Weinstein, A. M. Parfitt, S. C. Manolagas, T. Bellido. Endocrinology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Osteocytes, former osteoblasts entombed in the bone matrix, form an extensive cell communication network that is thought to detect microdamage and mechanical strains and to transmit signals leading to repair and compensatory bone augmentation or reduction. We report that mechanical forces control the integrity of this network by regulating osteocyte survival in vitro and in vivo. Specifically, mechanical stimulation by biaxial stretching of MLO-Y4 osteocytic cells activates the extracellular signal regulated kinases (ERKs), which in turn are responsible for attenuating osteocyte apoptosis. The effect of osteocyte stretching is transmitted by integrins and a signalsome comprising actin filaments, microtubules, caveolae, and Src kinases. Stretch-induced anti-apoptosis also requires ERK nuclear translocation and new gene transcription. Furthermore, knock-down or knock out of the estrogen receptor (ER) α and β abolishes ERK activation and survival induced by mechanical stimulation, indicating the requirement of a ligand-independent function of the ER for the transduction of mechanical forces. Consistent with these in vitro studies, bone unloading by tail suspension of 4 month-old Swiss Webster mice increases the prevalence of osteocyte apoptosis both in cancellous and cortical vertebral bone as early as 3 days, and this event precedes the decrease in bone mineral density and compression strength observed at 18 days. These findings are consistent with the contention that physiologic bone loading provides continuous survival signals that preserve osteocyte viability; and that lack of these survival signals in states of low or absent mechanical loading induces apoptosis and disruption of the osteocyte network, and hence increased bone fragility.

Disclosures: **T. Bellido**, None.

M34

Serum Protein Profiling by SELDI-TOF Mass Spectrometry for Biomarkers of PTH Response. A. K. Prahalad*[1], R. J. Hickey*[2,3], J. Huang*[4], S. Murthy*[1], T. Winata*[1], L. E. Dobrolecki*[2], J. M. Hock*[1,3]. [1]Anatomy & Cell Biology, Indiana Univ Sch. of Medicine, Indianapolis, IN, USA, [2]Dept of Medicine, Indiana Univ Sch. of Medicine, Indianapolis, IN, USA, [3]Indiana Cancer Research Institute, Indianapolis, IN, USA, [4]Dept of Computer Science, Indiana Univ, Indianapolis, IN, USA.

Parathyroid hormone [hPTH(1-34)] (PTH) is an anabolic agent which reduces fracture risk in osteoporotics. However, patient responsiveness to PTH varies. Distinctive serum protein profiles could be used as biomarkers to identify patients most likely to benefit from PTH therapy. Biomarker discovery using SELDI ProteinChip® platform offers great potential for monitoring response to treatment. Our goal was to determine if this technology can reliably detect changes in serum protein profiles in response to PTH in mice. Groups of 5-week old C57/BL-6 male mice were given once daily subcutaneous injection of PTH or vehicle for 3 and 11 days. At 6 hours after the last dose, blood samples were collected for serum protein analysis by SELDI-TOF-MS. Serum protein profiling of the low molecular weight proteome was done using strong anion exchange (SAX) surface chip. Mass spectral data were corrected by baseline subtraction to remove matrix and background noise on raw proteomic data, obtained from the sera of treated and untreated animals. Peaks with high signal-to-noise ratio after baseline subtraction were selected and grouped into bins with various intervals along m/z axis. Two-sample t-test was utilized to search candidate biomarkers among PTH and vehicle treated spectral patterns. Preliminary results indicated discernable changes in 7.4, 7.7, 14.8 and 15.4 kD proteins/peptides in PTH treated groups at both 3 and 11 days, compared to controls. This change was more pronounced in serum from mice treated with PTH for 11 days. Identification of proteins/peptides regulated by PTH will determine their utility as prospective biomarkers of PTH-responders.

Disclosures: **A.K. Prahalad**, None.

M35

Genetic and Activity Level Effects on Variation in BMD and BMC in a Human Genetic Isolate, the Schmiedeleut Hutterites. L. M. Havill[1], M. C. Mahaney[1], T. Binkley*[2], B. L. Specker*[2]. [1]Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA, [2]South Dakota State University, Brookings, SD, USA.

We conducted this study to detect and characterize the effects of genes and physical activity (mean % time per week spent moderately or vigorously active, miles walked per day, stair flights climbed per day) on BMD and BMC in a sample of 711 Schmiedeleut Hutterites from South Dakota, aged 8 to 85 years. Statistical genetic analyses of vBMD (obtained via pQCT) of the radius and aBMD and BMC (obtained via DXA) of the femoral neck, hip, lumbar spine, and total body, show heritability estimates ranging from 0.40 to 0.60. Mean % time per week of moderate or vigorous activity had the most notable effect on BMD and BMC and was associated with higher BMD at all sites ($p \leq 0.02$). Further, significant sex-by-activity interaction was detected for femoral neck BMD. The effect of increased activity is to increase BMD and is stronger in males ($p=0.01$). A similar interaction was observed for BMC. The most novel and interesting aspect of this study is the demonstrated effect of activity level on bone mineral and the sex-specificity of this effect in some cases. Systemically, higher mean time in at least moderate activity/week results in more bone mineral. In the femoral neck, the magnitude of this effect is dependent on gender. The lesser impact of activity level in females may point to a blunting of activity effects by other variables important to bone quality and is consistent with the hypothesis that the female skeleton's role in reproduction may make it less responsive to mechanical loading.

Disclosures: **L.M. Havill**, None.

T1

Role of a Stretch-activated Potassium Channel in Mechanically-induced PTHrP Gene Expression in Osteoblasts. X. Chen, C. M. Macica*, B. E. Dreyer*, A. E. Broadus. Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA.

PTHrP functions locally in an antocrine/paracrine manner in many tissues and is normally expressed in osteoblastic cells. Mechanical forces regulate bone mass and architecture, and PTHrP is mechanically-induced in smooth muscle. We tested the possibility that PTHrP might be a candidate as a local mediator of mechanical force in bone using UMR-201 osteoblast-like cells exposed to hypotonic induction of cell swelling, a surrogate for mechanical loading. Reduction of osmolality from 317 to 240 mosm produced a 3-fold increase in PTHrP mRNA. Addition of either gadolinium or nifedipine had no effect on this response. Furthermore, removal of extracellular calcium or depletion of intracellular calcium with thapsigargin also had no effect. These findings indicate that neither stretch-activated cation channels, L-type calcium channels, nor intracellular calcium is involved in the induction of PTHrP in response to hypotonicity.

TREK family members (two-pore domain potassium channels) are novel stretch-activated channels that can be activated by both stretch and intracellular acidosis. By PCR, we identified the TREK-2 gene expression in UMR-201cells. We found that intracellular acidification markedly increased PTHrP mRNA expression. Furthermore, we found that siRNA targeted against the TREK-2 gene reduced endogenous TREK-2 expression by 80% and that this was associated with 30% decrease of hypotonic induced PTHrP mRNA compared with control siRNA transfected cells. We also found PTHrP expression was induced by cyclic stretch in UMR-201 cells.

We propose PTHrP as a candidate mediator of the anabolic effects of mechanical force on bone, and that TREK-2 channels are involved in the induction of PTHrP.

Disclosures: **X. Chen**, None.

T2

CCAAT Enhancer Binding Proteins: Mediators of $1,25(OH)_2D_3$ and PTH Action that Affect Osteoblast Function. P. Dhawan*[1], X. Peng*[1], S. Williams*[2], S. Christakos[1]. [1]Biochemistry and molecular biology, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA, [2]Cell Biology and Biochemistry, Texas Tech University, Health Sciences Center, School of Medicine, Lubbock, TX, USA.

C/EBPβ is a $1,25(OH)_2D_3$ target in osteoblastic cells and one role for C/EBPβ as a target of $1,25(OH)_2D_3$ is as an enhancer of VDR mediated 24(OH)ase transcription. Our findings indicate that not only $1,25(OH)_2D_3$ but also PTH can induce C/EBPβ in osteoblastic cells. C/EBPβ was found to enhance PKA mediated transcription of hVDR, suggesting a role for C/EBPβ in the cross talk between PTH and $1,25(OH)_2D_3$ that involves enhancement of PKA induced VDR transcription. To examine the mechanism of activation of C/EBPβ by $1,25(OH)_2D_3$ and PTH, UMR 106 osteoblastic cells were transfected with different deletion constructs of the C/EBPβ promoter. These constructs were found to be unresponsive to $1,25(OH)_2D_3$, suggesting possible post transcriptional regulation of C/EBPβ by $1,25(OH)_2D_3$. However, a region located at -120/-60 of the C/EBPβ promoter was found to be required for the activation of the C/EBPβ promoter by PTH (10 nM). Mutation constructs of the C/EBPβ promoter demonstrated that activation of the C/EBPβ promoter by PTH is mediated through a CREB binding site at -111/-107. Transfection of osteoblastic cells with PKA expression vector resulted in activation of this site, indicating a PKA dependent effect of PTH on the induction of C/EBPβ transcription. These findings provide a mechanism for the first time for PTH induction of C/EBPβ and suggest that stimulation of C/EBPβ transcription may be an important mediator of other actions of PTH that can affect skeletal integrity and osteoblast function.

Disclosures: **P. Dhawan**, None.

T3

Role of Crem Gene in the Anabolic Effect of PTH on Bone. F. Liu*[1], S. Lee[1], G. A. Gronowicz[2], D. J. Adams[2], B. E. Kream[1]. [1]Endocrionology, University of Connecticut Health Center, Farmington, CT, USA, [2]Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, USA.

