Program and Abstracts

Targeting Bone Remodeling for the Treatment of Osteoporosis

ASBMR™
The American Society for Bone and Mineral Research

December 6-7, 2007
Omni Shoreham Hotel, Washington, DC, USA

A Meeting Sponsored by
American Society for Bone and Mineral Research

Co-Sponsored by
American Academy of Orthopaedic Surgeons
American Society for Nutrition
Foundation for Osteoporosis Research and Education
International Society for Clinical Densitometry
National Osteoporosis Foundation
Orthopaedic Research Society
Osteogenesis Imperfecta Foundation
The Endocrine Society
The Paget Foundation
U.S. Bone and Joint Decade

CME Sponsored Event
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Targeting Bone Remodeling for the Treatment of Osteoporosis

December 6–7, 2007
Omni Shoreham Hotel
Washington, DC, USA

SPONSORED BY:

American Society for Bone and Mineral Research (ASBMR)

CO-SPONSORED BY:

American Academy of Orthopaedic Surgeons (AAOS)

American Society for Nutrition (ASN)

Foundation for Osteoporosis Research and Education (FORE)

International Society for Clinical Densitometry (ISCD)

National Osteoporosis Foundation (NOF)

Orthopaedic Research Society (ORS)

Osteogenesis Imperfecta Foundation (OIF)

The Endocrine Society (ENDO)

The Paget Foundation

U.S. Bone and Joint Decade (USBJD)

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Washington, DC 20036-3309, USA
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E-mail: asbmr@asbmr.org
Website: www.asbmr.org

Welcome!

On behalf of the American Society for Bone and Mineral Research, we welcome you and thank you for your participation.

Over the last several years, we have gained new insights into how bone models and remodels, and this information has been applied to the treatment of osteoporosis and related skeletal disorders. Such knowledge has extended itself into areas that, just a few years ago, would be incomprehensible.

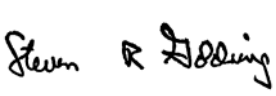
We believe that it is critical to bring together both clinical and basic investigators working in this field to encourage open discussion regarding the conflicts and goals of the osteoporosis field, to develop scientific approaches to resolve these conflicts, and to facilitate development of a reliable knowledge base. We believe that these discussions will facilitate translational research and transfer of information to the clinical arena.

This symposium will focus on evolving approaches and innovations that target bone remodeling. Our goal is to provide an opportunity for the participants in the field—researchers, clinicians, health policy experts, regulators, marketing personnel and others—to interact, to think collegially, to hypothesize, to argue constructively, and to brainstorm and prioritize the key questions researchers must address in moving forward to improve the diagnosis and treatment of osteoporosis.

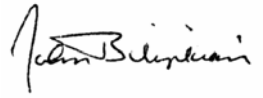
The organizers wish to thank the following U.S. National Institutes of Health Institutes for providing funding for this meeting through an R13 grant: the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute on Aging (NIA).

We are grateful for the co-sponsorship of the American Academy of Orthopaedic Surgeons (AAOS), American Society for Nutrition (ASN), Foundation for Osteoporosis Research and Education (FORE), International Society for Clinical Densitometry (ISCD), National Osteoporosis Foundation (NOF), Orthopaedic Research Society (ORS), Osteogenesis Imperfecta Foundation (OIF), The Endocrine Society (ENDO), The Paget Foundation and the U.S. Bone and Joint Decade (USBJD). We extend a special thanks to our many colleagues for ideas, recommendations, and guidance along the way. We also want to thank the companies that have helped to support this meeting. Finally, we wish to thank the ASBMR staff who provided continuous organizational support.

Sincerely,



Steven R. Goldring, M.D.
ASBMR Immediate Past President



John Bilezikian, M.D.
Organizing Committee Chair

Toshio Matsumoto, M.D.
Organizing Committee Co-Chair

*Funding for this conference was made possible in part by **AR055036** from the National Institutes of Health. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.*

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
National Institute on Aging (NIA)

American Society for Bone and Mineral Research
Targeting Bone Remodeling for the Treatment of Osteoporosis

Organizing Committee

John P. Bilezikian, M.D., *Chair*
Columbia University College of Physicians & Surgeons, New York, New York, USA

Toshio Matsumoto, M.D., *Co-Chair*
University of Tokushima Graduate School of Medical Sciences, Tokushima, Japan

Steven R. Goldring, M.D., *ASBMR Immediate Past President*
Hospital for Special Surgery, New York, New York, USA

Teresita M. Bellido, Ph.D.
University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

Sundeep Khosla, M.D.
Mayo Clinic College of Medicine, Rochester, Minnesota, USA

Jack Martin, M.D., D.Sc.
St. Vincent's Institute of Medical Research, Melbourne, Victoria, Australia

Joan A. McGowan, Ph.D.
National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, USA

Socrates Papapoulos, M.D.
Leiden University Medical Center, Leiden, The Netherlands

Robert R. Recker, M.D.
Creighton University Osteoporosis Research Center, Omaha, Nebraska, USA

ASBMR Young Investigator Award Recipients

Supported in part by educational grants from Alliance for Better Bone Health, Immunodiagnostic Systems and Kyphon Inc.

Tao Fan
Guri Grimnes
Kim Hendriksen
Nikolas H. Kazmers
Alia Khaled
Taeyong Lee
Brett A. Lenart
Xin-Ming Liu
Donald Lucas
Erin S. Mackinnon
Hideto Maruno
B. Kimani Mwaura
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General Information

VENUE

This meeting will take place in the Regency Ballroom of the Omni Shoreham Hotel located at:
2500 Calvert Street, NW, Washington, DC, USA.

REGISTRATION

All registration services will take place at the West Registration Desk of the Omni Shoreham Hotel

Registration Hours

Wednesday, December 5, 2007	4:00 p.m. – 7:00 p.m.
Thursday, December 6, 2007	7:00 a.m. – 2:00 p.m.
Friday, December 7, 2007	7:00 a.m. – 2:00 p.m.

SPEAKER READY ROOM

All speakers must check into the Speaker Ready Room, preferably 24 hours before presentation. At that time, you are encouraged to review your slides to ensure all Greek characters and graphs transferred successfully. The Speaker Ready Room is located in the Committee Room of the Omni Shoreham Hotel

Speaker Ready Room Hours

Wednesday, December 5, 2007	4:30 p.m. – 7:30 p.m.
Thursday, December 6, 2007	7:00 a.m. – 8:00 p.m.
Friday, December 7, 2007	7:00 a.m. – 3:00 p.m.

POSTER INFORMATION

Posters will be displayed in the Ambassador Ballroom of the Omni Shoreham Hotel. Poster presentation time is scheduled during the Welcome Reception and Poster Session on Thursday, December 6; from 6:45 p.m. to 7:30 p.m. Presenters must be at their posters during this time and available to answer questions.

	Thursday, December 6, 2007	**Friday, December 7, 2007**
Poster Set-Up	7:00 a.m. – 8:00 a.m.	
Welcome Reception/Poster Session	5:40 p.m. – 7:30 p.m.	
Presentation Time	6:45 p.m. – 7:30 p.m.	
Poster Dismantle		3:20 p.m. – 3:50 p.m.
Poster Viewing Schedule		
Morning Break	9:50 a.m. – 10:10 a.m.	10:20 a.m. – 10:40 a.m.
Lunch Break	11:50 a.m. – 1:00 p.m.	12:40 a.m. – 2:00 p.m.
Afternoon Break	3:00 p.m. – 3:20 p.m.	
Evening Hours	5:40 p.m. – 7:30 p.m.	

MEETING MEALS

Your registration for the meeting includes a continental breakfast and lunch on Thursday, December 6th and Friday, December 7th. Lunch will be served in the Palladian Room at the Omni Shoreham Hotel.

EXPECTATION OF PRESENTERS

Through ASBMR meetings, the Society promotes excellence in bone and mineral research. Toward that end, ASBMR expects that all authors and presenters affiliated with the ASBMR Meeting on Targeting Bone Remodeling for the Treatment of Osteoporosis will provide informative and fully accurate content that reflects the highest level of scientific rigor and integrity.

Furthermore, the ASBMR expects that authors and presenters will disclose any conflicts of interest, real or perceived; authors and presenters describing a study funded by an organization with a proprietary or financial interest must affirm that they had full access to all the data in the study. By so doing, they accept complete responsibility for the integrity of the data and the accuracy of the data analysis; the content of abstracts, presentations, slides, and reference materials must remain the ultimate responsibility of the authors and presenters; the planning, content, and execution of abstracts, speaker presentations, slides, abstracts, and reference materials should be free from corporate influence, bias, or control; and all authors and presenters (invited and abstracts-based oral and poster presenters) should give a balanced view of therapeutic options by providing several treatment options, whenever possible, and by citing the best available evidence.

CONTINUING MEDICAL EDUCATION (CME) CREDITS

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for CME through the joint sponsorship of the Federation of American Societies for Experimental Biology (FASEB) and the ASBMR. FASEB is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

Category I Continuing Medical Education (CME) credits toward the American Medical Association's (AMA) Physician Recognition Award will be offered at this meeting. FASEB designates this educational activity for a maximum of 13.25 Category I credits. Each physician should claim only those credits that he or she actually spent in the activity. A CME application form may be found on the last page of the *Program and Abstracts* book. CME forms should be sent to:

FASEB Office of Scientific Meetings and Conferences
9650 Rockville Pike
Bethesda, Maryland 20814, USA
Tel: (301) 634-7013 ◆ Fax: (301) 634-7007

MEETING OBJECTIVE

The ASBMR meeting on Targeting Bone Remodeling for the Treatment of Osteoporosis is designed to allow members and attendees to present new developments in education, research and clinical practice related to bone and mineral metabolism. The program objectives include identifying and discussing traditional and novel molecules and pathways that can be targeted to enhance bone formation, providing insights into traditional and novel approaches for inhibiting bone resorption, analyzing the mechanism of action and role of bisphosphonates and other agents that are available or are being developed to treat osteoporosis, defining the role of combination therapy (antiresorptive/anabolic) in the treatment of osteoporosis, and translating new basic concepts into new therapeutic paradigms.

As a result of their attendance, participants should have enhanced their knowledge of osteoporosis, the cellular and molecular mechanisms regulating bone remodeling, basic bone biology and its correlation to mineral metabolism, and their ability to treat and care for their patients. Attendees should have developed a clearer relationship among basic research, clinical research, and patient care though the discussions that are expected to take place. The program for the ASBMR Meeting on Targeting Bone Remodeling for the Treatment of Osteoporosis should produce and enhance appreciation of the investigative, diagnostic, and therapeutic approaches to osteoporosis, which is a major cause of disability in our aging population.

TARGET AUDIENCE

The program is designed for researchers, physicians, clinicians, and other allied health professionals with interests in endocrinology, physiology, cell biology, pathology, molecular biology, genetics, epidemiology, internal medicine, rheumatology, orthopedics, dentistry, and pharmacology.

DISCLOSURE/CONFLICT OF INTEREST

ASBMR is committed to ensuring balance, independence, objectivity and scientific rigor in all education activities. ASBMR requires that presenters inform the audience of the presenters' (speakers', faculties', authors', and contributors') academic and professional affiliations and disclose the existence of any financial interest or other relationships a presenter has with the manufacturer(s) discussed in an educational presentation.

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

Disclosure should include any relationship that may bias a presentation or that, if known, could give the perception of bias. These situations may include, but are not limited to the following:
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
6) Non-remunerative positions of influence such as officer, board member, trustee, spokesperson
7) Receipt of royalties
8) Speakers bureau

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DISCLAIMER

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AUDIO- AND VIDEOTAPING

ASBMR expects that attendees will respect a 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. The use of cameras, audio-taping devices, and videotaping equipment is strictly prohibited within all Oral Scientific Sessions and the Poster Sessions without the express written permission of the ASBMR. Unauthorized use of taping equipment may result in the confiscation of the equipment or the individual may be asked to leave the session. These rules will be strictly enforced.

ASBMR MEMBERSHIP

The ASBMR Membership Booth will be located in the Regency Ballroom Foyer of the Omni Shoreham Hotel. Stop by and meet the ASBMR staff and pick up information about the Society, the high-ranking *Journal of Bone and Mineral Research* (*JBMR*) and the upcoming 30th Annual Meeting in Montréal, Québec, Canada, September 12–16, 2008.

MEETING EVALUATION

An online evaluation form for the ASBMR Meeting on Targeting Bone Remodeling for the Treatment of Osteoporosis will be available on the ASBMR Website at www.asbmr.org after the meeting. Your participation in this evaluation is extremely important to us. Please take a moment to complete the evaluation of this meeting to aid in planning future meetings. Thank you in advance for your feedback.

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, logo and meeting logo may not be used without permission. Use of the ASBMR logo is prohibited without the express written permission of the ASBMR Executive Director. All corporate supporters should share their media outreach plans with the ASBMR Executive Director before any release.

No abstract presented at the ASBMR Meeting on Targeting Bone Remodeling 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 MEETING DATES

ASBMR 30th Annual Meeting
September 12–16, 2008
Palais des congrès de Montréal
Montréal, Québec, Canada

ASBMR 31st Annual Meeting
September 11–15, 2009
Colorado Convention Center
Denver, Colorado, USA

American Society for Bone and Mineral Research
Targeting Bone Remodeling for the Treatment of Osteoporosis

Schedule-at-a-Glance

Thursday, December 6, 2007

Time	Session	Location
7:00 a.m. – 8:00 a.m.	Breakfast	Regency Ballroom
8:00 a.m. – 8:10 a.m.	Introductions	Regency Ballroom
8:10 a.m. – 11:50 a.m.	Session 1: Overview of Bone Remodeling and Bone Modeling	
9:50 a.m. – 10:10 a.m.	Break/Exhibits Open	Regency Ballroom
11:50 a.m. – 1:00 p.m.	Lunch and Poster Viewing	Ambassador Ballroom
1:00 p.m. – 5:40 p.m.	Session 2: Bone Resorption	Regency Ballroom
3:00 p.m. – 3:20 p.m.	Break/Exhibits Open	Regency Ballroom
5:40 p.m. – 7:30 p.m.	Welcome Reception and Poster Session	Ambassador Ballroom

Friday, December 7, 2007

Time	Session	Location
7:00 a.m. – 8:00 a.m.	Breakfast/Exhibits Open	Regency Ballroom
8:00 a.m. – 12:40 p.m.	Session 3: Bone Formation	Regency Ballroom
10:20 a.m. – 10:40 a.m.	Break/Exhibits Open	Regency Ballroom
12:40 p.m. – 2:00 p.m.	Lunch and Poster Viewing	Ambassador Ballroom
2:00 p.m. – 3:00 p.m.	Session 4: Targets Affecting Both Resorption and Formation of Bone	Regency Ballroom
3:00 p.m. – 3:20 p.m.	Session 5: Wrap Up	Regency Ballroom
3:20 p.m.	Meeting Adjourns	

American Society for Bone and Mineral Research
Targeting Bone Remodeling for the Treatment of Osteoporosis

Thursday, December 6, 2007

BREAKFAST
7:00 a.m. – 8:00 a.m.