The cAMP response element modulator (Crem) gene encodes transcriptional activators and inhibitors. Osteoblasts express many Crem transcripts including inducible cAMP early repressor (ICER) isoforms, which are transcribed from an intronic promoter and induced by PTH. To determine whether Crem plays a role in the anabolic response of bone to PTH, 11-12 week old Crem knockout (KO) and wild type (WT) male mice (20 mice per group, C57BL/6/129 mixed background) were given daily subcutaneous injections of vehicle or hPTH(1-34) (160 μg/kg) for 10 days. Compared to vehicle-treated controls, PTH caused an 11% increase in femoral areal BMD in WT mice but only a 3% increase in KO mice. Similar results were observed in tibia and vertebrae. PTH significantly increased femoral cortical area and trabecular bone volume in WT but not in KO mice. Interestingly, PTH significantly increased the % osteoblast surface and bone formation rate in WT and KO mice to the same extent. However, PTH increased the % osteoclast surface and osteoclast number to a greater extent (1.8- and 3.4-fold, respectively) in KO compared to WT mice. In vitro osteoclast formation in response to PTH (100 ng/ml) was about 2-fold greater in bone marrow cultures from KO mice. However, there was no difference between the two genotypes in CFU-GM, the osteoclast precursor population in bone marrow. In conclusion, we suggest that the Crem gene plays an important role in the anabolic effect of PTH on bone, which may be mediated in part through an effect on osteoclast formation.

Disclosures: **F. Liu**, None.

T4

Individual and Combined Effects of Exercise and Alendronate on Material and Structural Properties of the Hip and Spine in Ovariectomized Rats. R. K. Fuchs[1,2], M. Shea[3], S. L. Durski*[2], B. Hanson*[3], B. K. Bay*[4], K. M. Winters[5], J. Widrick*[6], C. Snow[2]. [1]Anatomy and Cell Biology, Indiana University Medical School, Indianapolis, IN, USA, [2]Bone Researach Laboratory, Oregon State University, Corvallis, OR, USA, [3]Orthopedic Surgery, Oregon Health Science University, Portland, OR, USA, [4]Mechanical Engineering, Oregon State University, Corvallis, OR, USA, [5]Nursing, Oregon Health Science University, Portland, OR, USA, [6]Exercise and Sport Science, Oregon State University, Corvallis, OR, USA.

We examined the skeletal response to the individual and combined effects of alendronate and exercise on bone. Seven-month-old ovariectomized (ovx) rats were divided into five groups: sham, ovx-controls, ovx-alendronate, ovx-exercise and ovx-alendronate-exercise. Treatments commenced two weeks post-ovx and lasted 14 weeks. Alendronate groups received twice-weekly alendronate (1ml/kg), while sham, ovx-controls and ovx-exercise received vehicle. Exercise groups ran 60 min/day, 21m/min, 5 days/wk at a 5% grade. DXA, μCT and mechanical testing were used to examine femoral and vertebral material and structural properties. Ovx-controls had significant reductions in bone mass and mechanical strength compared to sham. Declines in bone mass and mechanical strength were prevented in each alendronate treated group. The superior treatment was a combination of alendronate and exercise. Ovx-alendronate-exercise had +13.2% and +16.2% femoral BMC, and +21.6% and +15.7% vertebral BMC compared to ovx-alendronate and ovx-exercise, respectively. At the femur, ovx-alendronate-exercise had +5.7% and +9.6% cortical area, +3.9% and +14% cortical thickness, and +8.5% and +12.2% moment of inertia compared to ovx-alendronate and ovx-exercise, respectively. Similarly, at the spine ovx-alendronate-exercise had +9.4% and +11.2% cross-sectional area compared to ovx-alendronate and ovx-exercise, respectively. Ultimate force at the femur did not differ between ovx-alendronate-exercise and ovx-alendronate; however, both alendronate treated groups had stronger bones than both ovx-controls and ovx-exercise. No differences were observed in femoral neck and vertebral ultimate force. In summary, the combined interventions of alendronate and exercise were more efficient in preventing declines in bone mass and strength following ovx than the introduction of either intervention alone.

Disclosures: **R.K. Fuchs**, None.

T5

A Mutation in the Osteoactivin/Gpnmb Gene Causes Osteopenia in Mice. M. C. Rico[1], M. G. Anderson*[2], A. Virgen*[1], S. W. M. John*[2], S. N. Popoff[1], F. F. Safadi[1]. [1]Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA, [2]HHMI and The Jackson Laboratory, Bar Harbor, ME, USA.

Osteoactivin/Gpnmb is a glycosylated protein implicated in bone matrix production and mineralization. Previous studies from our laboratory have demonstrated that a synthetic osteoactivin peptide induces osteoblast differentiation associated with increased alkaline phosphatase activity, osteocalcin production and calcium deposition in vitro, and stimulates bone formation in vivo. A mutation on chromosome 6 of the osteoactivin/Gpnmb gene in the mouse strain DBA/2J(D2) results in a premature stop codon leading to the production of a truncated osteoactivin/Gpnmb protein. Western Blot analysis confirmed the absence of osteoactivin/Gpnmb protein in the osteoactivin/Gpnmb mutant when compared to controls. Radiographic and histological analyses showed a decrease in cortical and trabecular bone area in osteoactivin/Gpnmb mutants compared to age- and gender-matched normal controls. Micro-CT measurements revealed a significant decrease in trabecular bone volume, and an increase of bone marrow cavity area in the osteoactivin/Gpnmb mutant compared to control animals. Total RNA isolated from long bones of mutant and control animals were analyzed for extracellular matrix production. Collagen type I expression was decreased in osteoactivin/Gpnmb mutants compared with controls. These data support the hypothesis that osteoactivin/Gpnmb is a novel bone protein that regulates osteoblast differentiation and augments bone formation. Further studies of this mutation will help elucidate the mechanism(s) of action and signaling pathways involved in osteoactivin/Gpnmb function in bone.

Disclosures: **M.C. Rico**, None.

T6

Skeletal Disease Accompanying High Bone Mass and Novel LRP5 Mutation. M. R. Rickels*[1], X. Zhang*[2], S. Mumm[2,3], M. P. Whyte[2,3]. [1]Division of Endocrinology, Diabetes and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA, USA, [2]Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA, [3]Shriners Hospitals for Children, St. Louis, MO, USA.

Gain-of-function mutation (*Gly171Val*) of the gene encoding LDL receptor-related protein 5 (LRP5) was discovered in 2002 in two American kindreds with dense bones and seemingly benign phenotypes. However, in 2003, 6 novel *LRP5* missense mutations affecting the same protein domain were reported in individuals from the Americas and Europe, some with clinically significant dense bone disease. Our 59-year-old patient has skeletal disease affecting her oropharynx. Three years earlier, extensive mandibular buccal and lingual exostoses (osseous "tori") necessitated surgical removal because of infection attributed to food trapping between the teeth and exostoses. Bilateral maxillary buccal and lingual exostoses have remained asymptomatic. Radiographic skeletal survey revealed marked thickening of the skull base and cortical widening of long bones reflecting endosteal hyperostosis. Bone mineral density Z-scores, assessed by DEXA (Lunar® DPXMD, Madison, WI), were + 8.5 and + 8.7 in the right total hip and L_1-L_4 spine (~ 195% average for age-matched women), respectively. Her brother was diagnosed with "osteopetrosis," and a first cousin required similar removal of excessive bone from her jaw. PCR amplification and sequencing of *LRP5* exons 2-4 and adjacent splice sites revealed heterozygosity for a new *LRP5* missense mutation, *Arg154Met*. This novel defect affects the same first "β-propeller" module as the 7 previously reported gain-of-function missense mutations. Because our patient's LRP5 mutation alters the region responsible for the receptor's antagonism by dickkopf (Dkk), her extensive oral exostoses and high bone mass likely reflect increased Wnt signaling through LRP5. Our patient illustrates that exuberant *LRP5* signaling may not be benign.

M.R. Rickels, None.

T7

BMP6 Regulation of Human Marrow-derived Mesenchymal Stem Cell Differentiation. M. S. Friedman*[1], M. W. Long*[2], K. D. Hankenson[3]. [1]Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI, USA, [2]Pediatrics, University of Michigan, Ann Arbor, MI, USA, [3]Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA.

Bone morphogenetic proteins (BMP) are members of the TGF-beta superfamily that are sequestered and released from bone and cartilage into the marrow cavity. We hypothesize that BMPs are factors that induce differentiation of pluriopotent human marrow-derived mesenchymal stem cells (hMSC) to the osteoblast lineage. Preliminary experiments indicated that BMP-6 alone, or in combination with BMP-7, strongly induced osteoblast differentiation of hMSC determined by an approximate 4.5-22.5 fold increase in alkaline phophatase activity (multiple donors). BMP-6 treated MSC exhibit a phenotype consistent with differentiated osteoblasts, forming a mineralized extracellular matrix composed of $CaPO_4$-hydroxyapatite, type I collagen, osteopontin, and BSP-II. A detailed analysis of gene expression in BMP-6 induced MSC was performed using quantitative RT-PCR. Increases in collagen 1A1 and osteocalcin expression are detected early in the differentiation program, while osteopontin expression is virtually unchanged. Cbfa1 expression increases 16-fold after 14 days of BMP induction and returns to basal levels by day 21. Osterix expression peaks at day 1, rising again at day 14, a pattern similar to Dlx5. Hedgehog signaling was not detected in BMP-6 treated MSC, but the wnt-associated transcription factor, LEF1, and the wnt co-receptor, LRP5, both show increased levels of expression, indicating that BMP signaling may activate a wnt autocrine loop. Contrary to studies with murine cells, we did not observe an increase in wnt-3a. Thus, we conclude that BMP-6 is a potent inducer of human MSC osteoblast differentiation, and that a wnt autocrine loop, independent of wnt-3a, is involved in this differentiation program.

Disclosures: **M.S. Friedman**, None.

T8

Osteogenic Oxysterols Inhibit the Adverse Effects of Oxidative Stress on Osteogenic Differentiation of Marrow Stromal Cells. F. Parhami[1], C. M. Amantea*[1], J. A. Richardson*[1], T. J. Hahn[2], D. Shouhed*[1]. [1]Medicine, UCLA, Los Angeles, CA, USA, [2]Medicine, West L.A. VA Medical Center, Los Angeles, CA, USA.

Oxysterols form a large family of oxygenated derivatives of cholesterol present in the circulation and in tissues of humans and animals. We reported that a specific oxysterol combination containing 22(R)-hydroxycholesterol (22R) and 20(S)-hydroxycholesterol (20S) (RS) has potent osteogenic activity in vitro when applied to osteoprogenitor cells including M2-10B4 (M2) marrow stromal cells. The osteogenic oxysterol combination also had synergistic osteogenic effects with BMP2 and BMP7, and potent anti-adipogenic effects in pluripotent mesenchymal stem cells. Recently we found that substitution of 22R with 22S stereoisomer, in combination with 20S, has even greater osteogenic activity, as determined by the greater induction of alkaline phosphatase activity, and Cbfa1 and osteocalcin mRNA expression in M2 cells. We previously demonstrated that oxidative stress induced by xanthine/xanthine oxidase (XXO) or by minimally oxidized LDL (MM-LDL) inhibited osteogenic differentiation of M2 cells. Pretreatment of M2 cells for 24 hours with SS completely protected them from the adverse effects of XXO and MM-LDL, as assessed by their intact levels of alkaline phosphatase activity, osteocalcin mRNA, and mineralization. Treatment with SS also rescued XXO- or MM-LDL treated M2 cells from their inhibitory effects on osteogenic differentiation. The protective effects of the osteogenic oxysterols were inhibited by cyclooxygenase-1 (COX-1) inhibitor, SC-560, but not by COX-2 inhibitor, NS-398, or MAPK inhibitor, PD 98059. Non-osteogenic oxysterols and oxidized lipids including 7-ketocholesterol and 4-hydroxynonenal did not have protective effects. In conclusion, the osteogenic oxysterols protect osteoprogenitor cells against oxidative stress and may be potentially important in enhancing bone formation in aging and osteoporosis.

Disclosures: **F. Parhami**, None.

T9

Decreased Estrogen May Contribute to Osteopenia in Unloaded Bones. J. C. Tou*, S. Arnaud*, R. Grindeland*, C. Wade*. Life Sciences Division, NASA Ames Research Center, Moffett Field, CA, USA.

Progressive loss of weight-bearing bone in astronauts is one of the most serious impediments to long-duration spaceflight. Estrogen deficiency in women is an established factor in bone loss. Reduced sex hormone levels have been reported in male astronauts, but no data is available regarding spaceflight effects on female sex hormones. The objective of our study was to determine the role of estrogen in disuse osteopenia. The NASA developed hindlimb suspension (HLS) model was used to simulate the unloading disuse of weight-bearing bones experienced in space. Female Sprague-Dawley rats (age 77d; n=20/group) were HLS or kept ambulatory (AMB) for 38 d and endocrine and bone indices determined. HLS of rats resulted in lower ($p<0.01$) bone mass (9%), bone mineral content (BMC 13%) and mechanical strength (28%) compared to AMB animals. Plasma estradiol (E2) was lower ($p=0.03$) in HLS (10.1 +1.4 pg/ml) compared to AMB rats (16.7 +2.6 pg/ml). E2 was positively correlated to BMC $r2=0.67$ and mechanical strength $r2=0.61$. These results suggest that reduced E2 plays a role in disuse osteopenia induced by HLS. Plasma or pituitary lutenizing hormone (LH) and follicle stimulating hormone (FSH) levels were not different in HLS versus AMB rats. However, pituitary LH was correlated to E2 ($r2=0.57$), suggesting change in E2 was exerted at the level of the hypothalamus-pituitary axis. Understanding the role of estrogen in disuse osteopenia is necessary to the development of efficacious therapies for female astronauts, bed rest patients and the increasing number of individuals in our sedentary population suffering bone loss.

Disclosures: **J.C. Tou**, NASA 2.

T10

Anabolic Effects of Lactoferrin in Bone. J. Cornish, A. Grey, D. Naot, I. R. Reid. Medicine, University of Auckland, Auckland, New Zealand.

Lactoferrin, an 80 kDa iron-binding glycoprotein that belongs to the transferrin family, is present in breast milk, epithelial secretions and in the secondary granules of neutrophils. We have recently discovered that human and bovine lactoferrin are anabolic to bone at physiological concentrations. Lactoferrin is a very potent stimulator of proliferation and differentiation of primary osteoblasts, and also acts as a survival factor, inhibiting apoptosis induced by serum withdrawal by up to 70%. In murine bone marrow culture, lactoferrin acts as a potent inhibitor of osteoclastogenesis. In vivo, local injection of lactoferrin above the hemicalvaria of adult mice results in substantial increases in the dynamic histomorphometric indices of bone formation and bone area.
We have demonstrated that the mitogenic effect of lactoferrin in osteoblast-like cells is mediated through members of the LDL receptor-related proteins. Lactoferrin is being endocytosed into the cells and also activates MAPK phosphorylation. These two pathways operate independently; MAPK signalling, but not endocytosis, is necessary for the mitogenic effect of lactoferrin.
We investigated lactoferrin's role in osteoclastogenesis by measuring changes in the expression levels of OPG and RANKL mRNA in primary human osteoblasts and in a mixed cell population derived from murine bone marrow. The results indicate that it is unlikely that changes in OPG and RANKL levels are mediating the effect of lactoferrin in bone. We speculate that lactoferrin may have a physiological role in bone growth and healing, and a potential therapeutic role as an anabolic factor in osteoporosis.

Disclosures: **J. Cornish**, None.

T11

Intermittent Parathyroid Hormone Treatment Enhances Guided Bone Regeneration in Rat Calvarial Bone Defects. T. T. Andreassen[1], V. Cacciafesta*[2]. [1]Department of Connective Tissue Biology, University of Aarhus, Aarhus C, Denmark, [2]Department of Orthodontics, The Royal Dental College, University of Aarhus, Aarhus C, Denmark.

Animal experiments have revealed that parathyroid hormone (PTH) treatment enhances fracture healing by augmenting callus formation and increasing strength of the fractures. We now have investigated the effects of intermittent PTH(1-34) treatment on bone regeneration and mechanical strength of critically sized rat calvarial bone defects covered with expanded membranes. A full-thickness bone defect (diameter 5 mm) was trephined in the central part of the parietal bones in 20-month-old female Wistar rats. The bone defects were covered with an exocranial and an endocranial expanded polytetrafluoroethylene membrane. The animals were killed 35 days after operation. 60 µg PTH(1-34)/kg was administered daily during the healing period, and control animals with calvarial bone defects were given vehicle. Mechanical testing was performed by a punch out testing procedure by placing a steel punch (diameter 3.5 mm) in the center of the healed defect. After mechanical testing, the newly formed tissue inside the defect was removed and the dry weight and ash weight were measured. PTH(1-34) increased dry weight by 48%, ash weight by 51%, and ash concentration by 26%. PTH(1-34)also augmented the mechanical strength of the new bone formed inside the defect by increasing ultimate stiffness by 87%. No differences in body weight were found between the vehicle-injected and the PTH-treated animals during the experiment. The experiment demonstrates that intermittent PTH(1-34) treatment increases bone deposition and enhances mechanical strength of healing rat calvarial defects covered with expanded polytetrafluoroethylene membranes.

Disclosures: **T.T. Andreassen**, None.

T12

Cyclical Treatment with High Dose Calcitriol Increases Vertebral Bone Mass in Normal and Osteopenic Rats. R. G. Erben, K. Nägele*. Institute of Animal Physiology, University of Munich, Munich, Germany.

It was our aim to test the hypothesis that remodeling period-based cyclical treatment with high dose calcitriol would increase bone mass in osteopenic ovariectomized (OVX) rats. Eighty-eight female 6-month-old Fischer 344 rats were either OVX or sham-operated (SHAM). Eight rats served as baseline controls. Three months postsurgery, 8 SHAM and 8 OVX rats were killed as pretreatment controls. Beginning 3 months postovariectomy, groups of SHAM and OVX rats ($n = 8$ each) were subcutaneously injected with either 0.2 µg calcitriol/kg/day or vehicle on 3 consecutive days. This treatment regimen was repeated every 3 weeks for a total of 3 cycles. Groups of vehicle- and calcitriol-treated SHAM and OVX rats ($n = 8$ each) were killed after the third treatment cycle, and after a 9-week therapy-free posttreatment interval. By 3 months postsurgery, OVX rats had developed marked vertebral and proximal tibial cancellous bone osteopenia. Although the rats were treated for only 9 days during this experiment, vertebral cancellous bone area and vertebral bone mineral density measured by pQCT were significantly increased in SHAM and OVX rats after 3 cycles of high dose calcitriol, relative to vehicle controls. However, the positive effects of cyclical calcitriol on vertebral bone mass were lost during the 9-week posttreatment period. There were no significant effects of cyclical calcitriol treatment in the tibia. We conclude that cyclical calcitriol treatment has anabolic effects on vertebral bone mass in both SHAM and OVX rats and can partially reverse estrogen deficiency-induced osteopenia in the axial skeleton of the rat.

Disclosures: **R.G. Erben**, ClinTrials BioResearch, Procter & Gamble Pharmaceuticals 5.

T13

The Acute Effects of a Novel Oral Formulation of Salmon Calcitonin on Bone Turnover in Healthy Postmenopausal Women. L. B. Tanko[1], Y. Z. Bagger[1], J. P. Devogelaer*[2], J. Y. Reginster*[3], L. Mindeholm*[4], M. Olson*[4], M. Azria*[4], C. Christiansen[1]. [1]Center for Clinical and Basic Research, Ballerup, Denmark, [2]Arthritis Unit, Université Catholique de Louvain, Brussels, Belgium, [3]3WHO Collaborating Center for Public Health Aspects of Osteoarticular Disease, Liege, Belgium, [4]Novartis, Basel, Switzerland.