INTRODUCTION
8:00 a.m. – 8:10 a.m.

8:00 a.m. **Opening Comments on Behalf of ASBMR**
Steven R. Goldring, M.D., Immediate Past President, American Society for Bone and Mineral Research and Hospital for Special Surgery, New York, New York, USA

Opening Comments on Behalf of NIH and NIAMS
Stephen I. Katz, M.D., Ph.D., Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland, USA

Opening Comments on Behalf of the ASBMR Organizing Committee
John P. Bilezikian, M.D., Organizing Committee Chair, Columbia University College of Physicians and Surgeons, New York, New York, USA

Toshio Matsumoto, M.D., Organizing Committee Co-Chair, University of Tokushima Graduate School of Medical Sciences, Tokushima, Japan

SESSION 1
8:10 a.m. – 11:50 a.m.
Overview of Bone Remodeling and Bone Modeling

Moderators:
Lee S. Weinstein, M.D., National Institute of Diabetes and Digestive and Kidney Diseases-NIH, Bethesda, Maryland, USA
Uri A. Liberman, M.D., Ph.D., Tel Aviv University, Tel Aviv, Israel

		Presentation Number
8:10 a.m.	**Basic Concepts: Modeling, Remodeling, and the Decline of Structural Strength** David W. Dempster, Ph.D., Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, USA	**1**
8:30 a.m.	**Clinical Concepts of Modeling and Remodeling** Robert P. Heaney, M.D., Creighton University, Omaha, Nebraska, USA	**2**
8:50 a.m.	**Mechanical Stimuli** Charles H. Turner, Ph.D., Indiana University-Purdue University, Indianapolis, Indiana, USA	**3**
9:10 a.m.	**Modeling, Remodeling, and the Assembly of Structural Strength** Ego Seeman, M.D., FRACP, Heidelberg Repatriation Hospital, West Heidelberg, Victoria, Australia	**4**

9:30 a.m. Question and Answer Period

BREAK/EXHIBIT VIEWING
9:50 a.m. – 10:10 a.m.

		Presentation Number
10:10 a.m.	**The Osteocyte as a Signaling Focus** Lynda F. Bonewald, Ph.D, University of Missouri, Kansas City, Missouri, USA	**5**
10:30 a.m.	**Neural Control of Bone Mass** Gerard Karsenty, M.D, Ph.D., Columbia University, New York, New York, USA	**6**
10:50 a.m.	**Bone and Fat** Clifford J. Rosen, M.D., St. Josephs Hospital, Bangor, Maine, USA	**7**
11:10 a.m.	**POSTER ORAL PRESENTATION** **Overexpression of Bcl-2 in Osteoblasts Prevents Bone Loss Induced by Glucocorticoids through Suppression of Osteoclast Activity in Col2.3Bcl-2 Mice** Gloria Gronowicz, Ph.D., University of Connecticut, Farmington, Connecticut, USA	**8**
11:20 a.m.	**YOUNG INVESTIGATOR AWARD PRESENTATION** **Regulation of Bone Remodeling through Brain-Derived Serotonin** Vijay K. Yadav, Ph.D., Columbia University, New York, New York, USA	**9**
11:30 a.m.	**Question and Answer Period**	

LUNCH AND POSTER VIEWING
11:50 a.m. – 1:00 p.m.

SESSION 2
1:00 p.m. – 5:40 p.m.
Bone Resorption

Moderators:
Joan McGowan, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, USA
Stavros Manalagas, M.D., Ph.D., University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

1:00 p.m.	**αvβ3 Integrin and c-Fms: Partners in the Osteoclast** Steven L. Teitelbaum, M.D., Washington University in St. Louis, St. Louis, Missouri, USA	**10**
1:20 p.m.	**The RANKL and OPG System and a New Role of Osteoblasts in Osteoclastogenesis** Naoyuki Takahashi, Ph.D., Matsumoto Dental University, Nagano, Japan	**11**
1:40 p.m.	**Mechanisms of Bisphosphonate Action** Graham Russell, M.D., Ph.D., University of Oxford, Oxford, United Kingdom	**12**

		Presentation Number
2:00 p.m.	**Clinical Aspects of Bisphosphonates** Socrates Papapoulos, M.D., Leiden University Medical Center, Leiden, The Netherlands	**13**
2:20 p.m.	**Oversuppression of Bone** Robert R. Recker, M.D., Creighton University, Omaha, Nebraska, USA	**14**
2:40 p.m.	**Question and Answer Period**	

BREAK/EXHIBIT VIEWING
3:00 p.m. – 3:20 p.m.

Moderators:
Meryl S. Leboff, M.D., Brigham and Women's Hospital, Boston, Massachusetts, USA
Bart L. Clarke, M.D., Mayo Clinic, Rochester, Minnesota, USA

3:20 p.m.	**Oversuppression of Bone Remodeling: Counterpoint** Susan M. Ott, M.D., University of Washington Medical Center, Seattle, Washington, USA	**15**
3:40 p.m.	**Osteonecrosis of the Jaw (ONJ)** Peter R. Ebeling, M.D., FRACP, University of Melbourne, Footscray, Victoria, Australia	**16**
4:00 p.m.	**Sex Steroids** Sundeep Khosla, M.D., Mayo Clinic College of Medicine, Rochester, Minnesota, USA	**17**
4:20 p.m.	**Mechanisms of SERM Action** Donald McDonnell, Ph.D., Duke University Medical Center, Durham, North Carolina, USA	**18**
4:40 p.m.	**New Members of the SERM Family** Henry U. Bryant, Ph.D., Eli Lilly and Company, Indianapolis, Indiana, USA	**19**
5:00 p.m.	**New Roles for Osteoclasts in Bone** Brendan F. Boyce, M.D., University of Rochester, Rochester, New York, USA	**20**
5:20 p.m.	**Question and Answer Period**	

WELCOME RECEPTION AND POSTER SESSION
With support provided by Novartis Pharmaceuticals

5:40 p.m. – 7:30 p.m.

ADJOURN
7:30 p.m.

Friday, December 7, 2007

BREAKFAST/EXHIBIT VIEWING
7:00 a.m. – 8:00 a.m.

SESSION 3
8:00 a.m. – 12:40 p.m.
Bone Formation

Moderators:
Michael P. Whyte, M.D., Shriners Hospital for Children, St. Louis, Missouri, USA
Julie Glowacki, Ph.D., Brigham and Women's Hospital, Boston, Massachusetts, USA

		Presentation Number
8:00a.m.	**Molecular Aspects of Osteoblast Regulation** Jane B. Lian, Ph.D., University of Massachusetts, Worcester, Massachusetts, USA	**21**
8:20 a.m.	**Targeting the Wnt Signaling Pathway for Osteoporosis Treatment** Roland Baron, D.D.S., Ph.D., Yale University School of Medicine, New Haven, Connecticut, USA	**22**
8:40 a.m.	**Mechanical Loading and the Wnt Signaling Pathway** Mark L. Johnson, Ph.D., University of Missouri, Kansas City, Missouri, USA	**23**
9:00 a.m.	**Sclerostin** Teresita M. Bellido, Ph.D., University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA	**24**
9:20 a.m.	**New Concepts in Bone Formation** Gregory R. Mundy, M.D., Oates Institute of Experimental Therapeutics, Nashville, Tennessee, USA	**25**
9:40 a.m.	**Molecular and Cellular Mechanisms of PTH Anabolic Action** Robert L. Jilka, Ph.D., University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA	**26**
10:00 a.m.	**Question and Answer Period**	

BREAK/EXHIBIT VIEWING
10:20 a.m. – 10:40 a.m.

Moderators:
Keith A. Hruska, M.D., Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
Benjamin Leder, M.D., Massachusetts General Hospital, Boston, Massachusetts, USA

10:40 a.m.	**New Concepts of PTHs: The Molecules, the Mimetics** Thomas J. Gardella, M.D., Massachusetts General Hospital, Boston, Massachusetts, USA	**27**
11:00 a.m.	**New Concepts of PTH: The Development of Calcimimetics and Calciolytics** Dolores M. Shoback, M.D., VA Medical Center, University of California, San Francisco, California, USA	**28**

Presentation Number

11:20 a.m. **Clinical Aspects of the PTHs** **29**
Felicia Cosman, M.D., Helen Hayes Hospital, West Haverstraw, New York, USA

11:40 a.m. **Sclerostin Inhibition** **30**
Chris Paszty, Ph.D., Amgen, Inc., Thousand Oaks, California, USA

12:00 p.m. **Bone Morphogenetic Proteins** **31**
Vicki Rosen, Ph.D., Harvard School of Dental Medicine, Boston, Massachusetts, USA

12:20 p.m. **Question and Answer Period**

LUNCH AND POSTER VIEWING
12:40 p.m. – 2:00 p.m.

SESSION 4
2:00 p.m. – 3:00 p.m.
Targets Affecting Both Resorption and Formation of Bone

Moderators:
Jonathan Reeve, D.M., D.Sc., Addenbrookes Hospital, Cambridge, Untied Kingdom
Robert M. Neer, M.D., Massachusetts General Hospital, Boston, Massachusetts, USA

2:00 p.m. **Concepts of Coupling Between Bone Resorption and Formation** **32**
Jack Martin, M.D., D.Sc., St. Vincent's Institute of Medical Research, Melbourne, Victoria, Australia

2:20 p.m. **Rationale and Data for Combination Antiresorptive and Anabolic Therapy** **33**
John P. Bilezikian, M.D., Columbia University College of Physicians and Surgeons, New York, New York, USA

2:40 p.m. **Question and Answer Period**

SESSION 5
3:00 p.m. – 3:20 p.m.
Wrap Up

3:00 p.m. **Summary and Conclusions**
John P. Bilezikian, M.D., Columbia University College of Physicians and Surgeons, New York, New York, USA

MEETING ADJOURNS
3:20 p.m.

Session 1: Overview of Bone Remodeling and Bone Modeling

1

Basic Concepts: Modeling, Remodeling and the Decline of Structural Strength

D. W. Dempster*. Regional Bone Center, Helen Hayes Hopital, West Haverstraw, NY, USA.

Bone loss with age is inevitable and universal. It occurs at most, if not all skeletal sites, in all races and cultures, and in both sexes. However, the pattern of bone loss, the magnitude, and the underlying cellular mechanisms differ significantly between men and women. In men, aging is associated with a gradual decline in the ability of the osteoblast teams in cancellous bone to refill the resorption cavity. Consequently, the thickness of the bone packets gradually declines and this, in turn, causes thinning of the trabeculae. The result is a linear reduction in cancellous bone volume with age. Although thinner, the trabeculae remain connected to each other. The reduction in wall thickness is associated with a decrease in the linear rate of matrix deposition and, as well, an increased duration of the bone formation phase of the remodeling cycle, which indicates a generalized decline in osteoblast activity with age. This may be the result of a decrease in the number of osteoblasts recruited during the reversal phase of the remodeling and/or in the amount of bone matrix synthesized by each osteoblast. Bone loss in women is more complex and more deleterious to its strength. Women experience gradual trabecular thinning and bone loss by the same mechanism as men until menopause when there is there is an abrupt acceleration in the rate of bone loss. Rapid, postmenopausal bone loss is not the result of osteoblast insufficiency. It is due to enhanced osteoclastic resorption. With the increase in bone turnover rate due to estrogen deficiency, the number of resorption sites that are active per unit time is increased and the osteoclasts dig deeper cavities, so deep that the trabeculae are perforated and, ultimately removed. This results in a greater age-related loss of trabecular number and connectivity with age in women than in men.

It is well established that tubular bones grow in size with age and the cortex gets thinner. This is due to small differences in the amount of bone removed and replaced in each remodeling cycle. The balance is negative on the endosteal surface and positive on the periosteal surface. Furthermore the magnitude of the amount of bone gained in each remodeling transaction on the periosteal surface is less than that lost on the endosteal surface. While these differences are quantitatively small in each remodeling unit, their effects accumulate throughout life. The endosteal surface moves outwards at a greater rate than the periosteal surface and, as a result, the cortex gets thinner. Again there is a sexual dimorphism in this process. Both men and women lose bone from the endocortex and. This is partially offset by net periosteal apposition, which occurs to a greater degree in men than women. The result is a greater loss in the cross-sectional moment of inertia with age in women and this, combined with smaller bone size, leads to a greater increase in fracture risk.

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***Disclosures: D.W. Dempster** , None.*

2

Clinical Concepts of Modeling and Remodeling

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From the clinical standpoint, modeling and remodeling are largely imperceptible, except perhaps, for their consequence, fracture. High remodeling conditions such as primary hyperparathyroidism are known to result in increased fragility. The significance of remodeling activity, particularly in cancellous bone, lies in the fact that it produces bony fragility out of proportion to the corresponding, temporary reduction in mass. In this regard, it is not the mass *rate* of remodeling (g/t), but the *number* of remodeling sites that is important, whether those sites are currently active or not. All agents that suppress remodeling and lead to filling in of the remodeling space reduce fragility, irrespective of whether bone mass or density changes, either up or down. In women the number of remodeling sites doubles across menopause and triples by age 65. This change is due to estrogen withdrawal and can be prevented by estrogen replacement. The menopausal augmentation in remodeling sites is the best available explanation for the large increase in fragility following menopause.

Bone remodeling biomarkers, which are rough surrogates for remodeling (measured as mass per unit time), are sensitive indicators of the activity of bone-specific agents in large treatment trials, but they are not sufficiently precise and reproducible to be very useful in the clinical evaluation of individual patients. Furthermore, there are currently no biomarkers for the *number* of remodeling sites (as contrasted with remodeling *rate*). Hence, while measurement of remodeling offers considerable potential for detection of those at risk of fracture, or in monitoring response to treatment, that potential is yet to be realized in practice.

***Disclosures: R.P. Heaney** , None.*

3

Mechanical Stimuli

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During growth, mechanical loading of the skeleton is essential for the development and maintenance of strong weight-bearing bones. Without sufficient mechanical loading appositional bone growth is suppressed resulting in thin, fragile bones. Conversely exercise enhances appositional bone growth. For instance, tennis players have increased bone density and stronger bones in their playing vs. non-playing arms. Bone remodeling rate increases when bone is underloaded and when it is overloaded. In overloaded bone, microdamage develops and this in turn stimulates local removal and replacement of the damaged bone. Removal of bone loading activates osteoclasts and allows bone remodeling to increase resulting in bone loss.

Our studies have shown that mechanical loads cause bone formation at the periosteal surface, which has a disproportionately positive effect on bone strength at an organ level. Most importantly, tissue added in regions of high mechanical stress provides the most efficient means for improving bone strength. Experiments have shown that small additions of bone mineral density (5-8%) caused by mechanical loading can improve bone strength by over 60% and extend bone fatigue life by 100-fold. Consequently, it is clear that bone tissue possesses a mechanosensing apparatus that directs osteogenesis to where it is most needed for improving bone strength.

Loading must be dynamic to stimulate bone formation. Static loads little effect on bone cells. The most likely sensors of mechanical loads, the osteocytes, are visco-elastically coupled to the bone matrix so that their biological response increases with loading rate. Loading must be applied for only a short time to stimulate bone formation, after which continued loading has little additional effect. Bone apparently desensitizes to continuous bone loading but, if loading is stopped for a short period, full mechano-sensitivity returns to the bone tissue.

The biological processes involved in bone mechanotransduction are becoming clearer. Several pathways are emerging from current research, including membrane ion channels, ATP signaling, second messengers such as prostaglandins and nitric oxide and Wnt signaling.

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***Disclosures: C.H. Turner**, Pfizer 2; Merck 5.*

4

Modeling, Remodeling and the Assembly of Structural Strength

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Bone must be stiff - resistant to deformation, yet sufficiently flexible to absorb energy imposed by loading. As energy cannot be destroyed, if not absorbed, it must be dissipated by structural failure - fracture. Bone must also be light to facilitate mobility. These contradictory properties are achieved by bone's material composition and structural design. As material composition is similar in land dwelling mammals, variability in bone strength is largely the result of structural diversity.

Long bones function more as levers than springs; stiffness is favoured over flexibility. As bone increases in length by endochondral apposition during growth, concurrent periosteal apposition and relatively less endocortical resorption increase net cortical thickness and displaces the cortex outwards from the neutral axis. As stiffness conferred by a unit volume of bone is proportional to the fourth power of the distance from this axis, larger cross sections can be assembled with less material than smaller ones (relative to their cross sectional sizes). vBMD is lower in wider bones as endocortical resorption excavates a larger marrow and a relatively thinner cortex; they are more 'empty'. vBMD is higher in more slender bones because less endocortical resorption excavates a smaller marrow leaving a relatively thicker cortex to offset the fragility of slenderness.

Variation in cellular activity at each point around the periosteal and endocortical perimeters fashions the diverse shapes of adjacent cross sections along the bone. The absolute and relative positions of these two envelopes determine cortical thicknesses, cortical area and mass distribution; there is no single periosteal or marrow diameter, or cortical thickness. Structures that function more like springs (vertebrae, metaphyses) favour flexibility over peak loading ability and are fashioned by endochondral apposition as trabecular plates of varying thickness and proximity forming porous 'honeycombs'.

Thus, modelling and remodelling establish structural diversity within and between sexes and races - in size, shape, and mass distribution at the macroscopic level, in osteonal density, lamellar structure and cement lines at the microscopic level and in the proportions of mineral and collagen at the nanometer level. This hierarchical organization prevents damage but also detects, confines and removes damage when it occurs. Strength is optimised and mass minimizes using 'nothing'; a single void (marrow space) for levers or many (spongiosa) for springs. The ability of this machinery to adapt structure to loads is Herculean during growth, but not during ageing when changes in its cellular constituents and regulators compromise bone's pristine material composition and structural design.

***Disclosures: E. Seeman** , Eli Lilly; Servier; Sanofia Aventis P and G; Novaritis, MSD.*

5

The Osteocyte as a Signaling Focus

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The transformation of a plump, polygonal, matrix producing osteoblast into a dendritic cell, the early osteocyte, surrounded by osteoid is striking. The transformation of osteoid into a hard mineralized matrix entombing the osteocyte is unique and just as dramatic. Connections and communication of emtombed cells with other cells are accomplished through the flow of bone fluid, gap junctions at the tips of connecting dendritic processes, and the extension of dendritic processes into marrow spaces and vasculature. Osteocytes are exquisitely sensitive to shear stresses, translating bone fluid flow shear stress into biochemical signals between themselves and with cells on the bone surface to affect modeling and remodeling. As 90-95% of all cells in the adult skeleton are osteocytes, it is highly likely that these cells play a significant role in adult bone disease. Osteocytes appear to be able to modify their local environment by modifying the size of their lacunae and the surrounding perilacunar matrix. In the young adult skeleton, osteocytes are viable and the lacuno-canalicular system has fewer canaliculi than the older skeleton. Some studies have shown larger or empty lacunae in the older skeleton. An increase in either size of lacunae and/or number of canaliculi dramatically changes the dynamics of fluid shear stress, actually reducing magnitude of strain. This may explain in part why the adult skeleton loses bone and why exercise fails to increase or even maintain bone mass with aging. Therefore, therapeutics that protect and maintain osteocyte viability and their capacity to modify their microenvironment may be targets for pharmaceutical research.
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***Disclosures: L.F. Bonewald** , Proctor & Gamble 1.*

6

Neural Control of Bone Mass

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The adipocyte-derived hormone leptin regulates bone remodeling by modulating bone formation as well as bone resorption. This biological function occurs through a hypothalamic relay and relied on two mediators linking hypothalamic neurons to osteoblasts, those are the sympathetic nervous system and CART (Cocaine amphetamine regulated transcript). Yet it is not known if leptin regulates the activity of these two mediators directly or indirectly, in other words whether additional mediators of leptin regulation of bone remodeling exist. Although the sympathetic tone and *Cart* hypothalamic expression are low in leptin-deficient (*ob*/*ob*) mice, inactivation of sympathetic tone or *Cart* does not affect the other major function of leptin i.e., the control of appetite. This observation suggest that looking at bone remodeling in mice lacking suspected leptin-regulated genes whose deletion does not affect appetite may be a tool to identify additional regulators of bone mass if they exist. Experiments will be presented at the meeting showing that by using this strategy at least one novel neuronal regulator of bone mass has been identified.
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***Disclosures: G. Karsenty** , None.*

7

Bone and Fat

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Endosteal remodeling occurs within a micro-environment of marrow containing elements composed of hematopoietic, osteoblastic and adipogenic cells. These cells play a role in bone turnover but their specific function is dependent on the developmental stage of the skeleton (i.e. growth, acquisition or maintenance). Since MSC can differentiate into osteoblasts, or adipocytes, defining the pathways and stages at which final commitment into a particular lineage occurs, has become a major research priority. Another area of interest is the physiologic role of marrow fat, beyond space occupation. For example, in aging mice and humans, low BMD and impaired bone formation is closely tied to enhanced marrow adiposity. During pubertal growth marrow fat replaces hematopoietic elements, even though the rate of peak trabecular bone acquisition is at its highest point in life. Certain drugs such as rosiglitazone, can stimulate marrow adipogenesis at the expense of osteoblastogenesis, suggesting that commitment down one lineage may prevent MSCs from entering another differentiation pathway. Other TZDs increase marrow fat but do not affect bone formation. In the *Ebf1* null mouse, marrow adiposity is associated with greater bone formation, despite a more generalized lipodystrophic phenotype. Similarly, mutant strains of mice with marked obesity do not have enhanced marrow adiposity,while states of protein calorie deprivation are associated with greater marrow fat. Finally, in the spontaneous mutant mouse, *small*, characterized by a loss of function mutation in the *Irs1* gene, bone formation is severely impaired but bone marrow fat is absent. These data suggest that fat storage and/or utilization is site specific and regulated by

several key systems including the Wnt/Lrp5 and IGF-I/ PI3K signaling networks. Newer imaging techniques have allowed us to characterize the developmental stages of marrow adiposity, and recent data have provided a preliminary roadmap for the expression patterns of marrow adipocytes during states of energy excess. Ultimately, our understanding of the physiologic role of marrow fat as it relates to bone remodeling will shed new light on skeletal homeostasis and may become an important target for therapies that will prevent the development of osteoporosis.

***Disclosures: C.J. Rosen**, None.*

8

ASBMR POSTER ORAL PRESENTATION Overexpression of Bcl-2 in Osteoblasts Prevents Bone Loss Induced by Glucocorticoids through Suppression of Osteoclast Activity in Col2.3Bcl-2 Mice

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In this study, we evaluated the effects of glucocorticoids on bone mass in mice in which apoptosis was blocked by overexpression of Bcl-2. A transgenic mouse, Col2.3Bcl-2, was developed with a 2.3 kb fragment of the type I collagen gene promoter driving the 1.8 kb region of human Bcl-2 (hBcl-2) cDNA. Three-month old wild-type (+/+) and Col2.3Bcl-2 (tg/+) mice were injected subcutaneously with slow release pellets of placebo and 2.1 mg/kg/day prednisolone, and sacrificed 35 days later. Calcein and xylenol orange were injected 7 and 2 days, respectively, before sacrifice, and both static and dynamic histomorphometry were performed. Osteoblast apoptosis was determined by terminal deoxynuleotidyl transferase-mediated dUTP nick end labeling (TUNEL). In +/+ animals, prednisolone produced a 3.9-fold increase in TUNEL-positive apoptotic osteoblasts per bone surface ($p<0.05$). Prednisolone significantly decreased % trabecular bone volume, % BV/TV (33%, $p=0.03$), with a significant increase in trabecular spacing, TbSp (42%, $p=0.03$), and a significant decrease in trabecular number, TbN (24%, $p=0.05$), in the +/+. Prednisolone also increased bone resorption in the +/+ animals, as indicated by 1.9-fold increase ($p=0.01$) in osteoclast number/bone surface. A 2.0-fold increase in the bone formation rate (BFR/BS) but not in the mineral apposition rate (MAR) were also found. At the time of sacrifice, no significant differences in static or dynamic parameters were found between control +/+ and tg/+ mice. However, in the prednisolone-treated tg/+ mice, Bcl-2 overexpression prevented the glucocorticoid-induced changes in all parameters, including the increase in osteoblast apoptosis, decrease in bone mass, and increase in osteoclast parameters. Analysis by Western blot, revealed that the Col2.3Bcl-2 mice had decreased levels of RANKL compared to +/+ mice with comparable levels of osteoprotegerin.
Thus, glucocorticoids induced bone loss by increasing bone resorption as well as elevating osteoblast apoptosis, whereas bone-targeted Bcl-2 overexpression prevented the induction of bone resorption and osteoblast apoptosis, thus maintaining bone mass in the Col2.3Bcl-2 mice.

***Disclosures: G. Gronowicz**, None.*

9

ASBMR YOUNG INVESTIGATOR AWARD Regulation of Bone Remodeling through Brain-Derived Serotonin

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Serotonin (5-hydroxytryptamine) is a biogenic amine that functions both as a neurotransmitter in mammalian central nervous system and as a hormone in the periphery where most of (95%) it is produced. Serotonin is generated through an enzymatic pathway in which L-tryptophan is converted into L-5(OH)-tryptophan by an enzyme called tryptophan hydroxylase (Tph), this intermediate product is then converted to serotonin by an aromatic L-aminoacid decarboxylase. In mammals brain-derived serotonin is synthesized by the gene Tph2 and peripheral-derived serotonin is synthesized by the gene Tph1. To analyse the spatio-temporal pattern of serotonin expression and the effect of brain serotonin depletion on bone mass, we introduced a LacZ allele in the mouse Tph2 locus by homologous recombination in embryonic stem cells. LacZ staining during embryonic development and postnatally delineated serotonergic neurons in the mouse brain and showed that in the adult, Tph2 neurons are clustered in to six groups in the brain stem, those neuronal groups are known as dorsal and caudal raphe nuclei. Furthermore, histological analysis of the vertebrae and long bones revealed that Tph2-deficient mouse develop a severe low bone mass phenotype early during adult life. Histomorphometric analysis revealed that this is due to a decrease in bone formation and an increase in bone resorption. Current investigations are aiming at elucidating the molecular bases of this newly discovered function of serotonin. In summary, our results already identify serotonin as a novel neuropeptide involved in the regulation of bone mass.

***Disclosures: V.K. Yadav**, None.*

Session 2: Bone Resorption

10

αvβ3 Integrin and c-Fms; Partners in the Osteoclast

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The osteoclast (OC) is a macrophage-derived polykaryon, which degrades the skeleton by forming an isolated microenvironment between itself and the bone surface. This activity requires intimacy between the OC and extracellular matrix, an event reflected by polarization of the cell's cytoskeleton towards the bone-apposed plasma membrane resulting in formation of resorptive organelles such as the ruffled membrane and actin rings. In other cells, integrins are key matrix- binding molecules. We find that the integrin, αvβ3, mediates OC/bone recognition and upon occupancy by matrix residing-ligands, transmits intracellular signals which polarize the cell and activate its resorptive machinery. In fact, OCs lacking the αvβ3 integrin fail to organize their cytoskeleton upon contact with bone and are thus dysfunctional. Consequently, β3 integrin-/- mice develop progressive osteopetrosis with age. Interestingly, high dose M-CSF, previously considered a proliferation and survival factor for OC lineage cells, substantially rescues the cytoskeletal defects of αvβ3-deficient OCs suggesting that its receptor, c-Fms, and the integrin, activate common intracellular signals, which organize the cell's cytoskeleton. We explored this signaling pathway and find that occupancy of either c-Fms or αvβ3 activates c-Src leading to recruitment and activation of a second tyrosine kinase, Syk, whose binding to either receptor requires its association with the ITAM proteins Dap12 and FcRγ. Phosphorylated Syk recruits the adaptor protein, SLP76, and activates the guanine nucleotide exchange factor, Vav3, which transits Rac from its inactive GDP- to its active GTP-bound state. Activated Rac, in turn, promotes organization of the OC cytoskeleton and its capacity to resorb bone. Importantly, deletion of any signaling molecule in the shared αvβ3/c-Fms pathway induces a common OC phenotype characterized by failure to organize the OC cytoskeleton and dysfunctional bone resorption. Thus, c-Fms and αvβ3 collaborate in skeletal biology.

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***Disclosures: S.L. Teitelbaum**, None.*

11

The RANKL and OPG System and a New Role of Osteoblasts in Osteoclastogenesis

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Osteoclasts develop from the monocyte-macrophage lineage. We have established a mouse coculture system of osteoblasts and hemopoietic cells in which osteoclasts are formed in response to bone-resorbing factors (1). A series of experiments using this coculture system have established the concept that osteoblasts are crucially involved in osteoclast development. Osteoblasts express two cytokines essential for osteoclast formation, macrophage colony-stimulating factor (M-CSF) and receptor activator of NF kappaB ligand (RANKL) (2). RANKL, a member of the TNF-ligand family, is expressed as a membrane-associated protein in osteoblasts in response to many bone-resorbing factors. Osteoclast precursors that possess RANK (a receptor for RANKL) recognize RANKL through cell-cell interaction with osteoblasts and differentiate into osteoclasts in the presence of M-CSF. Osteoprotegerin (OPG) mainly produced by osteoblasts is a soluble decoy receptor for RANKL. Thus, osteoblasts play a central role in the regulation of osteoclast differentiation and function. Using RANKL-deficient mice and a system involving bone morphogenetic protein 2 (BMP-2)-induced ectopic bone formation, we examined how the site of osteoclastogenesis is determined (3). Collagen disks containing BMP-2 (BMP-2-disks) or vehicle were implanted into RANKL-deficient mice, which were i.p. injected with RANKL for 7days. Osteoclasts and osteoblasts simultaneously appeared in the BMP-2-disks but not in the control disks. Osteoclasts were located in close proximity to osteoblasts. Recently, we have identified "cell cycle-arrested quiescent osteoclast precursors (QuOPs)" as the committed osteoclast precursors in vitro (4). In vivo studies have revealed that osteoblasts prepare the osteoclast niche, in which QuOPs are maintained for a long period in the undifferentiated state. Distribution of the osteoclast niche determines the correct location of osteoclast formation. These results suggest that osteoblasts also play important roles in osteoclastogenesis by providing a suitable microenvironment for osteoclastogenesis.