The purpose of this study was to investigate the acute effects of a novel oral salmon calcitonin (sCT) on bone formation and resorption in 278 healthy elderly women (55-85 years old) in a randomized and placebo-controlled setting. Participants received sCT 0.15, 0.4, 1.0, or 2.5 mg daily or 1.0 mg every other day combined with an eligen technology-based carrier (200 mg), or placebo. All participants received 1000 mg Ca plus 400 IU of vitamin D daily. Study parameters were serum C-terminal telopeptide of collagen type I (sCTx), osteocalcin (OC), calcium, and PTH measured in fasting samples taken from 8 to 12 a.m at hourly

intervals. Acute responses were re-tested at Month 1 and 3. The 24-hour profile of sCTx showed marked dose-dependent decreases with nadirs ranging from -61.2 to -82.7% ($p<0.001$). Serum OC measured at corresponding time points indicated no significant changes, yet there was a trend toward increasing values at lower and decreasing values at the highest doses. The parallel changes of serum calcium and PTH indicated transient decreases and increases, respectively. These responses showed high degree of reproducibility when re-evaluated at Month 1 and 3. Oral salmon calcitionin inhibits bone resorption without inhibiting bone formation (uncoupling) thereby opening for relative predominance of bone formation. Further investigations are needed to clarify whether the pulsatile stimulation of PTH at each drug intake, which has been shown to provide anabolic effects in animal experiments, have implications for the effects of calcitonin in humans.

Disclosures: **L.B. Tanko**, None.

T14

Sclerostin Is an Osteocyte-expressed Negative Regulator of Bone Formation, but Not a Classical BMP Antagonist. C. W. Lowik*[1], P. ten Dijke*[2], R. L. van Bezooijen[1]. [1]Endocrinology, Leiden University Medical Center, Leiden, Netherlands, [2]Endocrinology, The Netherlands Cancer Institute, Division of Cellular Biochemistry, Netherlands.

Sclerosteosis, a skeletal disorder characterized by high bone mass due to increased osteoblast activity, is caused by loss of the *SOST* gene product, sclerostin. The localization in bone and the mechanism of action of sclerostin are not yet known, but it has been hypothesized that it may act as a bone morphogenetic protein (BMP) antagonist. We show here that *SOST*/sclerostin is expressed exclusively by osteocytes in mouse and human bone and inhibits the differentiation and mineralization of murine pre-osteoblastic cells (KS483). Although sclerostin shares some of the actions of the BMP antagonist noggin, we show here that it has also actions distinctly different from it. Sclerostin, in contrast to noggin, did not inhibit basal ALP activity in KS483 cells neither did it antagonize BMP-stimulated alkaline phosphatase activity in mouse C2C12 cells. In addition, sclerostin had no effect on BMP-stimulated Smad phosphorylation and direct transcriptional activation of MSX-2 and BMP response element (BRE) reporter constructs in KS483 cells. Its unique localization and action on osteoblasts suggest that sclerostin may be the previously proposed osteocyte-derived factor that is transported to osteoblasts at the bone surface and inhibits bone formation. These observations suggest that inactivation of sclerostin by small molecules or humanized neutralizing antibodies may induce a positive bone balance, an effect that may have therapeutic implications for patients with osteoporosis.

Disclosures: **C.W. Lowik**, None.

T15

Increased Osteoblastic Differentiation in Cultured Marrow Cells After Blood Loss or Surgery. S. Odoi*, J. Burford*, R. Da Souza*, L. Parry*, T. Skerry. VBS, Royal Veterinary College, London, United Kingdom.

Ex vivo studies of bone marrow cell differentiation following drug treatments or surgical procedures correctly use sham treated/operated control animals. In ovariectomy studies we have found a consistent significant increase in fibroblastic colony forming units (CFU-Fs) when bone marrow is cultured from sham-operated animals than cells from ovariectomised animals. The following experiments test the hypothesis that surgical trauma and/or blood loss increases the pool of CFU-Fs or their precursors in bone marrow.

Bone marrow was extracted post-mortem from the femora and tibiae of female Wistar rats weighing approximately 200g. Five experimental groups were used (see table). Sham ovariectomy was performed by exposing both ovaries via a flank incision but not removed. Anaesthesia was by intra-peritoneal injection of ketamine (90mg.kg^-1) and xylazine (10mg.kg^-1). Marrow cells were removed from the bones by gentle centrifugation and were cultured for 18 days in osteogenic conditions, after which they were stained for osteoblastic markers. The total colony area and number of colonies were measured by image analysis.

Sham surgery or blood removal had greater numbers of larger colonies than intact		
	area (cm2)	colony number
Intact, non-anaesthetised control	0.236 s.e.m+/- 0.0106	17.7 s.e.m+/- 0.76
Control, anaesthetised 2w prior to euthanasia	0.096 s.e.m+/- 0.0095	10.1 s.e.m+/- 0.085
ovariectomised 2 weeks before euthanasia	0.125 s.e.m+/- 0.0130	14.3 s.e.m+/- 0.19
anaesthetised 2 weeks before euthanasia and 5ml.kg-1 blood removed by direct cardiac aspiration	0.261 s.e.m+/- 0.0061	20.1 s.e.m+/- 1.39
sham ovariectomy control 2 weeks before euthanasia	0.272 s.e.m+/- 0.0098	22.7 s.e.m+/- 1.39

The effects of the ketamine/xylazine anaesthetic was consistent with our other studies showing that these agents produce an inhibitory effect on CFU-Fs.

It is possible to speculate that blood loss or surgery upregulates haematopoietic cell lineage differentiation. This concomitantly upregulates the precursors of osteoblastic lineage cells. Alternatively the precursor pool affected by trauma/blood loss may remain sufficiently plastic that ex vivo, under osteogenic conditions, they can be induced to transdifferentiate towards an osteoblastic phenotype.

Disclosures: **S. Odoi**, None.

T16

Msx2 Regulates Mesenchymal Cell Lineage and Body Composition via Paracrine Wnt-Dkk Signals. S. L. Cheng, N. Charlton-Kachigian, J. S. Shao, A. P. Loewy*, D. A. Towler. Dept of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA.

Msx2 promotes osteogenic differentiation of vascular progenitors while suppressing adipogenic potential. Along with cell autonomous actions, conditioned media (CM) from Msx2-transduced cells controls cell fate; Msx2 CM enhances alkaline phosphatase (ALP) activity of C3H10T1/2 cells by 50%, but inhibits adipogenesis by >90%. Since Wnts exert similar activities, we studied effects of Msx2 on Wnt and Dkk signaling. Msx2 transduced 10T1/2 cells and myofibroblasts express significantly elevated Wnt1, Wnt3a, Wnt5a, and Wnt5b levels. In contrast, Dkk expression is decreased to <24% of control (Dkk1 in myofibroblasts, Dkk2 in 10T1/2 cells). Msx2 CM stimulates canonical Wnt-regulated LEF/TCF transcription along with ALP activity. Importantly, 1 ug/ml recombinant Dkk1 suppresses Msx2-dependent ALP induction. To confirm these results, we generated CMV-Msx2 transgenic mice. Msx2 is significantly over-expressed in aorta, bone marrow cells, and osteoblasts isolated from transgenic mice (2.9, 1.6, and 2.6 fold, respectively) as compared to wild type littermates (WT). Wnt3a levels in aorta and osteoblasts are increased in Msx2 transgenics (2.7 and 3.8 fold), with a concomitant decrease in Dkk1 in aorta and bone marrow cells (27% and 64% of WT). The BMD of Msx2 transgenic mice is significantly higher than WT littermates after 4 and 8 weeks of a high fat diet challenge (2.7 % and 3.5% increase). Total body fat is significantly decreased in Msx2 transgenic mice as compared to WT, and serum leptin levels are concomitantly lower (45.2 ± 174.3 vs. 1773.1 ± 383.6 pg/ml). Thus, Msx2 regulates mesenchymal cell fate and body composition in part via paracrine Wnt-Dkk signals.

Disclosures: **D.A. Towler**, Pfizer 2.

T17

Physical Activity Is Associated with the Size but not with the Volumetric Mineral Density of the Cortical Bone in Young Adult Men. M. Lorentzon[1], D. Mellström[2], C. Ohlsson[1]. [1]Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg, Sweden, [2]Department of Geriatric Medicine, Göteborg University, Göteborg, Sweden.

Physical activity has been reported to enhance bone mass accretion but little is known about its differential influence on the separate bone compartments, i.e. trabecular and cortical bone.
The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study consists of 1075 Swedish men, age 18.9±0.6 yrs, and was initiated with the aim to find both environmental and genetic determinants for bone and fat mass. Questionnaires were used to collect information about current and previous physical activity (hours/week and duration in years), dairy product intake and smoking. 670 men (62%) were currently physically active and 761 (71%) had previously participated in any sports. Bone parameters were measured using both DXA and pQCT.
Both current and previous physical activity were independent predictors (multivariate analysis including age, height, weight, dairy product intake and smoking) of areal BMD of the total body, femoral neck, and lumbar spine as measured by DXA. To determine the associations between physical activity and the different bone compartments pQCT was utilized, demonstrating that current physical activity was an independent predictor of cortical bone mineral content (radius β=0.14, $p<0.001$; tibia β=0.22, $p<0.001$), cortical bone area (radius β=0.15, $p<0.001$; tibia β=0.22, $p<0.001$), and periosteal circumference (radius β=0.16, $p<0.001$ tibia β=0.17, $p<0.001$), but not of cortical volumetric BMD in the long bones. These results demonstrate that physical activity is associated with the size but not with the volumetric mineral density of the cortical bone in young adult men, suggesting that physical activity increases the amount but not the material quality of the cortical bone.

Disclosures: **M. Lorentzon**, None.

T18

Pathophysiology of Osteoporosis: Is the Major Defect (Bone Loss) Due to Metabolic Imbalance Between Bone Resorption and Formation, or to Insufficient Bone Collagen (Matrix) Formation? L. Klein. Biochemistry, Case Western Reserve Univ School of Medicine, Cleveland, OH, USA.

To evaluate that osteoporosis is a systemic disease where the diurnal rhythm of bone turnover is regulated by systemic multifactors: (1) Muscle activity; (2) Nutrition: Vitamins A, C, D, proteins, amino acids, minerals (calcium [Ca] and phosphorus [Pi]); and (3) Hormones: anabolic: parathyroid hormone (PTH), growth hormone (GH)/insulin-like growth factor (IGF/I); catabolic: calcitonin (CT) gluco- and adreno-corticoids; insulin, thyroid, androgens, and integrated by estrogens *a/b.* Systemic multifactors are involved at different levels of biological organization: (1) System, organ, cell, receptor; (2) Multi-organs: bone, intestine, kidney, liver, (3) Multi-hormonal: PTH, CT, Vit D, GH/IGF I, etc.; and (4) Multi-factorial: environment, genetic, nutrition, vascular, nervous, hormonal, etc.
Of two major pathways, one major pathway is inorganic, catabolic, and bone resorptive (induced by calcitonin, solubilization of Ca and Pi, and proteolysis of collagen to peptides, amino acids, and their combustion). The renal reabsorption of Ca and Pi results in their efficient conservation via their uptake by mineralization of new osteoid and old bone. The other major pathway is organic, anabolic, and hormonally regulated. Collagen is biosynthesized de novo from amino acids to macromolecules. The new collagen structure is specifically modified by vitamin C to bone osteoid which in turn is specifically mineralized to bone by osteoblasts. Thus, organic collagen can only be used once, while bone minerals can be reused repeatedly in bone formation. In addition, the therapy of osteoporosis can be bolstered by a larger intake of vitamin C (citrus fruit), vegetable proteins, and physical exercise.

Disclosures: **L. Klein**, None.

T19

Fluoroaluminate Stimulates and RGD Peptides Inhibit the Cellular Attachment and Spreading of Osteoblasts. C. J. C. Boersma*[1], R. J. Arends*[2], B. L. H. van Lith*[3], K. McGurk*[4]. [1]Target discovery unit Oss, NV Organon, Oss, Netherlands, [2]Pharmacology Unit Oss, NV Organon, Oss, Netherlands, [3]Target Discovery Unit Oss, NV Organon, Oss, Netherlands, [4]Lead Discovery Unit Oss, NV Organon, Oss, Netherlands.

Bone formation involves the processes of recruitment of mesenchymal precursor cells, followed by attachment to the bone surface and further differentiation of the pre-osteoblasts in mature osteoblasts and formation of new bone. Stimulation of pre-osteoblast attachment to the bone surface is expected to stimulate the process of osteoblast differentiation and mineralization. For this purpose a model was developed in which the attachment of C2C12 and MC3T3-E1 cells could be quantified. C2C12 cells especially attached very efficiently to fibronectin or vitronectin coated plates, and to a much lesser degree to collagen coated plates. In contrast, attachment of MC3T3 cells was very efficient to fibronectin, vitronectin as well as collagen coated plates.
Cellular attachment of MC3T3 or C2C12 cells to fibronectin or vitronectin coated plates could be inhibited by RGD peptides. In contrast, fluoroaluminate gave a small but significant increase in cellular attachment. The described model can be used for studying the role of different genes, including cSrc and integrins, in attachment of osteoblast precursor cells to the bone surface.

Disclosures: **R.J. Arends**, None.

T20

Bone Mass Has Reached its Peak in the Spine and Hip but Continues to Increase in the Cortices of the Long Bones in 18-20-Year-Old Men. M. Lorentzon[1], D. Mellström[2], C. Ohlsson[1]. [1]Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Department of Geriatric Medicine, Göteborg University, Göteborg, Sweden.

In men, peak bone mass is believed to be achieved by the end of the second decade in life. The aim of the present study was to determine if the peak bone mass, at different localities, is reached in 18-20-year-old men. The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study consists of 1075 Swedish men, age 18.9±0.6 yrs, and was initiated with the aim to find environmental and genetic determinants for bone and fat mass. Questionnaires were used to collect information about physical activity, dairy product intake and smoking. Bone parameters were measured using DXA and pQCT.
DXA measurements demonstrated that age was correlated to areal BMD of the radius ($r=0.16$; $p<0.001$) and the ulna ($r=0.15$; $p<0.001$) but not to the total body, femoral neck, or lumbar spine. pQCT measurements revealed that age was correlated to cortical BMC in both the radius and tibia ($p<0.05$). Age was found to be an independent predictor (in a multiple linear regression analysis including height, weight, physical activity, and smoking) of both the cortical volumetric BMD (radius β=0.29, $p<0.001$; tibia β=0.14, $p<0.001$) and the cortical thickness (radius β=0.15, $p<0.001$; tibia β=0.08, $p<0.01$) in the long bones. Trabecular volumetric BMD of the radius (β=0.08, $p<0.05$) but not of the tibia was associated with age.
These results demonstrate that in 18-20-year-old men peak bone mass has been attained in the femoral neck and lumbar spine, but not yet in the cortical bone of the long bones.

Disclosures: **M. Lorentzon**, None.

T21

Free Serum Estradiol Levels Correlate with both Trabecular and Cortical Volumetric Bone Mineral Density in Young Adult Swedish Men. M. Lorentzon, S. McGovern*, E. Svensson*, S. Heigis*, A. Eriksson, M. Svensson*, C. Ohlsson. Center for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden.

Estrogens regulate skeletal growth and mineralization in males. The aim of the present study was to determine the associations between serum levels of

estradiol (E2) and skeletal size and mineralization in young adult males. The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study consists of 1075 men, age 18.9±0.6 yrs, and was initiated with the aim to find both environmental and genetic determinants for bone and fat mass. Bone parameters were measured using both DXA and pQCT. Serum levels of SHBG and E2 were measured using RIA and free E2 (fE2) levels were calculated.
Regression models using physical activity, smoking, age and fE2 as covariates showed that fE2 was an independent predictor of areal BMD in the total body, the total femur, the femoral neck and the trochanter ($p<0.01$) but not in the spine as measured by DXA. pQCT analysis demonstrated that fE2 was an independent predictor of both trabecular (radius $\beta=0.13$, $p<0.001$; tibia $\beta=0.11$, $p<0.001$) and cortical (radius $\beta=0.10$, $p<0.001$; tibia $\beta=0.12$, $p<0.001$) volumetric BMD but not of cortical periosteal circumference or cortical cross sectional area. The subjects with the highest tenth percentile of fE2 (n=107) had 9.5 % ($p<0.01$) higher trabecular volumetric BMD in the radius than the subjects with the lowest tenth percentile of fE2 (n=108). These findings demonstrate that fE2 is a predictor of both the trabecular and the cortical volumetric BMD but not of the size of the cortical bone in young adult Swedish men.

Disclosures: **M. Lorentzon**, None.

T22

Mice Deficient in ÃŸ-AR Signaling Have Increased Bone Mass Despite Increased Leptin Levels. M. L. Bouxsein, V. Glatt*, H. Dhillon*, E. Bachman*. Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, USA.

Mice devoid of leptin (ob/ob) or the signaling form of its receptor (db/db) have increased trabecular bone mass, despite reduced gonadal function (1). Recent evidence suggests that the inhibitory effects of leptin on bone may be mediated by the ÃŸ-adrenergic system (2). We hypothesized that absence of ÃŸ-adrenergic signaling will lead to increased bone mass, despite increased leptin levels. To test this we evaluated male mice that lack the three known ÃŸ-adrenergic receptors (ÃŸ-less mice)(3). We used in vivo bone densitometry (PIXImus) to assess BMD and body composition between 6 and 16 weeks of age, and ex vivo ÂµCT to assess trabecular and cortical bone morphology at 6 and 16 weeks (n=7-9/group). As expected, weight and % fat were increased in ÃŸ-less mice after 8 weeks ($p<0.0001$ for both). Total body BMC was 14-22% higher in ÃŸ-less mice ($p<0.001$), but was similar to WT after correcting for their higher body weight. At 6 weeks ÃŸ-less mice had 1.3 and 3.5-fold higher vertebral and distal femoral trabecular BV/TV ($p<0.001$ for both). These differences were less, but maintained at 16 wks. In comparison, mid-femoral cortical geometry was similar at 6 wks, but at 16 wks ÃŸ-less mice had increased cross-sectional area, bone area and cortical thickness ($p<0.01$ for all). Leptin levels were approximately two-fold higher in ÃŸ-less mice (3). Altogether these data support a primary role for ÃŸ-adrenergic signaling in the regulation of bone mass.
1) Ducy, Cell 2000; 2) Takeda, Cell 2002; 3) Bachman, Science 2002

Disclosures: **M.L. Bouxsein**, None.

T23

Osteogenic Potential of Joint-Loading Modality. H. Yokota[1,2], S. M. Tanaka[1], H. B. Sun[1]. [1]Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Biomedical Engineering, Indiana University - Purdue University Indianapolis, Indianapolis, IN, USA.

The purpose of the current study was to evaluate osteogenic potential of a novel joint-loading modality using mouse ulnae as a model system. Animal studies support that mechanical loading stimulates bone formation, and *in vitro* studies show that bone cells are responsive to shear stress induced by fluid flow. Although a minimum effective strain or strain rate in bone for osteogenesis has been investigated, little is known about possible induction of fluid flow and osteogenic potential by loads applied laterally through a synovial joint. Since mechanical loads to the skeleton are transmitted to bone through joints, we addressed a question about whether lateral deformation of a joint would stimulate formation of trabecular and cortical bone through remote induction of fluid flow. Using mouse ulnae as a model system, we applied 2-Hz sinusoidal loads to an elbow joint with a peak-to-peak amplitude of 0.5 N for 3 min per day for 3 consecutive days. The histomorphometric results showed that this joint-loading modality elevated formation of trabecular and cortical bone 3- to 8-fold compared to control ulnae (no loading). The axial strain with the joint-loading modality was smaller than 30 µstrain in the ulnar cortical bone. The same loads, applied axially to ulnae in the ulna-loading model, induced ~ 250 µstrain, which was shown in the previous studies insufficient to enhance bone formation. Based on these results, we propose that the novel joint-loading modality has osteogenic potential.