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***Disclosures: N. Takahashi**, None.*

12

Mechanisms of Bisphosphonate Action

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Bisphosphonates (BPs) are well established as the leading drugs for the treatment of osteoporosis. There is new knowledge about how they work.

The ability of bisphosphonates (BPs) to inhibit bone resorption was discovered nearly 40 years ago and this is the basis of their use in osteoporosis. The classical pharmacological effects of BPs depend on two key properties; their affinity for bone mineral, and their inhibitory effects on osteoclasts. In the case of the nitrogen-containing BPs (such as alendronate, ibandronate, pamidronate, risedronate, and zoledronate), they appear to act principally by inhibiting the enzyme farnesyl pyrophosphate synthase (FPPS) in the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are utilized for the post-translational modification of small GTP-binding proteins essential for osteoclast function. The recently elucidated crystal structure of the human FPPS enzyme reveals how BPs bind to and inhibit at the active site via their critical N atoms. The heterocyclic BPs (risedronate and zoledronate) are characterized by particularly strong and sustained inhibition as a result of induced conformational changes in FPPS. In trying to understand how BPs exert their important clinical effects, actions on osteocytes (eg, increasing life span) also merit further investigation.

As a class, it is clear that the BPs share a number of common properties. However, as with all other classes of drugs, there are obvious chemical, biochemical, and pharmacological differences among the various BPs. Each BP has a unique profile, particularly in terms of mineral binding and FPPS inhibition that may help to explain observed clinical differences among the BPs in terms of speed of onset and anti-fracture efficacy, and the degree and duration of suppression of bone turnover.

***Disclosures: R.G.G. Russell** , Novartis 5; Amgen 5; GlaxoSmithKline 5; Alliance for Better Bone Health (Procter & Gamble, sanofi-aventis) 2, 5.*

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Clinical Aspects of Bisphosphonates

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Bisphosphonates (BPs) decrease the activity and life span of osteoclasts and are used extensively in the management of common skeletal disorders that are characterized by absolute or relative increase in bone resorption. There is a long list of approved or potential indications for BP treatment. However, for the effective use of BPs in clinical practice, issues related to their pharmacology as well as to the nature of the bone remodeling disturbance caused by the underlying disease need to be considered. BPs have unique pharmacological properties that include selective uptake by the skeleton, decrease of osteoclast-mediated bone resorption and long-term retention in the skeleton. The amount of BP taken up by the skeleton depends on the prevalent rate of bone turnover, renal function as well as of the affinity of the BP for hydroxyapatite which may also affect its retention and elimination from bone. The first measurable effect of BPs is the decrease of the rate of bone resorption that is followed by a slower decrease of the rate of bone formation, due to the coupling of the two processes, and the attainment of a new steady state at a lower rate of bone turnover. The duration of this new steady state varies markedly and depends on the underlying disease as well as the dose and the mode of administration of the BP. These principles of BP therapy were initially examined in patients with Paget's disease of bone which has also served as a model for the study of the clinical pharmacology of BPs, currently the treatment of choice of the disease. Better understanding of the pharmacology of BPs and of the pathophysiology of skeletal disorders together with the development of more potent compounds that allow the safe administration of effective doses at variable drug-free intervals, have led to the design of therapeutic regimens that are specific for relevant indications. These include malignancy-associated hypercalcemia, metastatic bone disease, multiple myeloma and osteoporosis.

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***Disclosures: S. Papapoulos**, Merck & Co 2, 5; Procter & Gamble 2, 5; Novartis 5; Roche/GSK 5.*

14

Oversuppression of Bone

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The introduction of bisphosphonates in 1995 for treatment of postmenopausal osteoporosis demonstrated significant reduction in fractures out of proportion to the increase in bone mass[1]. It became clear that the majority of the anti-fracture effect was attributable to reduction in bone remodeling, and that bone remodeling rates in patients with osteoporosis were far in excess of that needed to maintain skeletal integrity[1]. The ~70% reduction in bone remodeling by bisphosphonates has generated concern. Could bisphosphonate treatment suppress remodeling too much[2]? Since remodeling repairs micro-damage, the result of over suppression would be return of fracture risk. Further bisphosphonate may accumulate in the skeleton occurs over the entire period of treatment. Thus, should bisphosphonate therapy be discontinued after some period of continued treatment? There are no data available to decide on the timing and length of a proposed "drug holiday". Much remodeling does not seem to be targeted to sites of microdamage. For example, remodeling rates, as measured activation frequency (Ac.f) by histomorphometry, increase by two-fold in the 12 months following last menses at menopause[3] in healthy women, and by 3-fold in healthy postmenopausal women at age 60[4], remaining elevated in patients with

osteoporosis. Remodeling rates in excess of that required to repair microdamage can only weaken the skeleton by removing trabecular elements, thinning trabeculae, and converting a plate-like to a rod-like structure. This excess remodeling is driven by stimuli other than mechanical need, perhaps to provide for replenishment of apoptotic osteocytes, or defend plasma calcium concentration. The practical clinical question is whether bisphosphonate treatment can actually result in long-term loss of antifracture efficacy because of excessive suppression of remodeling. Is the anti-fracture effect of standard bisphosphonate treatment only transient, destined to be lost over time when the microdamage "catches up" to the new lower level of remodeling on treatment? The osteoporosis experience suggests that this is not the case since the remodeling rates on bisphosphonate treatment, as measured by histomorphometry, are reduced to the range seen in healthy pre-menopausal women who are not fracturing[5]. Continued observation of the long-term antifracture efficacy with continued treatment is needed to answer the question, "is there over-suppression of bone remodeling with bisphosphonate treatment.

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***Disclosures: R.R. Recker** , Merck 5; Eli Lilly 5; Wyeth 5; Proctor & Gamble 5.*

15

Oversuppression of Bone Remodeling: Counterpoint

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In patients with osteoporosis, excessive bone resorption increases the risk of trabecular plate perforation. This leads to loss of scaffolding and conversion of plates to rods, which decreases the strength of the bone and puts more stress on the remaining trabecular structures, which suffer microdamage, which causes even more resorption in an attempt to repair the damage. This vicious spiral of bone deterioration eventually results in catastrophic material failure. Bisphosphonates halt the vicious spiral and prevent fractures that would occur if the spiral is allowed to continue. However, bisphosphonates also inhibit bone formation to markedly low levels, which limits the effectiveness of these drugs. After one formation period, the nearly zero bone formation makes it impossible to increase bone volume via normal remodeling pathways. With prolonged suppression, the mineralization density increases and bone becomes more brittle. Furthermore, macrodamage can't be repaired if the damaged area can't be resorbed and replaced. Alternative or escape pathways, such as micro-callus formation or de novo bone formation, could theoretically attenuate any harm caused by inhibition of the normal remodeling cycle. An important unanswered question in clinical medicine is whether prolonged use of bisphosphonates can cause enough damage to the bone to outweigh the beneficial effects. This is especially important because the drugs have a long half life and there is no way to remove them from the skeleton. The longest controlled trials have a duration of five years, and the longest observational studies are ten years, but many women without osteoporosis are prescribed bisphosphonates with with the hope of preventing fractures that are not expected for two decades. These patients, who have bone to spare, would probably not experience adverse effects until sufficient time had passed. Findings of concern include: evidence of micro-crack accumulation and decreased toughness in animal models, persistence of woven bone in animal models of fracture repair, development of osteopetrosis in bisphosphonate-laden bone, anecdotal reports of unusual fractures and sub-trochanteric fractures in patients treated with bisphosphonates, lack of efficacy in osteoporotic women with low bone formation markers prior to starting bisphosphonates, and the association of jaw osteonecrosis with bisphosphonate use. Patients treated with bisphosphonates show an attenuated response to intermittent parathyroid, but the fact that they are able to respond to anabolic agents provides hope for the future treatment and prevention of osteoporotic fractures.

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

16

Osteonecrosis of the Jaw (ONJ)

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Bisphosphonate-associated osteonecrosis of the jaw (ONJ) is defined as the presence of exposed bone in the maxillofacial region, not healing within 8 weeks after its identification by a health care provider. Based on both published and unpublished data, the risk of ONJ associated with oral bisphosphonate therapy for osteoporosis appears to be low, estimated between 1 in 10,000 and less than 1 in 100,000 patient-treatment years. However, the true incidence is unknown and may be higher. The ONJ risk in patients with cancer treated with high doses of intravenous bisphosphonates is higher, in the range of 1-10 per 100 patients and depends on duration of therapy.

Suppression of bone remodeling allows accumulation of physiological microdamage in the jaws, a region associated with high levels of trauma. There is also an increased risk of infection from oral bacteria associated with caries and periodontal disease. Both trauma and infection increase demand for osseous repair, which the hypodynamic bone cannot meet, resulting in osteonecrosis. An exacerbating factor is reduced angiogenesis, associated with several factors including bisphosphonates; other anti-angiogenic agents such as glucocorticoids, thalidomide and the proteasome inhibitor, bortezimib; diabetes mellitus and peripheral vascular disease. Smoking has negative effects by both reducing angiogenesis and contributing to poor dental hygiene. Prolonged bisphosphonate-induced secondary hyperparathyroidism may contribute in some patients.

An important area is whether monitoring of bone turnover markers to help avoid over-suppression of bone turnover could reduce the incidence of ONJ, and whether salivary or dental crevicular fluid markers could be used as indicators of local bone metabolism. Many bone markers exhibit large intra-individual and diurnal variability, which needs to be carefully considered in the selection of markers for use in individual patients. However, it is possible bone turnover markers, such as serum PINP, could add to existing imaging technology (^{99m}Tc-MDP bone and cone beam CT scans) to detect early changes of ONJ that could identify those patients most likely to develop a clinical lesion if oral trauma or extractions occur. Prospective studies of this hypothesis are now required.

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***Disclosures: P.R. Ebeling**, Amgen 2, 5; Novartis 2; Merck, Sharp and Dohme 5; Eli-Lilly 8.*

17

Sex Steroids

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Sex steroids play a critical role in regulating bone turnover, and sex steroid deficiency in women and in men is associated with increased bone resorption. Bone formation is also increased following sex steroid deficiency, presumably due to the "coupling" between bone resorption and bone formation, although the precise mediators of this coupling remain unclear at present. Interestingly, however, studies in women[1] and in men[2] with acute induction of sex steroid deficiency have identified a transient phase of "uncoupling" between bone resorption and formation; thus, early (3 weeks) following sex steroid withdrawal in either sex, bone resorption markers are increased, whereas bone formation markers are decreased. These findings provide perhaps the most convincing evidence *in vivo* in humans of a role for sex steroids in maintaining bone formation, and also suggest that following the transient decrease in bone formation, factors possibly produced by osteoclasts may be stimulating osteoblast development and/or activity as part of the recoupling process.

Despite extensive studies in rodents, the precise mediators of the increase in bone resorption following sex steroid deficiency in humans remain unclear. It has been suggested that it is not sex steroid deficiency *per se*, but rather the concomitant increase in FSH secretion[3] that is responsible for the increase in bone resorption following sex steroid withdrawal. However, at least in humans, bone resorption markers increase following suppression of sex steroids and FSH secretion with a GnRH analog[4], indicating that sex steroid deficiency alone, despite suppression of FSH secretion, leads to increased bone resorption. Evidence from studies in women[5]and in men[4] does suggest that increased RANKL production by bone marrow osteoblastic and other cells plays an important role in stimulating bone resorption following sex steroid deficiency. However, it has been difficult to demonstrate changes in pro-resorptive cytokines (IL-1α or -β, IL-6, TNF-α, others) as upstream mediators of increased RANKL production following sex steroid deficiency in humans. Nonetheless, blockade of TNF-α action with a specific inhibitor and, to a lesser extent, abrogating IL-1 action appears to attenuate (by about 50%) the increase in bone resorption markers following estrogen deficiency in women[1]. Moreover, studies in men indicate that estrogen (but not testosterone, in the absence of aromatization to estrogen) tends to suppress T-cell numbers in bone marrow[4]. Collectively, these human findings are consistent with a potential role for expansion of T-cells and increased TNF-α action possibly leading to increased RANKL production by bone marrow

osteoblastic and other cells following estrogen deficiency, as appears to occur in mice[6]. Nonetheless, given the difficulty of studying the relevant responsive cells in humans, further studies are needed to definitively establish the mediators of sex steroid action on bone in humans.

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***Disclosures: S. Khosla** , GSK 5; Novartis 5.*

18

Mechanisms of SERM Action

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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.

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19

New Members of the SERM Family

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The widespread distribution of estrogen receptors (ER), and their critical role in normal physiology and various pathophysiologic states when estrogen levels decline indicate the importance of ER targeted therapies for use in postmenopausal women. Controversy and concern over the use of estrogen replacement therapies creates the opportunity to design molecules to selectively modulate estrogen action in those tissues where estrogen agonism is the desired goal while simultaneously producing an estrogen neutral or antagonistic effect in tissues where estrogen-related side effects are a concern. Selective estrogen receptor modulators, or SERMs, are currently in clinical use for the treatment and prevention of osteoporosis (raloxifene), breast cancer prevention (tamoxifen and raloxifene) and treatment (tamoxifen and toremifene), and for the induction of ovulation (clomifene). To date, seven different SERMs have reached advanced clinical evaluation for postmenopausal osteoporosis with three molecules (droloxifene, idoxifene, levormeloxifene) withdrawn for unfavorable risk/benefit profiles and three additional molecules (lasofoxifene, bazedoxifene, arzoxifene) currently in phase 3 status or under regulatory review. Experience with these molecules reveals several key themes for chronic use of SERMs. 1) Each SERM generates a unique complex with the ER that influences co-factor recruitment in estrogen-target tissues responsible for the tissue selective pharmacological profile, which translates to each SERM generating potentially an entirely unique overall safety and efficacy profile - indicating the need for thorough evaluation of each individual SERM across multiple tissue types for efficacy and safety determination. 2) Uterine safety historically has been the critical safety feature for chronic SERM use in osteoporosis therapy and careful assessment of the potential for uterine stimulation is a key element in the consideration of new molecules in this class. 3) Pharmacokinetic and distribution properties of SERMs offer an additional aspect influencing the magnitude of the overall biologic response by either improving systemic bioavailabilty or altering uptake into important estrogen responsive tissues. A deeper look into the future of SERMs reveals a number of new molecules designed to address various aspects of women's health, including molecules generated to address: menopausal vasomotor symptoms, estrogen-dependent gynecological diseases, vaginal atrophy, osteoarthritis as well as various possible uses in males.