Disclosures: **H. Yokota**, None.

T24

Loaded Bone Is the Target of the Anabolic Action of PTH. Y. Mikuni-Takagaki[1], K. Aoki[2], M. Takahashi*[2], K. Ohya[2]. [1]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan, [2]Department of Hard Tissue Engineering/Pharmacology, Tokyo Medical and Dental University, Graduate School, Tokyo, Japan.

While daily injections of parathyroid hormone (PTH) reduce incidence of fractures in ambulant patients (Neer et al. 2001), the effect in disuse osteoporotic patients has never been studied systematically. The purpose of this study was to characterize synergy between PTH and walking in the tibia of a new rat model for disuse osteoporosis (osteopenia) in bed rest/sedentary individuals. Experimental rats (DISUSE) were restricted by housing them in cages of 95 mm x 140 mm x 55-110 mm (W x L x H). The food pellet holder prevents rats from standing. Control animals (WALKING) were housed in institutional standard cages. Rats were injected subcutaneously with 10 µg/kg human PTH (1-34) (Asahi Chemical Co.) or saline three times a week for 6 weeks. Calcein (20mg/kg; Sigma) was injected twice, with a ten-day interval, for dynamic histomorphometry, and the animals were euthanized 3 days later. BMD, measured by pQCT in tibial cortical bone showed that the anabolic effect of PTH was synergistically upregulated by walking; (WALKING+PTH) − (WALKING) was significantly larger ($p<0.05$) than (DISUSE+PTH) − (DISUSE). In non-weight bearing clavicles, however, PTH did not alter BMD significantly, regardless of the conditions. Bone formation rate, BFR, was variable depending on the area of cortical bone suggesting that the local mechanical environment is reflected. Exposure of cells in tibial cortical bone to a walking-level strain seems to be a prerequisite for PTH to function in an anabolic manner.

Table 1. Effects of PTH and walking on parameters of 30-week female Wistar rats

	Disuse	**Walking**	**Disuse + PTH**	**Walking + PTH**
Initial body weight (g)	**196.8±4.7**	**204.4±6.2**	**197.6 ±5.0**	**200.0±8.2**
Body weight at 3 w (g)	**200.0±2.4**	**204.2±4.7**	**200.0±2.4**	**203.3±2.9**
Final body weight at 6 w (g)	**205.0±0.8**	**210.4±4.9**	**205.0±9.1**	**211.5±9.0**
Final liver weight (g)	**7.44±0.16**	**6.71±0.39 [a]**	**7.09±0.38**	**7.14±0.32 [c]**
Final gastrocnemius muscle weight (g)	**1.13±0.04**	**1.30±0.02 [a]**	**1.26±0.02[b]**	**1.31±0.03[c]**
Final soleus muscle weight (g)	**0.642±0.010**	**0.693±0.017[a]**	**0.693±0.053[b]**	**0.707±0.016[c]**
Proximal tibia BMD by pQCT (mg/cm^3)	**862±42**	**890±10**	**901±17[b]**	**948±12[ce]**

Disclosures: **Y. Mikuni-Takagaki**, None.

T25

Exogenously Applied rhTGF-beta2 Enhances Bone Regenration and Implant Fixation by Altering Gene Expression in a Rat Model. A. De Ranieri*[1], A. S. Virdi[1], S. Kuroda[1,2], D. R. Sumner[1]. [1]Anatomy & Cell Biology, Rush University Medical Center, Chicago, IL, USA, [2]Tokyo Dental and Medical University, Tokyo, Japan.

Transforming growth factor beta (TGF-β) enhances implant fixation in animal models. The purpose of this experiment was to determine if local application of rhTGF-β2 altered the temporal pattern of gene expression in a rat model. Two experimental groups of 21 animals each were studied in an IACUC-approved protocol. All rats received unilateral titanium implants coated with hydroxyapatite/tricalcium phosphate (Zimmer) ± 1μg rhTGF-β2 (Genzyme). Three animals per group were killed at d1, d3, d5, d7, d10, d14 or d28. Real-time PCR was used to measure gene expression for 21 genes (normalizing to GAPDH). Loading of implants with rhTGF-β2 accelerated expression of three growth factor receptors (TβRI, TβRII, and IGF-1R), three growth factors (IGF-1, VEGF, and TGF-β1) and osteocalcin. BMP-2, BMP-7, TGF-β2, and Cbfa1 at early time points, Flt-1 and Cox-2 at most time points, and osteonectin, Col I and osteopontin at later time points had elevated, but not accelerated expression. TNF-α, Noggin, TGF-β3 and alkaline phosphatase gene expression was delayed. These in vivo data are consistent with in vitro studies showing that TGF-β is a known mitogen with pleiotropic actions and the study demonstrates that TGF-β's action in enhancing bone regeneration is brought about by modulating the levels as well as time of expression of relevant genes.

Disclosures: **D.R. Sumner**, Depuy 2.

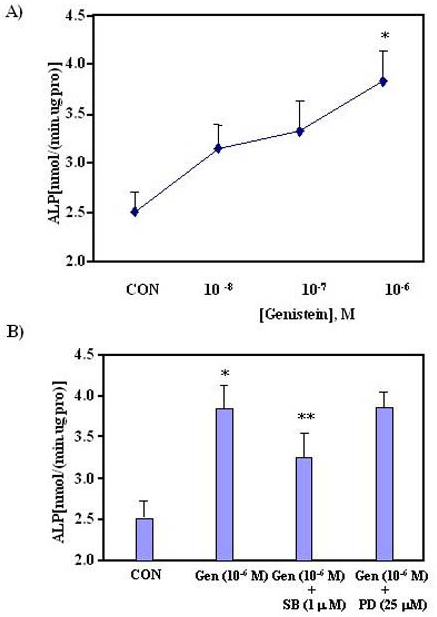


Fig 1. Inhibition of p38 MAPK blocked the genistein-increased ALP activity in BMSCs cultures.

T26

The Phytoestrogen Genistein Enhances Osteoblastic Differentiation of Mouse Bone Marrow-derived Mesenchymal Stem Cells Through p38 MAPK Pathway. Q. C. Liao*[1], T. Liu*[1], L. D. Quarles[2], Y. F. Qin*[1], W. Pan*[1], H. H. Zhou*[1], Z. S. Xiao[1,2]. [1]Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, China, [2]Medicine, Duke University Medical Center, Durham, NC, USA.

Genistein, an isoflavone structurally resembling 17ß-estradiol, has been shown to stimulate osteoblast-mediated bone formation. In the present study, we investigate the role of mitogen-activated protein kinases (MAPKs) in genistein-induced osteoblastic differentiation using mouse bone marrow-derived mesenchymal stem cells (BMSCs). BMSCs were cultured in a-minimal essential medium supplemented with ascorbic acid (25 mg/ml) and ß-glycerolphosphate (5 mM) treated with genistein in the absence or presence of SB203580 (1 μM), a p38 MAPK-specific inhibitor, or PD98059 (25 μM), a p42/44 MAPK-specific inhibitor. Genistein (10^{-8}~10^{-6} M) exhibited a dose-dependent effect on osteoblastic differentiation as evidenced by increasing alkaline phosphatase (ALP) activity (Fig. 1) and mineralization (Fig. 2 and 3) in mouse BMSCs cultures. This genistein-dependent effect was blocked by SB203580, but not PD98059. Genistein (10^{-6} M) treatment resulted in rapid and sustained activation of p38 MAPK in the BMSCs cultures, which was also blocked by the p38 MAPK inhibitor (Fig. 4). In contrast, genistein treatment resulted in inactivation of p42/44 MAPK, an effect that was further attenuated by adding the p42/44 MAPK inhibitor (Fig. 5). These results indicated that the p38 MAPK pathway plays an important role in genistein-induced osteoblastic differentiation of mouse BMSCs cultures.

Disclosures: **Q.C. Liao**, None.

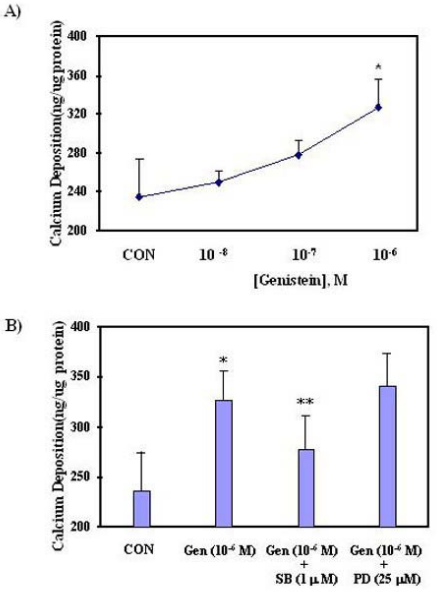


Fig 2. Inhibition of p38 MAPK abolished the genistein-enhanced calcium deposition in BMSCs cultures.

T27

PTH-stimulated Cortical Bone Remodeling Is Differentially Regulated by Estrogens and Arrestins. D. Pierroz[1], S. L. Ferrari[1], V. Glatt*[2], R. Rizzoli[1], M. L. Bouxsein[2]. [1]Bone Diseases, Geneva University Hospital, Geneva, Switzerland, [2]Orthopedic Biomechanics Lab, Beth Israel Deaconess Medical Center, Boston, MA, USA.

Intermittent PTH increases cancellous bone mass, but its effects on cortical bone remain poorly understood. PTH-stimulated cAMP signaling is regulated by cytoplasmic ß-arrestin2 and ß-arrestin2 KO mice have decreased cortical cross-sectional area compared to wild type (WT). Estrogen too regulate cortical bone remodeling, therefore we examined their interaction with arrestins in regulating PTH activity on bone.

Mid-femoral geometry following intermittent rhPTH-(1-34) (20, 40 and 80 mg/kg/d) or vehicle (VEH) for 4 wks were evaluated in intact and ovariectomized (OVX) WT and ß-arrestin2 KO female mice (N=8-11/group) using ex vivo mCT. In intact WT, PTH marginally increased cortical thickness and decreased marrow area (-5%, $p<0.05$ vs VEH). In contrast, in KO, PTH increased thickness, total, bone and marrow areas (up to +20%, $p<0.005$ vs VEH). OVX decreased cortical thickness (-8%, $p<0.005$ vs Sham) and marginally increased marrow area in both WT and KO mice. In OVX-WT, PTH significantly increased cortical thickness and bone area (+8,4%, $p<0.05$ vs VEH), decreased marrow area ($p=0.036$ vs VEH), but did not alter total area. In OVX-KO mice, cortical thickness was also significantly increased by PTH. However, contrarily to intact KO mice, OVX-KO mice responded to PTH with a modest decrease in marrow area and a non-significant increase in total area.

These data indicate that estrogens and arrestins differentially regulate cortical bone remodeling. Thus, the normal expansion of cortical bone is inhibited by estrogens but favored by arrestins. In addition, estrogen inhibits endosteal apposition while arrestins prevent periosteal apposition in response to intermittent PTH.

Disclosures: **D. Pierroz**, None.

T28

Use of a Simple Computerized Technique to Assess the Anabolic Effects of IGF-I in Mouse Bone Marrow Stromal Cells. T. L. Chen. Medicine/Endocrinology, V.A. Palo Alto Health Care System, Palo Alto, CA, USA.

We have applied a simple computerized Colcount program for the analysis of colony formed in primary cultures of bone marrow stromal cells (BMSC). BMSC were harvested from long bones of young (4-5 months) and old (22-25 months) C57BL/6 male mice and treated with varying concentrations of IGF-I to study how donor age affects growth and differentiation of osteoblasts and their sensitivity to IGF-I. We assessed changes in the number and area of alkaline phosphatase positive colonies (CFU-ALP) and in the total number of colonies (CFU-F).
In the Colcount program, colonies are counted by their gray level contours above background. The number of colonies counted was adjusted by setting the visibility parameter to visual acuity of a trained human counter. Overlapping colonies were discriminated by analyzing their density contours. The file obtained in Colcount was imported into Microsoft Excel for data processing.
We found that the number of osteoprogenitor cells in the BMS cell from old mice was much less than the young ones. IGF-I increased both the number and total area of the CFU-ALP and CFU-F dose-dependently. The effects on area were more pronounced than in the number of colonies formed. There was no significant difference between the responses of young and old. Further experiments are needed to find out if subtle differences exist. IGF-I stimulated ALP activity in young cells but not old cells. However, the stimulatory effect is cell density dependent as the young cells lost their response with an increase in cell density.

Disclosures: **T.L. Chen**, None.

T29

An Acceleration-based Anabolic Countermeasure to Bone Loss. R. Garman, C. Rubin, S. Judex. Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA.

Bone's ability to accommodate changes in its mechanical environment can be exploited for developing anabolic mechanical countermeasures. Recently, we have shown that low-magnitude (0.3*g*) high frequency (115Hz) mechanical vibrations can increase bone's anabolic activity. The physical mechanism by which loading induces tissue deformations smaller than those typically associated with exercise is unknown. Here, we developed a novel mechanical signal to test the hypothesis that vibratory signals can be anabolic in the absence of *any* tissue deformation.
The left hindlimb of three female F2 mice (C3H/HeJxBALB/cByJ) was subjected to a novel loading regime applying vibrations (0.3*g*, 115Hz) without inducing mechanical strain for 10min/d. The right hindlimb served as contralateral control. Strain gages on the tibial bone surface revealed that the loading device produced signals indistinguishable from those in unloaded bone.
Application of this loading regime, producing *only* accelerations and not deformations, for 3wk increased anabolic activity in the proximal tibia. The trabecular mineralizing surface (MS/BS) was increased by 20% while endocortical bone formation rate were two-fold greater in stimulated tibiae when compared to contralateral controls.
In contrast to exercise or external loading regimes relying on large forces and bone deformations to initiate an anabolic response, these data demonstrate that mechanical signals accelerating the bone without tissue deformation can be anabolic. Thus, the anabolic effects of low-level mechanical vibrations observed previously may be independent of bone matrix deformations, instead relying on physical events at the bone surface. Further development of this unique mechanical signal may provide a non-pharmacological and safe biomechanical countermeasure.

Disclosures: **R. Garman**, None.

T30

Connective Tissue Growth Factor (CTGF) Promotes Skeletogenesis. J. J. Song*, R. A. Kanaan*, F. F. Safadi, S. N. Popoff. Anatomy & Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA.

Cellular condensation is a stage of skeletogenesis involving the congregation of mesenchymal stem cells and is considered to be the critical step in this process. CTGF is a matricellular protein that has been shown to be highly expressed in cellular condensations by *in situ* hybridization studies during embryonic development. Interestingly, CTGF-deficient mice demonstrated a misshapen skeleton attributed to endoskeletal abnormalities and abnormal cartilage development. CTGF has been found to be highly up-regulated with TGF-β stimulation due to the presence of a novel TGF-β response element in its promotor. In addition, CTGF has also been found to enhance receptor binding of TGF-β. We propose that CTGF may promote condensation through the TGF-β pathway, a known regulator of cellular condensation. In this study, we established high-density micromass cultures using the C3H10T1/2 murine mesenchymal stem cell-line. These cells have been shown to form prechondrocytic nodules as a result of condensation following TGF-β treatment. C3H10T1/2 cells were micromass cultured in HamF12 containing 10% fetal bovine serum. With TGF-β treatment, nodules formed within 72 hours. Next, we down-regulated CTGF expression using an antisense oligonucleotide and confirmed its expression by RT-PCR and Western blot analyses. CTGF attenuation was sufficient to prevent nodule formation upon TGF-β treatment. In conclusion, CTGF may be an important regulator of cellular condensation *in vitro* and play an important role in proper skeletal development. The precise nature of how CTGF promotes cellular condensation will be elucidated with further experimentation.

Disclosures: **J.J. Song**, None.

T31

Androstene Immune Regulating Hormones: A New Class of Potent Anabolic and Catabolic Regulators of Bone Resorption. N. H. Urban*[1], M. Holmes*[1], R. M. Loria*[2], M. J. Beckman[3]. [1]Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA, USA, [2]Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA, USA, [3]Biochemistry, Virginia Commonwealth University, Richmond, VA, USA.

Androstenediol (5-androsten-3 beta-17 beta-diol, AED) and androstenetriol (5-androstene-3 beta-7 beta-17 beta-triol beta AET) restore myelopoiesis within two weeks of treatment after 90% bone marrow ablation. Understanding the effect of AED and AET on the signaling pathways involved in bone resorption could lead to novel therapies for bone diseases. We utilized Real-Time RT-PCR to examine the effect of AED and AET on regulation of RANKL and OPG gene expression in fetal osteoblast (FOB-9) cells. Treatment of FOB-9 cells with PTH (200 ng/ml) repressed OPG and stimulated RANKL gene expression in a time-dependent manner. Treatment with the PPAR-γ agonist, WY14, decreased OPG gene expression slightly, but treatment with the antagonist, GW9662, stimulated OPG gene expression 9-fold. Conversely, WY14 increased RANKL and GW9662 had no effect on RANKL gene expression. PPAR-γ is also influenced by AED and AET in many cell systems. Therefore, FOB-9 cells were incubated with DHEA, AED, or AET at either concentrations of 10^{-7} to 10^{-8} M. DHEA and AED significantly decreased OPG expression at 10^{-8} M, but the decrease effect was relieved at 10^{-7} M. Interestingly, AET showed a potential to stimulate OPG expression at both 10^{-8} and 10^{-7} M. RANKL expression decreased in response to DHEA 10^{-8} M and AED 10^{-8} and 10^{-7} M, whereas DHEA 10^{-7} M increased RANKL expression 2-fold, and both AET concentrations 10^{-8} and 10^{-7} M potently increased RANKL expression by 7.5-fold. In conclusion, AED and AET exhibit exquisite structure-specific regulation of divergent bone remodeling pathways with relation to RANKL and OPG gene expression.

Disclosures: **N.H. Urban**, None.

T32

An Adynamic Osteodystrophy and Vascular Calcification Associated with the Metabolic Syndrome Is Worsened by CKD and Successfully Treated with Exogenous BMP-7. K. A. Hruska, R. J. Lund, M. R. Davies*, S. Mathew*. Pediatrics, Washington University, St. Louis, MO, USA.

An osteodystrophy has not been defined in an animal model of the metabolic syndrome with hypercholesterolemia, hyperglycemia, vascular calcification and chronic kidney disease (CKD). We hypothesized the vascular calcification seen in these animals may be associated with alterations in bone remodeling, and changes in Pi. 10 wk old low density lipoprotein receptor deficient (LDLR-/-) mice were randomized into groups: Sham/Chow, Sham/Fat (15%), Sham/F/ BMP-7 (10mcg/kg q week), CKD/Fat, CKD/ F/BMP-7. The high fat fed mice developed aortic calcification. After 12 weeks BUN levels were equally high in the CKD groups; iPTH levels were high only in the CKD/Fat animals. The underlying osteodystrophy in both of the LDLR-/- high fat groups was consistent with an adynamic bone disorder (decreased OV/TV, ObN, MS/BS, and BFR/TV). BMP-7 normalized the osteodystrophy, by improving ObN, MS/BS, and BFR. Pi levels were reduced from 16.4±0.4mg/dl to 10.1±0.4mg/dl with BMP-7 treatment ($p<0.01$) (Sham Pi 9.9±0.6). This study demonstrates altered bone remodeling and relatively high iPTH levels in LDLR-/- animals with CKD fed a high fat diet consistent with an adynamic bone disorder and PTH resistance. The ABD was reversed with BMP-7 treatment, without change in iPTH. The hyperphosphatemia observed in the LDLR-/- fat mice may have been caused by the ABD and diminished exchangeable Pi and may have contributed to the calcification observed. Improving the mineralizing and bone formation parameters with BMP-7, normalized Pi and decreased vascular calcification. Thus, the ABD is associated with vascular calcification and a skeletal anabolic treated both the ABD and vascular calcification.