***Disclosures: H.U. Bryant** , Eli Lilly and Company 1; Eli Lilly and Company 3.*

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Other Novel Concepts That could Lead to Inhibition of Bone Resorption

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Osteoclasts (OCs) resorb bone in normal and pathologic bone remodeling. OC formation and activity are regulated predominantly by osteoblastic cells. There is growing evidence that OCs and their precursors (OCPs) have functions in and around bone other than resorption. For example they regulate osteoblast (OB) differentiation positively by signaling through their ephrinB2 ligand and the EphB4 receptor in OBs and negatively through the function of Atp6v0d2, a subunit of v-ATPase, a component of the V-type H^+ ATP6i proton pump complex. Ephrin/Eph signaling mediates arterial-venous link up and neuronal axon pathfinding through cell process interactions. OBs and OCs also interact through long cytoplasmic extensions. Interestingly, so-called reverse signaling through ephrinB2 back into OCPs inhibits OCP differentiation by down-regulating c-Fos activation of NFATc1[1] and thus could augment RANKL-induced interferon β-mediated inhibition of OCP formation. Atp6v0d2-deficient mice[2] have increased bone mass due to defective OCP fusion and decreased bone resorption plus enhanced bone formation. How Atp6v0d2 inhibits bone formation is known, but it may be through a product secreted by OCPs or OCs. The above findings suggest that single therapeutic agents could be developed to inhibit OC activity and increase bone formation.

Osteoblastic cells on endosteal surfaces in mice also support the self-renewal potential of hematopoietic stem cells (HSCs) and HSC entry into the blood. A direct role for OCs in HSC mobilization was identified unexpectedly by Kollet et al. who found that HSC mobilization accompanied OC formation induced by RANKL *in vivo*[3]. However RANKL did not mobilize HSCs in protein tyrosine phosphatase-epsilon-/- mice which have dysfunctional OCs, suggesting that functional OCs are required for HSC mobilization. These findings define a role for OC activation in HSC mobilization presumably through interaction with osteoblastic cells and suggest that OCs have a role to enhance recovery from marrow ablation and bone marrow transplantation.

Recent studies also identify OCs and OCPs as immune cells. For example in mice with TNF-induced inflammatory arthritis, OCPs proliferate in bone marrow and are mobilized from there to inflamed joints[4]. There they respond to cytokines such as TNF, IL-1 and RANKL and secrete factors that not only up-regulate OC formation and activation but also affect functions of other cell types. RANKL stimulates OCs and OCPs to produce and release the lymphatic growth factor VEGF-C which induces self-amplifying autocrine cycles in OCs through VEGFR3 signaling and also is associated with increased lymphangiogenesis. Thus RANKL and OCs may directly affect lymphangiogenesis through VEGF-C, which will require further investigation given the importance of the lymphatic system in inflammation.

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Disclosures: ***B.F. Boyce*** *, Merck 5.*

Session 3: Bone Formation

21

Molecular Aspects of Osteoblast Regulation

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Bone formation in the adult skeleton requires integration of developmental and growth factor signals and hormone responses for physiologic control of gene expression during osteoblast growth and differentiation. The bone morphogenetic proteins and canonical Wnt pathways support formation and mineralization of an organized bone matrix. Transduction of these signals results in the control of gene expression through post-translational modifications of their intracellular receptors which interact with transcription factors that are targeted to gene promoters. Among the required transcriptional regulators for bone formation, downstream of these signaling factors, are Runx2, Osterix, Homeodomain and TCF/Lef proteins. At each stage of osteoblast maturation, one also finds exquisite autoregulatory controls by these transcription factors for both maintaining the osteoprogenitor population and promoting their differentiation. From characterization of the multifunctional roles of Runx2 in epigenetic marking of genes for osteogenesis and chromatin remodeling for activation and repression of genes for bone formation, we have come to a better understanding of targeted approaches for stimulating bone renewal and remodeling. Human disorders and genetic mouse models have further identified approaches for developing anabolic treatments that will stimulate bone formation. An overview of the levels of regulation for promoting bone formation will be presented with specific examples for targeting osteoblast lineage cells by the Wnt signaling pathway and Runx2 bone-related transcription factor. Supported by grants from the National Institutes of Health (NIDCR, NIAMS, and NCI).

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Disclsosures: J.B. Lian, *Wyeth Research 2.*

22

Targeting the Wnt signaling pathway for osteoporosis treatment

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The need for a safe and effective anabolic treatment for osteopenic conditions such as osteoporosis (OP) or osteogenesis imperfecta is high. The most important recent finding in anabolic approaches has been the identification of LRP5 and the Wnt signaling pathway as major regulators of bone mass in humans. Gain-of-function mutations in the LRP5 receptor in humans lead to the High Bone Mass (HBM) trait whereas loss-of-function mutations lead to the Osteoporosis Pseudo-Glioma (OPPG) syndrome. In mice, LRP5 gene deletion mimics the OPPG syndrome whereas the gain-of-function mutation mimics the HBM phenotype. Knockout of the closely related LRP6 receptor is embryonic lethal but mice heterozygote for LRP6 deletion (LRP6+/-) exhibit an osteopenic phenotype. Double mutants missing one allele of LRP6 and both alleles of LRP5 have a more severe osteoporotic phenotype than LRP5-/- mice, suggesting that both receptors participate, in part redundantly, in the regulation of bone mass. LRP5 and 6 are co-receptors with Frizzled for Wnt ligands. Wnt signaling leads to the inhibition of GSK3B, decreased phosphorylation of beta-catenin and activation of beta-catenin-dependent genes, including several osteoblast marker genes. Several Wnt signaling targets are being explored in drug discovery: blocking the activity of endogenous antagonists (Sclerostin, Dkk1, sFRPs), agonists of the LRP5 or LRP6 receptors and inhibitors of signaling events downstream of LRP5/6 activation (GSK3B inhibitors, LiCl). These targets are all validated in mice: DKK1+/- mice show a marked increase in bone mass, sclerostin antibodies increase bone mass in rats, inhibition of GSK3beta activity and targeted overexpression of a constitutively active beta Catenin all increase bone mass by activating Wnt signaling. Some molecules are close to entering the first clinical trials where activation of the Wnt pathway will be tested in OP. A major question remains safety issues based on the reported link of Wnt with oncogenesis. However, the observations that some endogenous inhibitors are partially restricted to the bone microenvironment (Sost, Dkk1) suggest the possibility to increase Wnt signaling only in bone, avoiding side effects in other organs. Thus, activation of the Wnt pathway in bone currently remains the most promising approach to bone anabolic treatment.

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Disclosures: R. Baron*, None.*

23

Mechanical Loading and the Wnt Signaling Pathway

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The discovery that mutations in the low-density lipoprotein receptor-related protein 5 (*LRP5)* are the cause of human diseases of low bone mass *and* diseases of increased bone mass has dramatically shown that Lrp5 and the Wnt/β-catenin signaling pathway is critical for bone mass regulation (*1-3*). We originally hypothesized that Lrp5 and the Wnt/β-catenin signaling pathway were involved in bone responsiveness to mechanical loading (*4*). Several studies now support this hypothesis including those demonstrating that *Lrp5*$^{-/-}$ mice do not form new bone in response to mechanical load (*5*) and that activation of the Wnt/β-catenin pathway and changes in the expression of its target genes occur in response to mechanical loading (*6-8*).

Our recent studies suggest a 2-stage model for the role of the Wnt/β-catenin signaling pathway in the mechanoresponsiveness of bone. In the first stage osteocytes sense mechanical strain and release PGE_2, which acts in a paracrine/autocrine fashion. Subsequent binding of PGE_2 to EP2 (and/or EP4) receptors in osteocytes leads to GSK-3β phosphorylation and nuclear translocation of β-catenin. This results in an initial change in the expression of key target genes including positive (increased expression) and negative regulators (decreased expression) of Lrp5 function. In the second stage Wnt(s) bind to the Lrp5-frizzled co-receptor complex and further activate the Wnt/β-catenin pathway. This results in a positive amplification loop that enhances and sustains the β-catenin signaling response to loading.

The model is consistent with much of the published literature in terms of the spatial and temporal sequence of activation events that occur following mechanical loading. Our *in vivo* studies indicate that the osteocyte is the primary mechanosensory cell in bone. Our model proposes a temporal sequence of events in which target osteocytes respond to strain and the perceived load signal is propagated to neighboring osteocytes and eventually to lining cells and/or osteoblasts on the bone surface, thereby stimulating new bone formation. It also accounts for the Lrp5 dependence of bone formation in response to loading.

Understanding the pathway of mechanoresponsiveness in bone is likely to reveal new targets for development of novel drugs to treat skeletal disorders such as osteoporosis. Also, the application of mechanical load to the skeletal in combination with these new pharmaceuticals may represent a novel, safe and efficacious paradigm for increasing bone mass.

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

24

Sclerostin

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Sclerostin, the product of the Sost gene exclusively expressed in osteocytes in bone[1;2], represents the most compelling paradigm by which osteocytes influence the function and number of the executive cells of remodeling. Genetic evidence indicates that sclerostin acts in a paracrine fashion to inhibit osteoblast generation. Thus, loss of Sost/sclerostin in humans causes the high bone mass disorders Van Buchem's disease and sclerosteosis[3]. In mice, deletion of the Sost gene leads to increased bone mass[4]; and, conversely, mice overexpressing Sost exhibit low bone mass[1;5].

Sclerostin binds to several BMPs and to the Wnt co-receptors LRP5 and LRP6. Transcriptional analysis demonstrates that sclerostin specifically affects BMP and Wnt signaling out of other signaling pathways in osteoblasts. However, sclerostin is not a classical BMP antagonist and the interference with BMP action appears to result from inhibition of the Wnt pathway.

Sclerostin expression is negatively regulated by hormonal and mechanical stimuli that lead to increased bone formation. Thus, daily injections of PTH - the only approved bone-building therapy for osteoporosis - as well as chronic excess of this hormone suppress sclerostin expression in mice[6;7]. Moreover, exclusive activation of PTH receptor 1 signaling in osteocytes in transgenic mice is sufficient to inhibit Sost expression and to activate the Wnt signaling pathway; and leads to a remarkable increase in bone mass due to elevated osteoblast production[8]. Sclerostin expression is also dramatically decreased by mechanical loading; and this inhibition correlates with the levels of strain[9]. The therapeutic potential of controlling sclerostin levels is demonstrated by the effectiveness of an anti-sclerostin antibody to increase bone mass and restore bone lost upon sex steroid deficiency in experimental animals[4].

In summary, through sclerostin, osteocytes - the ultimate progeny of the osteoblast differentiation pathway - exert a negative feedback control of mesenchymal stem cell differentiation towards the osteoblast lineage. Unraveling the mechanisms by which sclerostin, and potentially other yet to be discovered osteocyte-specific genes, control skeletal homeostasis opens new possibilities for the treatment of osteoporosis by targeting osteocytes.

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

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New Concepts in Bone Formation

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As every bone biologist knows, bone remodeling is a cyclical process (Mundy GR, Elefteriou F. Boning up on Ephrin Signaling. Cell. 126(3):441-3, 2006). The important signal transduction cascades responsible for osteoclastic resorption and osteoblast differentiation are now well-described. In the case of bone resorption, these include the role of the cytokines RANK ligand and M-CSF, with contributions from TGF-beta (Mundy GR, Edwards J. A Non-cell Autonomous Osteoclast Defect in Osteopetrosis. Cell Metabolism. in press). In the case of osteoblast differentiation, they involve the signal transduction cascades mediated by the Hedgehog pathway, by BMP2 and the Wnt/beta-catenin pathways. Despite a marked increase in knowledge based on the widespread use of genetic mouse models over the last decade, there are still a number of critical unanswered questions in bone cell biology, including the nature of the mechanisms responsible for initiation of bone remodeling, the control mechanisms mediating the transition from osteoclastic resorption to osteoblast proliferation and

differentiation, the regulatory controls of the bone formation process and what stops it, and the mechanisms for the disruption in bone remodeling which occur in disease states, and specifically the disruption in the normal coupling of bone formation to previous bone resorption. These processes will be discussed, including their relationship to other remodeling processes such as in the hair follicle, where similar molecular pathways are utilized, in particular during the growth or anagen phase (Mundy G, Gutierrez G, Garrett R, Gallwitz W, Rossini G, Christiansen C, Langenberg A. Proteasome Inhibitors Stimulate both Bone Formation and Hair Growth by Similar Mechanisms. Annals of the New York Academy of Science. in press).

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Molecular and Cellular Mechanisms of PTH Anabolic Action

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The anti-fracture efficacy of intermittent PTH therapy is due to an increase in osteoblast number leading to increased bone formation and thickening of trabecular and cortical bone. In human cancellous bone, the increase in bone formation occurs within pre-existing basic multicellular units (BMUs) as well as on surfaces adjacent to the BMU, perhaps due to movement of osteoblasts outside the normal boundary of the bone surface being remodeled [summarized in (*1*)]. Thus, PTH appears to over-ride the mechanisms that normally limit the number of osteoblasts in the BMU to that needed to replace the bone previously removed by osteoclasts. Activation of the PTH receptor in cells of the osteoblast lineage rapidly stimulates multiple interconnected pathways resulting in increased survival signaling, decreased replication, and increased production and/or activation of osteoblastogenic growth factors. PTH directly stimulates survival signaling in osteoblasts, and delay of osteoblast apoptosis is a major contributor to the increase in osteoblast number in cancellous bone, at least in mice (*2*). PTH also promotes exit of replicating progenitors from the cell cycle, which may set the stage for pro-differentiating and pro-survival effects of locally produced factors [summarized in (3)]. Studies using genetically modified mice indicate that PTH-induced anabolism requires insulin-like growth factor-I, fibroblast growth factor-2, and perhaps Wnt signaling, each of which is known to promote the differentiation and survival of osteoblasts. PTH may also increase osteoblastogenesis by inactivating PPARγ, which normally exerts a suppressive effect on this process. Intermittent PTH increases osteoblast number and bone formation on periosteal bone surfaces where remodeling is low or absent [summarized in (*1*)]. Our recent studies in mice have shown that, in contrast to cancellous bone, the prevalence of osteoblast apoptosis in periosteal bone is extremely low. Thus, the importance of pro-survival effects of PTH on osteoblasts for anabolism at this site is minimal if any. Specific genetic ablation of osteoblast progenitors blocks PTH-induced bone formation in periosteal bone, indicating that pro-differentiating and pro-survival effects of PTH on osteoblast progenitors are required. The pleiotropic effects of intermittent PTH, each of which alone can increase osteoblast number, may explain why this therapy reverses bone loss regardless of the underlying pathologic mechanism. Further elucidation of molecular and cellular mechanisms underlying the anabolic actions of PTH may aid in the development of targets for new anabolic agents.