Disclosures: **K.A. Hruska**, Johnson & Johnson Pharmaceutical Research and Development, L.L.C. 2.

T33

Dutasteride, a Potent 5 alpha Reductase Inhibitor, Does Not Effect Bone Density and Bone Metabolism in Healthy Men. R. V. Clark[1], A. M. Matsumoto*[2]. [1]Clinical Pharmacology, GlaxoSmithKline R & D, Research Triangle Park, NC, USA, [2]Internal Medicine, Univ of Washington School of Medicine, Seattle, WA, USA.

Dutasteride is a potent, dual 5 ARI which is an effective treatment for benign prostatic hyperplasia. The objective of this study was to determine whether the marked suppression of dihydrotestosterone (DHT) observed with dutasteride has an effect on bone metabolism and bone density (BMD).

In this randomized, double-blind, placebo-controlled study, 99 healthy men, aged 18-25, received 0.5mg dutasteride, 5.0mg finasteride, or placebo for 52 weeks, and were followed for an additional 24 weeks. BMD was determined by DEXA at screening, end of treatement (48-52 wk) and 20-24 wk later at follow-up (F/U). Markers of bone metabolism, osteocalcin, bone alkaline phosphatase, and urinary n-telopeptide, were measured at baseline, wk 8, 16, 24, and 52 of treatment, and wk 8,12, and 24 of F/U.

The mean reduction in DHT in the dutasteride group was > 90% at each treatment phase visit compared with 70% for the finasteride group ($p<0.001$). There were no clinically, nor statistically, significant changes in BMD from baseline, or between groups at end of treatment or end of follow-up. There were no consistent changes or trends in the bone markers in any treatment group during the treatment period. At F/U wk 24, mean urinary n-telopeptide levels were greater in the finasteride group compared to the placebo and dutasteride groups (p values 0.017 and 0.003 respectively).

In conclusion, the marked suppression of DHT observed with dutasteride, compared with other 5 ARIs, had no clinically nor statistically significant effect on BMD or bone metabolism.

Disclosures: **R.V. Clark**, GlaxoSmithKline R & D 3.

T34

Targeted Overexpression of Androgen Receptor in Osteoblasts Results in Complex Skeletal Phenotype. K. Wiren[1], M. Gentile*[2], S. Harada[2], K. Jepsen[3]. [1]VA Medical Center, Oregon Health & Science Univ, Portland, OR, USA, [2]Merck Research Laboratories, West Point, PA, USA, [3]Mt. Sinai School of Medicine, NY, NY, USA.

We have genetically engineered transgenic mice in which androgen receptor (AR) expression is skeletally targeted, using the rat 3.6-kb α1(I)-collagen promoter fragment, to better understand the role of androgen signaling in the bone microenvironment. Bone quality was assessed at 2 months of age by micro-computed tomography, static and dynamic histomorphometry, biomechanical and gene expression analyses. Analysis of trabecular bone architecture of femur documented significantly increased bone volume and trabecular number in AR-tg mice. Dynamic histomorphometric analysis demonstrated reduced bone turnover on trabecular bone as well as on endosteal surfaces, indicating that increased bone mass results from suppression of bone resorption. In contrast, a small increase in labeling was observed at the periosteal surface which is in agreement with thickening of the calvaria, supporting the stimulatory effects of androgens on cortical bone growth. Analysis of gene expression in tibia confirmed the decreased levels of markers of osteoclasts, cathepsin K and TRAP as well as receptor activator of NF-kB ligand (RANKL). Levels of osteoprotegerin (OPG) are increased in bone tissue as well as in serum, suggesting the role of RANKL/OPG signaling in suppression of osteoclast activity. Overexpression of AR throughout the osteoblast lineage thus resulted in increased trabecular bone mass and reduced bone turnover resulting from suppressed osteoclast activity, and in anabolic thickening of calvaria (intramembranous) and enhanced periosteal apposition in cortical bone. These findings offer valuable insight into the role of androgen in bone metabolism, and provide proof of principle for direct androgen actions mediated by osteoblastic expression of AR.

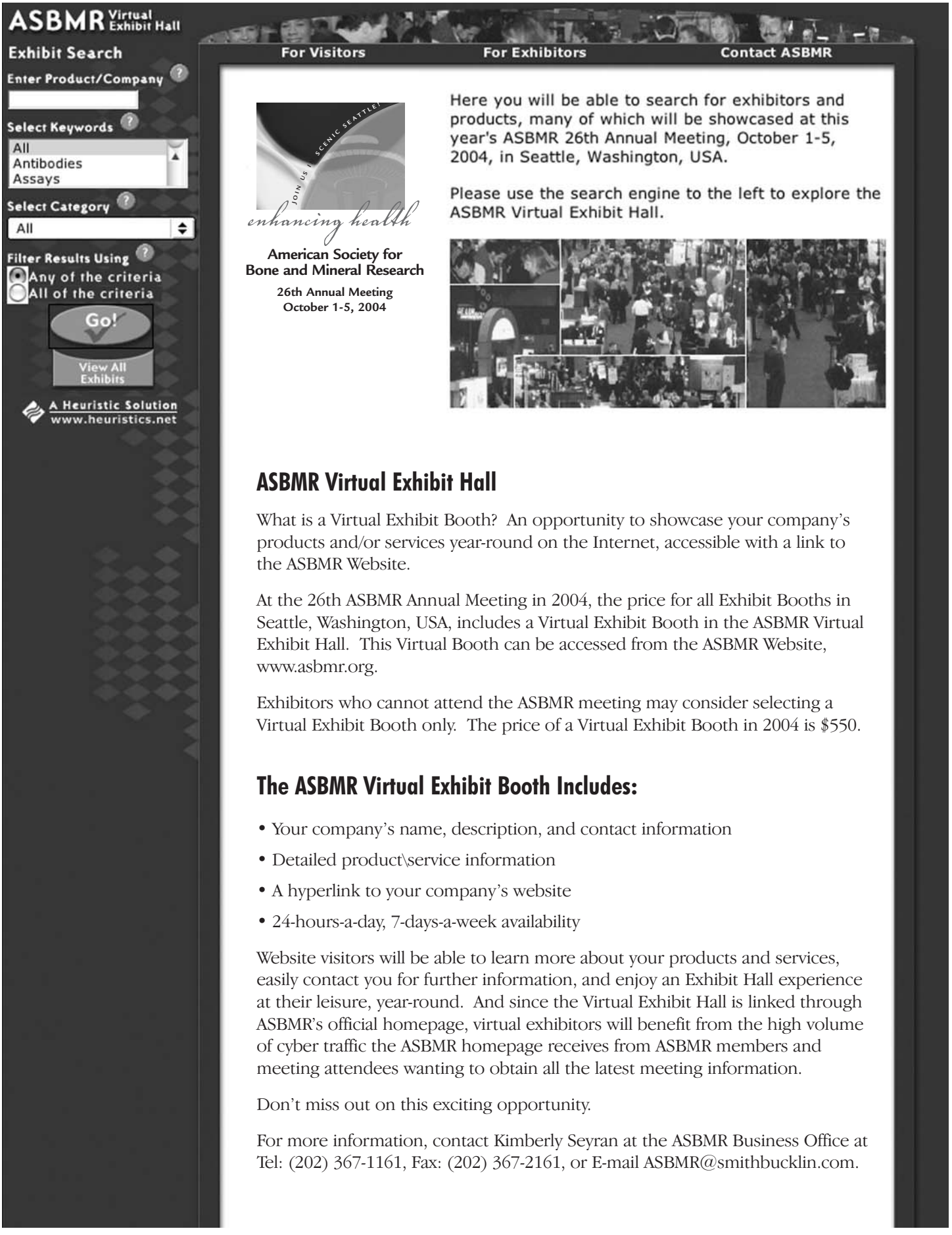
Disclosures: **K. Wiren**, None.

T35

Inorganic Phosphate Causes Rapid Changes in Gene Expression Through an ERK1/2 Dependent Pathway in MC3T3-E1 Osteoblasts. G. R. Beck, K. A. Simpson*. Center for Cancer Research, National Cancer Institute, Frederick, MD, USA.

The generation of inorganic phosphate during osteoblast differentiation and mineralization may represent an important signaling molecule in the process of bone development. We have previously identified the requirement of the mitogen activated signaling kinase ERK1/2 in the induction of osteopontin gene expression in inorganic phosphate stimulated MC3T3-E1 osteoblasts. Additionally, we have determined that elevated inorganic phosphate causes a biphasic phosphorylation of ERK1/2, with an initial activation at 15 to 45 minutes followed by a more gradual activation starting at 8 to 12 hours (J. Biol. Chem. 278: 41921-9). To determine the effect of the early inorganic phosphate induced ERK/12 phosphorylation on gene expression, MC3T3-E1 cells phosphate treated with 10 mM inorganic phosphate for various time points over a period of 4 hours and microarrays analysis performed. Within 30 to 60 minutes of exposure to 10 mM inorganic phosphate the expression of numerous genes were increased by more than 2 fold. The products of these genes range in function from transcriptional activators to effectors of signaling pathways and include, among others the AP-1 transcription factor members c-fos, JunB and c-jun and the zinc finger protein Egr1. The increased expression of the majority of these genes does not require protein synthesis and is ERK1/2 dependent. These studies demonstrate the rapid cellular response to elevated inorganic phosphate in osteoblasts and emphasize the potential significance of this signaling molecule in bone development.

Disclosures: **G.R. Beck**, None.



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