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***Disclosures: R.L. Jilka**, Radius Health, Inc. 1.*

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New Concepts of PTHs: The Molecules, The Mimetics

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The PTH receptor is well recognized as a top candidate target for developing new drugs for the treatment of osteoporosis. Human PTH(1-34), Forteo, the only PTHR ligand now in use to treat this disease, has proven efficacy, but also some limitations, including the need for daily injection, and the dose-limiting potential for hypercalcemia. Despite intense efforts by many groups, a potent small molecule ligand for the PTHR has not yet been found. Such a ligand, particularly if orally active, and, ideally, devoid of bone-resorbing/hypercalcemic effects, would represent a major breakthrough in the field. Two low-affinity small molecule ligands for the PTHR have been reported: one, an agonist and the other, an antagonist . These ligands, despite their low micro-molar affinity, and the fact that their modes of action at the receptor are largely undefined, suggest that it is at least possible, through the compound library screening approach, to obtain a non-peptide agonist ligand for the PTHR. Another approach is to optimize and minimize the pharmacophoric agonist domain of PTH(1-34). Our structure-activity studies suggest that this domain resides within the PTH(1-9) region, and that valine-2, isoleucine-5 and methionine-8 play key roles. The challenge now is to "morph" this pharmacophoric peptide domain into a mimetic structure that maintains affinity and potency on the receptor, as well as efficacy *in vivo*.

It is now appreciated that most, if not all, G protein-coupled receptors are conformationally dynamic and hence pleiotropic

in their capacities to activate various cellular signaling pathways in response to structurally distinct ligands. For the PTHR, such conformational pleiotropy could conceivably offer the opportunity to selectively activate those signaling pathways that mediate bone formation, but not those that mediate bone-resorption. In beginning to explore this concept, we have found that certain PTH ligands, including PTH(1-34), can bind with high affinity to a recently defined PTHR conformation, called R^0, that is apparently not coupled to heterotrimeric G protein, as the ligand-R^0 complexes are stable in the presence of GTPgammaS, or in the absence of Gs-alpha . This stable binding to R^0 is associated with prolonged cAMP signaling responses in cells, which suggests that the ligand-bound R^0 conformation can isomerize, over time, to the biologically active G protein-coupled conformation, RG. Interestingly, PTHrP(1-36) binds less well to R^0 than does PTH(1-34) and thus produces a shorter-lived cAMP response in cells. We have recently found a new modified PTH(1-28) analog that binds to R^0 with even higher affinity than does PTH(1-34), and produces a more prolonged cAMP responses in cells, as well as more prolonged calcemic and phosphaturic responses in mice than does PTH(1-34) . This new long-acting PTH ligand may not be suitable for treating osteoporosis, as the bone formation response is likely to be favored by a more transient action of the ligand at the receptor. However, such a long-acting PTH ligand might be useful for treating congenital or acquired forms of hypoparathyroidism. The findings overall suggest that exploring *in vitro* and *in vivo* the pleiotropic conformations of the PTHR, and obtaining further knowledge about the pharmacophoric domains of PTH and PTHrP, will provide important guidance for discovery strategies aimed at the next generation of PTHR-based therapeutics for osteoporosis and other diseases of bone and mineral metabolism.

References:

***Disclosures: T.J. Gardella**, Chugai Pharmaceutical 2.*

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New Concepts of PTH: The Calcimimetics and Calciolytics

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The calcium-sensing receptor (CaR) cDNA was identified in 1993 by Brown and colleagues (1) and determined to be the pivotal molecule responsible for the control of PTH secretion and parathyroid cell proliferation. Shortly after this identification, agents termed calcimimetics were reported whose cellular actions were to activate the CaR and suppress PTH secretion and parathyroid cell proliferation in states of uremic secondary hyperparathyroidism (HPTH) in animal models. They, thereby, mimicked the action of high extracellular [Ca] ($[Ca]_e$), the key physiologic activator of the CaR, and could be used to treat states of PTH excess such as primary and secondary HPTH and parathyroid carcinoma. One such agent cinacalcet was approved in the US in 2004 to treat secondary HPTH and refractory parathyroid carcinoma based on large placebo-controlled trials in dialysis patients (2). A smaller compassionate use study in patients with severe hypercalcemia due to parathyroid cancer showed efficacy in the control of hypercalcemia and an improvement in quality of life (3). Patients with mild primary HPTH were treated for 4 years with cinacalcet, and hypercalcemia was consistently controlled in these patients (4). Thus, within a short period of time from identification of the CaR cDNA a clinically useful receptor agonist has emerged. Considerable interest is building regarding the putative utility of an orally active agent that can antagonize the CaR, termed a calcilytic, in the treatment of osteoporosis. Theoretically, a calcilytic with the proper pharmacodynamic responses in vivo would induce a short-term burst of endogenous PTH secretion that would have an anabolic profile on bone (5) and effectively restore bone mass in osteoporotic individuals without the need for PTH injection therapy. Such calcilytics have proved challenging to develop because of these requirements and the fact that our understanding of the detailed cellular mechanisms whereby small molecules interfere with CaR signal transduction in vivo in intact parathyroid cells is limited. The ability to study PTH secretion and parathyroid cell proliferation in an in vitro model system is further hampered by the lack of appropriate in vitro models. Despite these challenges, the development of calcilytics is progressing with agents now in early clinical trials in postmenopausal osteoporosis.

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***Disclosures: D.M. Shoback** , Merck 8; Amgen 9; Novartis 8; GlaxoSmithKline 5.*

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Clinical Aspects of the PTHs

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PTH exerts skeletal effects distinct from all other available osteoporosis therapies. This agent stimulates bone formation and improves bone structure, producing increments in trabecular connectivity and cortical thickness[1-2]. The effect of PTH on bone formation is very rapid, within 4 weeks[3], and in treatment naïve patients, the most efficient bone building opportunity appears to be the first several months of therapy. The suggestion that PTH not be used for longer than 24 months derives from the duration of the clinical fracture trial, which was curtailed as a result of the development of osteosarcoma in rats. However, this duration might make sense from an efficacy perspective also, since both biochemical[4] and histomorphometric evidence[2] indicate that the stimulatory effect of PTH on bone formation and bone remodeling is largely over by this time. The etiology of this skeletal tachyphylaxis to PTH is not at all understood. These observations suggest that shorter pulses of PTH, given cyclically, perhaps with an antiresorptive agent in between,

might be the optimal way to use this compound. One study has provided some proof of this principle[5].

Currently, the prevailing view is that PTH should be reserved for those who have already had osteoporosis-related fractures or who are failing other therapies. However, the actions of this agent on bone structure provide a rationale for considering use in all patients with osteoporosis, perhaps just short-term, before initiating an antiresorptive agent. The fracture efficacy data for teriparatide, including both spine and nonspine fracture, are available for patients with osteoporosis on no prior treatment. Little is known about the anti-fracture efficacy in those on prior bisphosphonates because there have been no studies large enough to evaluate fracture incidence in this population. Several studies suggest that the effect of PTH on BMD in those treated with prior bisphosphonates is of lower magnitude than those who are treatment naïve; however data showing improvement in bone density and microstructure in these patients were presented at ASBMR this year. Nevertheless, from a clinical perspective, it would be reasonable to consider using teriparatide as first line therapy, prior to any other therapy in patients who have osteoporosis. The drawbacks are cost, subcutaneous administration and concerns related to the rodent toxicology studies, all of which pose realistic constraints on what should otherwise be more widespread use of this agent. Trying to improve the fundamental integrity of the skeleton initially with PTH, followed by skeletal maintenance with an antiresorptive, could provide a more substantial long-term impact on fracture risk than treating with an antiresorptive alone. Unfortunately, it is unlikely that we will ever have direct data, with fracture outcomes, to prove this thesis.

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***Disclosures: F. Cosman** , Eli Lilly 5, 8; Novartis 5, 8; Merck 5, 8; Amgen 5.*

30

Sclerostin Inhibition

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Genetic mapping and phenotypic studies of the rare, high bone mass human genetic disease, sclerosteosis, led to the discovery of sclerostin and its characterization as an inhibitor of osteoblast-mediated bone formation. Sclerostin is a secreted, cystine-knot protein expressed primarily in bone, specifically by osteocytes. Similar to humans with sclerosteosis, sclerostin knock-out mice have a high bone mass phenotype, demonstrating conservation of sclerostin function from human to mouse. *In situ* hybridization of bone samples from older rats and older humans reveals robust expression of sclerostin in osteocytes, further implicating sclerostin as a key regulator of bone mass throughout life. Based on the genetic, phenotypic and expression data, sclerostin likely represents a key inhibitory signal used by the mechanosensory osteocyte network for controlling bone mass. The discovery of this fundamental bone homeostatic pathway has generated significant interest in the area of potential therapeutic applications. In this regard, administration of a sclerostin neutralizing monoclonal antibody (Mab) in aged ovariectomized rats and in intact female cynomolgus monkeys resulted in increased trabecular and cortical bone mineral density, increased periosteal and endocortical bone formation, and increased bone strength. These preclinical studies suggest that a sclerostin neutralizing Mab could represent a clinically useful, and novel, therapeutic agent for the anabolic treatment of disorders in which bone loss is a significant component.

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

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Bone Morphogenetics Proteins

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Bone morphogenetic proteins (BMPs) are a family of evolutionarily conserved, dynamically expressed intercellular signaling molecules that regulate bone formation. During embryogenesis, BMPs influence the coordinated cell proliferation, apoptosis and differentiation required for normal skeletal development.[1] Loss of BMP activity during postnatal skeletal growth leads to osteopenia, bone fragility and spontaneous fracture.[2] In the adult skeleton where BMPs provide the endogenous bone regeneration signals necessary for fracture healing[3], local delivery of exogenous BMPs is currently used to augment bone repair in patients undergoing spine fusions, tibial fracture reconstructions, and oral maxillofacial surgeries. In each of these settings, BMPs increase osteoblast differentiation, leading to a site-specific gain in bone mass. These findings suggest that increasing endogenous skeletal BMP activity could be a potent anabolic therapy for osteoporosis. In my presentation I will focus on BMP signaling in skeletal target cells, detailing the stringent

negative regulation of BMP activity present in the adult skeleton, and highlighting recent data that underscore the potential of novel bone anabolic therapies that target BMP activity.

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***Disclosures: V. Rosen** , Musculoskeletal Transplant Foundation 2.*

Session 4: Targets Affecting Both Resorption and Formation

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Concepts of Coupling Between Bone Resorption and Formation

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Local signalling that results in bone formation during remodelling takes place in several ways. Growth factors released from resorbed bone matrix can contribute to preosteoblast differentiation and bone formation. The preosteoblasts themselves, growing in the resorption space, can communicate through cell contact and paracrine signalling mechanisms to differentiate. Osteocytes can sense the need for bone repair by detecting damage and pressure changes, and signalling to surface cells to respond appropriately. Now that it has been shown through mouse genetics that PTHrP generated locally in bone is a crucial physiological regulator of bone formation, and probably also of resorption, we need to understand how local PTHrP release is controlled in bone in the remodelling process, so that it can both promote differentiation of osteoblasts and inhibit their apoptosis

There is some evidence to support a view that osteoclasts in the BMU might also generate activity that contributes to bone formation, and could even complement the direct effect that PTH has in promoting differentiation of committed osteoblast precursors. First, both human and mouse genetics provide evidence supporting the view that osteoclasts, despite in some circumstances being unable to resorb bone, e.g. failure of acidification or of cathepsin K activity, can nevertheless be associated with normal, or even increased bone formation. An implication is that it may be possible to design resorption inhibitors that do not block bone formation. Second, PTH administered intermittently in an anabolic regime results in transient activation of osteoclasts, and prevention of the latter in a number of experimental approaches has been associated with blunting of the PTH anabolic effect. It is possible that osteoclasts, transiently activated by PTH can contribute to the coupling of bone formation to resorption by producing activity that influences preosteoblast participation in bone formation.

Among the processes to be considered, possible contributors to intercellular communication in bone remodelling are ephrins, with osteoclast-derived ephrinB2 acting in a cell contact-dependent manner on osteoblasts to promote bone formation. The complexity of ephrin/Eph involvement is increased with the finding that PTH rapidly increases the production by osteoblasts *in vitro* and in *vivo* of ephrinB2, which can act within the osteoblast lineage by phosphorylating its receptor, Eph B4. Thus location within the BMU would determine whether ephrin action at any point originates from osteoblast or osteoclast.

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

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Rationale and Data for Combination Antiresorptive and Anabolic Therapy

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Therapeutic agents that reduce bone resorption have become a mainstay of therapy for osteoporosis. By impairing processes that are associated with bone resorption to a greater extent than processes associated with bone formation, these drugs alter bone remodeling balance favorably. While less well studied, the anabolic class of agents for osteoporosis, typified by hPTH(1-34)- teriparatide- or the full length molecule PTH(1-84) realize their therapeutic effects in osteoporosis by stimulating processes associated with bone formation, to a greater extent than they stimulate bone resorption (1-2. It would seem logical and attractive, therefore, to consider that combination antiresorptive and anabolic therapy might provide greater therapeutic efficacy than treatment with an antiresorptive or anabolic agent alone. This question has been addressed in a prospective clinical trial (PaTH) in which PTH(1-84) was used alone or in conjunction with alendronate (3). Gains determined either by DXA or QCT were greater in the PTH(1-84) alone arm than in the combination therapy or alendronate alone arm. The combination therapy arm showed changes in bone turnover markers that fell, similar to the reduction in bone turnover markers observed when alendronate was used alone. It would seem that alendronate dominated the effects on bone turnover in the context of combination therapy with an anabolic agent. Similar data have been reported when combination therapy with alendronate and teriparatide was studied in men (4). In contrast, Deal et al. showed that when raloxifene, an antiresorptive agent not as powerfully antiresorptive as alendronate, was used in combination with teriparatide, changes in hip bone density were greater than

teriparatide alone (5). Bone marker changes were consistent with an additive effect. In addition to attempts to develop settings in which simultaneous combination therapy is advantageous, sequential combination therapy needs attention. Over 50% of patients who are treated with teriparatide in the USA have received a bisphosphonate, while in Europe, this figure approaches 100%. It is important to consider, therefore, whether previous exposure to an antiresorptive agent might influence subsequent actions of teriparatide, as well as to consider possible differences among and between classes of antiresorptives in this regard. When estrogen or raloxifene is used as the antiresorptive, teriparatide seems to be able to stimulate bone formation and to increase bone mass to levels that are similar to the response of patients who have not previously been treated with any drug. With alendronate, the data are more controversial. One hypothesis that has been raised in connection with these observations is that the level of baseline turnover after antiresorptive therapy is a determinant to subsequent responsivitiy to teriparatide. A recent study compared previous exposure to alendronate or risedronate with regard to subsequent changes in bone density and bone turnover induced by teriparatide. Greater changes in DXA and QCT-based BMD were seen when teriparatide was administered after risedronate than after alendronate therapy (6). Since teriparatide is approved for only 18-24 months, it is important to consider whether the gains achieved by anabolic therapy are sustained without subsequent antiresorptive therapy. Building upon observational data that support the idea the maintenance antiresorptive therapy is important, the PaTH study, in a prospective experimental design, showed that with subsequent antiresorptive therapy, gains achieved with PTH are maintained while they are lost if treatment with PTH is not followed by antiresorptive therapy (7). Finally, there is interest in considering protocols in which anabolic therapy is provided for limited time periods against a backdrop of continuous antiresorptive therapy (8).

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***Disclosures: J.P. Bilezikian** , Merck 5; Alliance for Better Bone Health 5; Amgen 5; Eli Lilly 5.*

POSTERS

Integrative Functions of Cells and Organs Controlling Bone Remodeling

P1

ASBMR YOUNG INVESTIGATOR AWARD
The Association between Thyroid Stimulating Hormone and Bone Mineral Density in Men and Postmenopausal Women - a Cross-Sectional Population Study

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In this study, we wanted to evaluate the relation between serum thyroid stimulating hormone (TSH) and bone mineral density (BMD) in a healthy population based on data from the Tromsø Study, which is a prospective, population based follow-up study in Northern Norway. With an attendence rate of 80%, 8130 men and women aged 30-89 years participated in the 5th Tromsø Study in 2001. All subjects filled in a questionnaire regarding health, medication and life style factors in addition to a basic physical examination including blood samples. A random selection of 5939 persons attended a second study phase including bone mineral density of forearm by SXA (DTX-100; Osteometer Medi Tech, Inc., Hawthorne, CA, USA) and hip by DEXA (GE Medical Systems Lunar). After exclusion of subjects reporting thyroid disease or use of thyroxine, the 2,5 and 97,5 percentiles of TSH were calculated in the remaining 6163 subjects with valid measurements. Quartiles were calculated in the normal TSH range, resulting in 6 different subgroups of TSH. BMD measurements of the femoral neck, trochanter, distal and ultradistal forearm were available in a random sample of 968 men and 993 postmenopausal women after further exclusion of subjects reporting diabetes, cancer, angina, heart attack, stroke, use of statins, antidiabetic medication or antiresorptive drugs. After adjustment for age, weight, height, physical activity score, current smoking and for women also current use of hormonal replacement therapy, TSH levels below the 2,5 percentile predicted lower BMD at all four measurement sites in both sexes, significantly at the ultradistal and distal forearm in postmenopausal women, and at the ultradistal forearm in men (p= .028, .032 and .036, respectively). In postmenopausal women, adjusted BMD at all the four measurement sites were higher in the highest TSH-group, significantly only at the femoral neck (p=.029). In men there was no such association. Within the normal range of TSH, the adjusted BMD was remarkably constant at all measurement sites both in men and postmenopausal women. TSH as a continuous variable did not predict BMD level. In conclusion, we have demonstrated that serum TSH below the 2.5 percentile is a negative predictor at the BMD ultradistal forearm in both sexes, and also at the distal forearm in postmenopausal women. Serum TSH above the 97,5 percentile predicts higher BMD in postmenopausal women, but only at the femoral neck. In contrast to previous studies, we could not find any relationship between TSH and BMD in the normal range at any measurement site, neither in postmenopausal women nor men.

***Disclosures: G. Grimnes**, None.*

P2

ASBMR YOUNG INVESTIGATOR AWARD
Hormone Therapy Enhances the Effects of Physical Activity on The Femur in Postmenopausal Women from the Observational Cohort of the Women's Health Initiative

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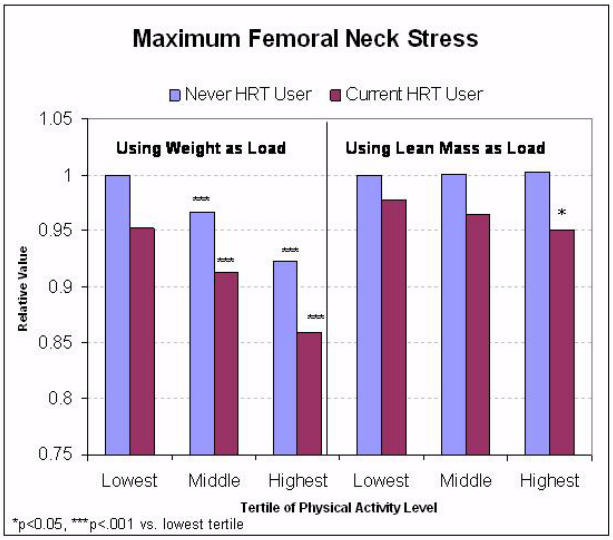
We hypothesized that load stresses in femurs of postmenopausal women would decrease with higher physical activity levels with greater effects with hormone therapy (HT). We studied women over 50 of all races in the observational cohort of the Women's Health Initiative (WHI) study with DXA scans of the hip and total body measured at the Universities of: Alabama at Birmingham, Pittsburgh, and Arizona at Phoenix and Tucson. After excluding 875 past users 2457 current HT users were compared to 2697 never-users after division into activity tertiles using estimates of weekly energy expended in exercise in metabolic units. We simulated a one-legged stance using: an abductor force acting on the superior aspect of the greater trochanter, a joint force through the femoral head and a ground reaction through the knee, adjusted to achieve static equilibrium. Stresses at the infero-medial femur neck at its narrowest point were computed in an engineering model incorporating dimensions and geometry from DXA scans using the Hip Structure Analysis (HSA) method. Stresses for HT groups broken down by tertile of activity level and plotted relative to the least active tertile are shown in the left using body weight as load, and on the right using lean mass. When stresses are computed with body weight they generally decline (indicating strength improvement) with increasing activity and the effect is greater among HT users ($p < 0.001$ for HT and activity effects). When computed using lean body mass (mostly muscle) effect magnitude is eliminated in non HT users and diminished but remains significant among HT users. We conclude that physical activity effects in estrogen-deficient women improve both lean mass and

femur strength, but there is an additional independent effect of activity on femur strength in HT users.



***Disclosures: A. Khaled**, None.*

P3

ASBMR YOUNG INVESTIGATOR AWARD Association between Serum Parathyroid Hormone (PTH) and Total Fat Mass, and Impact of Smoking. The fifth Tromsø Study

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The aim of the present study was to explore the relation between serum PTH and total fat mass, and to assess the impact of smoking. In the fifth Tromsø Study in 2001, 8130 subjects aged 30-89 years participated (attendance rate 80 %). All subjects filled in a questionnaire covering health, smoking habits and physical activity in spare time. Measurements of total body fat, total bone mineral content (BMC) and total lean mass were done in 1555 persons.The percentages of fat, BMC and lean mass were calculated. We had complete data sets for 502 men and 724 women (117 and 199 smokers, respectively). There was a signficant interaction between smoking status and serum PTH regarding measurements of body composition, and smokers and non-smokers were therefore analysed separately. The analyses were perfored with linear regression with age, gender, serum calcium, and physical activity as covariates. In non smokers we found a positive and significant linear trend across increasing PTH quartiles for total fat mass % ($p<0.01$), and a signficant negative linear trend for both total BMC % ($p<0.001$) and total lean mass % ($p< 0.001$). The adjusted mean total fat, BMC, and lean body mass percentages were in the lowest serum PTH quartile 30.5 %, 3.7 %, and 65.8 %, and in the highest serum PTH quartile 32.7 %, 3.5 %, and 63.8 %, respectively. No such trends were seen in the smokers. In conclusion, there is a positive association between PTH and percentage body fat, which seems to be modified by smoking.

***Disclosures: M. Sneve**, None.*

Pathways Controlling Bone Resorption

P4

Matrix Metalloproteinase-12 (MMP-12) Knockout Mice Have Increased Trabecular and Cortical Bone Mass

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Matrix metalloproteinase-12 (MMP-12) is member of a family of Zn2+-dependent endopeptidases that degrade nearly all proteins of the extracellular matrix (ECM). MMP-12 is expressed in hypertrophic chondrocytes and osteoclasts in developing bone. However, there is no reported evidence of a role for MMP-12 in bone mass regulation. MMP-12 knockout (KO) mice were generated (Shipley *et al* 1996 PNAS 93:3942-3946) and bone mineral density (BMD) was evaluated longitudinally up to 20 weeks of age using peripheral dual-energy x-ray absorptiometry (pDXA). Whole body, femoral midshaft and lumbar spine BMDs increased in an age-dependent manner ($p<0.005$) to a greater extent in the MMP-12 KO mice, compared to wild-type littermates. Mice were euthanized at 20 weeks to enable a more detailed analysis of the bone micro-architecture. Trabecular bone volume (BV/TV) of the distal femora was increased in both male (45%) and female (184%) KO mice compared to wild-type littermates (WT) ($p<0.005$), as determined by three-dimensional micro computed tomography (microCT) and histomorphometry. Trabecular thickness and number were also increased. Interestingly, a 6-8% increase in the tissue mineral density of trabeculae was also observed in KO compared to WT mice by microCT. Furthermore, in the femoral shaft, the cross sectional area of cortical bone was also increased by 18% (female) and 26% (male) in KO mice, ($p<0.004$). Surprisingly, MMP-12-deficient mice had less double-labeled trabecular bone surface, suggesting a low bone turnover phenotype. In addition, serum markers of bone formation (osteocalcin) and resorption (CTx, type I collagen degradation products) were significantly lower in KO mice compared to their WT littermates. In summary, these data suggest that the absence of MMP-12 results in a reduction in both bone resorption and bone formation. We hypothesize that bone resorption is reduced to a greater extent compared to bone formation, in MMP-12 KO mice, which results in the increased bone mass and may explain in part, the increased mineral density.

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***Disclosures: J.M. Owens**, None.*

P5

ASBMR YOUNG INVESTIGATOR AWARD
The Negative Role of PDE4 in PTH -Induced Osteoclast Formation.

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Parathyroid hormone (PTH) induces osteoclast formation via expression of TNF-related activation-induced cytokine (TRANCE, identical to RANKL, ODF, and OPGL), and cAMP-PKA signaling cascade mediates this catabolic effect of PTH in osteoblast. Although cAMP concentration is determined by the activities of both adenylate cyclase (AC) and the phosphodiesterase (PDE) enzymes, little is known about the involvement of PDEs in PTH-induced osteoclast formation. Thus we investigated the role of PDEs in PTH-induced cAMP-PKA activation and TRANCE expression, which in turn increase osteoclast formation. To identify the PDE isozymes that regulate TRANCE-mediated osteoclast formation by PTH, various PDE isozyme specific inhibitors were added in coculture system in the presence of PTH. PTH-induced osteoclast formation was dramatically increased only with a presence of a PDE4 inhibitor, rolipram. TRANCE expression was also enhanced when osteoblasts were treated with rolipram in addition to PTH. We previously demonstrated that cyclooxygenase-2 (COX-2) mediates PTH-induced osteoclast formation by regulating the TRANCE expression in osteoblasts. We found that PTH significantly activated the COX-2 promoter in a manner sensitive to deletion of the cAMP-responsive element sequence (CRE) in osteoblasts. Moreover, PDE4 inhibition enhanced the CRE-dependent COX-2 expression by PTH. Finally, further study revealed that PDE4 exclusively degrades intracellular cAMP increased by stimulus of PTH in osteoblasts. Taken together, we conclude that PDE4 negatively regulates PTH-induced osteoclast formation via cAMP-PKA-COX-2-TRANCE signaling axis.

***Disclosures: H. Park**, None.*

P6

ASBMR YOUNG INVESTIGATOR AWARD
BMP Signaling Controls both Bone Formation and Resorption for Remodeling in vivo. ~Genetically Activated BMP receptor using Tamoxifen Inducible System~

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Bone morphogenetic proteins (BMPs) were discovered as bone inductive growth factors due to their ability to induce ectopic bone formation when transplanted into soft tissue. In vitro studies using primary osteoblasts or osteoblastic cell line have revealed the osteogenic function of BMPs, and several including BMP2, BMP4, BMP6 and BMP7 have been shown to induce ectopic bone formation. Although this ectopic bone formation follows endochondral ossification, which occurs mostly in long bone, it is unclear how these proteins affect regular long bone formation during developmental stages and bone remodeling during adult life. BMPs phosphorylate Smads through BMP type I receptors, and BMPRIA (ALK3) and ACVRI (ALK2) are the most active forms of this receptor in bone. To reveal the function of Smad-dependent BMP signaling in bone remodeling, we generated mouse lines using the Cre-loxP system that conditionally express constitutively activated Bmpr1a (caBmpr1a) or Acvr1 (caAcvr1). Since both caBmpr1a and caAcvr1 have mutations in the GS domain, tissues will receive ligand-independent Smad signaling after Cre expression. Using a 3.2 kb promoter of type I collagen with Cre-ERTM, we can control the timing of the constitutive activation in osteoblasts in long bones at different life stages by administration of tamoxifen. When caBmpr1a was induced at adult stages, gross morphology, body size, and body weight were normal compared with controls. Histological analysis revealed that long bones of caBmpr1a mice appeared to be similar to controls, and bone density measured by micro CT showed no significant difference in the long bones of caBmpr1a mice compared to controls. However, expression levels of both bone formation and resorption markers were elevated in caBmpr1a mice. Expression of Rankl was significantly increased and the ratio of Rankl to Opg was also increased in caBmpr1a mice. Similar results were observed when caAcvr1 was induced in adults. These results suggest that activation of the Smad pathway in osteoblasts upregulates both bone formation and resorption in vivo, and bone morphology appears to be unchanged, perhaps due to the proper balance between bone formation and resorption. Because bone formation is not predominant in long bone in caBmpr1a mice, this observation suggests that the effect of BMPs on osteoblasts is different between remodeling in long bone and formation of ectopic bone.

***Disclosures: N. Kamiya**, None.*

P7

ASBMR YOUNG INVESTIGATOR AWARD
Integrated Responses of the Hypothalamic-Pituitary-Gonadal Axis Hormones, FSH and Inhibins, on Human Osteoclastogenesis

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We have demonstrated that decreased serum Inhibin A (InhA) and InhB levels correlate with markers of increasing bone turnover, regardless of changes in sex steroids or follicle stimulating hormone (FSH) in pre-, peri-, and post-menopausal women. In fact, serum InhA is a better predictor of bone formation and resorption markers in premenopausal women than either FSH or bioavailable E2. These correlations with bone turnover are consistent with our finding that Inh suppresses both osteoblast and osteoclast (OCL) development in vitro. Others have recently reported a direct stimulation of OCL differentiation by FSH. Given

that both FSH and Inh are endocrine hormones in the hypothalamic-pituitary-gonadal (HPG) axis, we sought to understand the integrated/net effect of changes in FSH and Inh exposure on OCL development. Using human cultures consisting of either purified CD14+/CD11B+ cells or whole unselected peripheral blood mononuclear cells (hPBMCs), a cell-type specific effect of FSH on OCL differentiation was observed. In purified human CD14+/CD11B+ cells a 40% increase in RANKL-dependent osteoclastogenesis was observed following FSH stimulation, as previously reported. However, when cultures of whole hPBMCs were treated with FSH, RANKL-dependent OCL differentiation was suppressed by >50%. These data suggest that the selection of CD14+/CD11B+ cells from hPBMCs isolates a pool of FSH-responsive progenitors that cultured alone demonstrate FSH enhancement of RANKL-induced OCL differentiation. In contrast, cultures of normal hPBMCs contain a mixed population of unselected cells whose integrated paracrine/autocrine response to FSH results in a significant decrease in RANKL-induced OCL formation. In addition, treatment of FSH treated PBMC cultures with InhA, the primary endogenous regulator of FSH secretion, significantly enhanced FSH suppression of RANKL-induced OCL formation. The addition of the soluble form of the Inh-specific receptor betaglycan to PBMC cultures abrogated only the InhA-induced suppression in OCL differentiation, and not the FSH-mediated decrease. In vivo, osteoclastogenesis is the result of integrated responses to a variety of stimuli. Although a sub-population of PBMCs capable of a stimulatory response to FSH exists, these data suggest that, the net effect of FSH on OCL development in intact heterogeneous cell populations is likely to be suppressive, which can be further suppressed by Inh. These data provide insight into the action of FSH and InhA on OCL, and demonstrate the important role of the non-steroidal hormones of the HPG axis on the regulation of OCL formation.

***Disclosures: K.M. Nicks**, None.*

P8

Multifunctional protein FUS is a p38-dependent positive co-activator of MITF

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The microphthalmia-associated transcription factor (MITF) is required for terminal osteoclast differentiation and is a target for signaling pathways engaged by colony stimulating factor (CSF-1) and receptor-activator of nuclear factor-kappaB ligand (RANKL). Activation of MITF leads to an increase in osteoclast-specific gene expression. To clarify the mechanism by which MITF transcriptional activity is regulated, we focused our attention on MITF-interacting proteins. Using MALDI-TOF mass spectrometry we identified FUS, a nuclear protein with RNA-binding motifs and putative transcriptional activation domain. We confirmed the specificity of the interaction between FUS and MITF by GST pull down assay and co-immunoprecipitation followed by Western blotting. The functional contribution of FUS-MITF binding, using the RAW 264.7 C4 preosteoclast cell line stably expressing FLAG-MITF, was demonstrated, showing that interaction of FUS-MITF binding dramatically increased after RANKL/CSF-1 treatment. The ability of FUS to affect MITF transcriptional activity using Q RT PCR assays was shown when co-expression of FUS/shFUS led to increase/decreases activation of MITF target gene expression. Additionally, FUS and MITF interaction on chromatin remodeling complexes using CHIP assay was performed in bone marrow cells treated with CSF/RANKL. Analysis of MITF-FUS interaction domain revealed that C-terminal domain of FUS is necessary for such interaction and depends on MITF phosphorylation on Ser 73 and Ser 307. Surprisingly, catalytic subunit of the ATPase SWI/SNF complex, BRG-1 promotes FUS-MITF interaction and rescued interaction of FUS-MITFS73A but not FUS-MITFS307A. Finally we show that phosphorylation of MITF on 307Ser depends on p38 binding to the docking sites on MITF. Binding p38 to MITF and subsequent phosphorylation Ser 307 increases co-binding of MITF with BRG-1 and FUS. The results presented here show FUS as a novel interacting partner of MITF. Ability of FUS to affect MITF's activity is cytokine dependent and reveals that FUS play a critical role in promoting the assembly of proteins required for the formation of the transcription initiation complex and chromatin remodeling.

***Disclosures: A. Bronisz**, None.*

P9

ASBMR YOUNG INVESTIGATOR AWARD
Analysis of Signal Dependent Epigenetic Modulation of Osteoclast Specific Gene *Cathepsin K* During Osteoclast Differentiation

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Transcription factors NFATc1, PU.1 and MITF collaborate to regulate specific genes in response to CSF-1 and RANKL signaling during osteoclast differentiation. OCL precursors exhibit association of MITF and PU.1 at *Cathepsin K* promoter in absence of RANKL stimulus but without transcriptional activity. This inactive state of *Ctsk* promoter was partly explained by interaction of MITF and PU.1 with an *Ikaros* family zinc finger transcriptional repressor Eos. Eos formed a complex with MITF and PU.1 and suppressed transcription through recruitment of co-repressors CtBP and Sin3A. The combination of RANKL and CSF-1 concurrently increased the levels of phospho MITF, p38 MAP Kinase and SWI/SNF chromatin remodeling complexes bound to these target promoters while alleviated the association of Eos and co repressors. These results indicated epigenetic modulations might control the transcription of cell specific genes such as in closely related cell types such as macrophages and osteoclasts.

Here using ChIP and qPCR based tiling array we demonstrate that changes in histone signature during osteolcast differentiation precedes recruitment of activators and dissociation of repressors.ChIP assays were performed with three antibodies that recognize specific histone H3 modifications such as phospho tri methyl modification at T3/ K4, T22/K24, tri methyl phospho modifications at K9/S10; K27/S28 and mono methyl K4 modification. Bone marrow precursors both non treated or treated for 0.5 day and 3 days were used for ChIP assays. Pulled chromatin was analyzed by real-time PCR using four sets of oligonucleotides that spanned approximately 2.5 kb of the *CtsK* promoter. In non treated murine OCL precursors the *CtsK* promoter exhibited relatively high enrichment of K4 mono methylated H3 when the gene was in repressed state. The treatment of RANKL for 0.5 days showed a 50% reduction in this mark at sites that harbor MITF/ PU.1/EOS sites and 3' regions. This mark spread to the distal region 5' of MITF/PU.1 binding sites by 3 days of RANKL treatment when the *CtsK* gene expression is highly induced. A rapid increase in enrichement by 4-6 fold was observed with phospho trimethyl marks during by 0.5 days during differentiation which correlated well with the reduction in transient repressive mark of trimethyl phospho modification. These changes in histone signature preceded the changes in association of corepressor-coactivators during differentiation with Cathepsin K promoter.

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

P10

MicroRNA gene expression profiles during murine osteoclast differentiation

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MicroRNAs (miRNAs) are endogenous 22 to 25 nt non-coding RNAs that have been implicated to control many aspects of cellular functions such as development, differentiation, proliferation, apoptosis, metabolism and hematopoiesis. Mature miRNAs negatively regulate target gene expression at the post-transcriptional level, and it is estimated that miRNAs may regulate up to 20-30% of the protein-coding genes. Expression of miRNAs has been shown tissue specific patterns and time dependent dynamics. These expression patterns and dynamics are providing insights into their possible functions. Hematopoiesis is a highly regulated multistage process where a pluripotent self-renewing hematopoietic stem cell gives rise to all blood cell lineages. MiRNAs play important roles in normal hematopoiesis and hematopoietic malignancies. Macrophages can be differentiated to osteoclasts, which play an important role in bone remodeling. Despite accumulating information for the role of miRNAs in hematopoiesis, miRNA expression profile during osteoclast differentiation has not been explored. In this study, we investigated the expression profile of miRNAs in RAW264.7 cells treated with or without recombinant receptor activator of nuclear factor Kappa B-ligand (RANKL) using microRNA microarrays. Our studies showed that 42 of 380 miRNAs were differentially expressed during osteoclast differentiation. 15 of them were further validated by Northern blots, including miR-233 and miR-146. miR-223 was drastically down-regulated during differentiation, and miR-146 was upregulated at early stage but down-regulated at late stage. Our data indicated that miRNAs are probably involved in osteoclast development. Further experiments are needed to define the function of these regulated miRNAs in regulating osteoclastogenesis.

***Disclosures: Q. Mi**, None.*

Anti-resorptive Agents

P11

PADRE-RANKL Vaccination to Treat Osteoporosis

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The TNF superfamily member RANKL regulates osteoclast activity and bone resorption and its over-production results in the pathological bone loss that characterizes osteolytic diseases. We are developing a vaccine against autologous RANKL that bypasses immunological tolerance and induces antibodies that effectively neutralize endogenous RANKL. This can be achieved using the AutoVac™ technology where immunodominant foreign T helper cell epitopes are inserted into recombinant human proteins while retaining their tertiary structure.

Recombinant human RANKL was produced with the highly immunogenic universal PADRE (Pan HLA-DR Epitope) epitope inserted into the primary sequence. Structural integrity of the PADRE-RANKL trimer protein was determined by CD spectroscopy, light scattering and MALDI-TOF MS while biological activity was measured as a function of *in vitro* osteoclastogenisis. Mice and ovarectomized monkeys were immunized with PADRE-RANKL formulated with adjuvants and antibody responses were analysed using competitive ELISA and inhibition of osteoclastogenesis.

Modification of recombinant human RANKL resulted in a PADRE-RANKL protein that was correctly folded, trimeric and glycosylated. PADRE-RANKL immunizations induced antibodies capable of neutralizing the function of human RANKL *in vitro*. The *in vivo* effect of the antibodies on bone degradation is under study in ovarectomized monkeys.

We successfully designed, produced and tested a human PADRE-RANKL protein for use as a vaccine. Immunogenicity testing in rodents and monkeys support our view that tolerance can be bypassed using proteins modified through this approach. Active immunization using PADRE-RANKL AutoVac™ vaccine is a novel immunotherapeutic approach for the treatment of bone loss diseases.

***Disclosures: T. Bratt**, Pharmexa 3.*

P12

ASBMR YOUNG INVESTIGATOR AWARD
Mechanism of N-containing bisphosphonate effects on the actin cytoskeleton of osteoblastic cells

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Cytoskeletal elements are critical for cell shape and motility, for intracellular transport and for signal transduction. The actin cytoskeleton is stabilized by the small GTP-binding protein, RhoA. A lipid modification, geranylgeranylation, allows RhoA to anchor to the cell membrane and become activated by interaction with the guanine nucleotide exchange factor, Rho-GEF. N-containing bisphosphonates/ aminobisphosphonates could potentially affect the cytoskeleton since they block steps in the mevalonate pathway that are critical for the synthesis of geranylgeranyl groups. To investigate the mechanism of the effects of a N-containing bisphosphonate on the osteoblast actin cytoskeleton, we determined the responses of actin stress fibers and the edge actin bundle of MC3T3-E1 cells to the bisphosphonate alendronate in comparison with those to a geranylgeranyl transferase I inhibitor, GGTI-2166, and a Rho kinase inhibitor, Y-27632. Cells were stained with rhodamine phalloidin to visualize the actin cytoskeleton and examined by confocal microscopy. Actin stress fibers and the edge actin border were quantified by a program developed with Matlab® software. GGTI-2166, Y-27632 and alendronate all significantly decreased actin stress fibers. GGTI-2166 (3-30 uM) and Y-27632 (1-30 uM) had significant inhibitory effects within 6 hr. Alendronate (50 uM) significantly decreased actin stress fibers after 22 hr incubation. None of the treatments affected the actin edge border, cell area or circularity (relationship between the area and the perimeter). The geranylgeranyl group donor, geranylgeraniol (40 uM), antagonized the effects of alendronate, but did not significantly affect the responses to GGTI-2166 or Y-27632. Constitutively active RhoA antagonized the effects of alendronate and GGTI-2166, but not those of Y-27632. The effects of alendronate were slightly but significantly antagonized by PTH 3-34 amide (500 nM), but not by PTH 1-34 (30 nM). The results indicate that the bisphosphonate alendronate has effects on the internal actin cytoskeleton of osteoblastic cells, and that these effects involve geranylgeranyl groups and RhoA. The response shows only a limited interaction with parathyroid hormone peptides. In view of the importance of the actin cytoskeleton, the findings constitute further evidence that N-containing bisphosphonates, when they attain certain concentrations, have effects on osteoblasts that could influence bone remodeling.

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

P13

ASBMR YOUNG INVESTIGATOR AWARD
Low Energy Femoral Diaphyseal Fractures Associated with Prolonged Alendronate Use: A Case-Control Study

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Purpose: Recent evidence has suggested that bisphosphonates cause accumulation of microdamage through severe suppression of bone turnover, potentially compromising bone strength. Data on the long-term effects of bisphosphonate use are unknown. The purpose of this study was to elucidate a correlation between prolonged alendronate use and low energy subtrochanteric/shaft fractures in postmenopausal women.
Methods: We performed a retrospective case-control study of postmenopausal women who presented with low energy femoral fractures from 2000-2007. Forty-one subtrochanteric/ shaft fracture cases were identified, and matched by age, race and body mass index (BMI) to 82 hip fracture controls, one intertrochanteric and femoral neck fracture each. Patients with co-morbidities or taking medications known to affect bone metabolism were excluded.
Results: Bisphosphonate use was observed at a rate of 36.6% in subtrochanteric/shaft fracture cases, compared to 11% in hip fracture controls (odds ratio (OR), 4.68 [95% CI 1.83-11.98]; *P*=.001). Alendronate was the bisphosphonate prescribed in each of the cases, different from published estimates of proportion of bisphosphonates use (*P*=.042). Alendronate was significantly associated only with cases, logistic regression (*P* =.003). A common x-ray pattern was identified in cases on alendronate, defined as a simple oblique fracture with cortical thickening and beaking of the cortex on one side. This x-ray pattern was highly associated with alendronate use (OR, 15.33 [95% CI 3.06-76.90]; *P*<.001). Ten patients with the x-ray pattern had an average duration of alendronate use of 7.3 years, compared to 2.8 years for those on alendronate without the x-ray pattern (*P* <.001). Wilcoxon survival analysis yielded significantly different cumulative survival without fracture between these two groups (*P* =.003). Duration of alendronate use in subtrochanteric/shaft cases differed from both hip fracture controls (*P* =.001).
Conclusions: We found that a greater percentage of patients with uncommon low energy fractures were receiving alendronate therapy than intertrochanteric and femoral neck fractures. Clinical data confirm that prolonged bisphosphonate treatment prevents hip fractures, but a small subgroup of patients may be susceptible to long-term effects of bisphosphonates by developing subtrochanteric/shaft fractures. Additional studies are needed to confirm whether prolonged bisphosphonate use increases the risk of these fractures.

***Disclosures: B.A. Lenart**, None.*

P14

ASBMR YOUNG INVESTIGATOR AWARD
Osteoclast Inhibitory Peptide-1 (OIP-1/hSca) Binding to FcRγ Membrane Receptor Results in Inhibition of Osteoclast Differentiation

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We previously characterized the osteoclast inhibitory peptide-1 (OIP-1/hSca) as an autocrine/paracrine inhibitor of osteoclast differentiation. OIP-1 is also known as TSA-1, a member of Ly-6 gene family. We have recently demonstrated that mice targeted with the OIP-1/hSca expression to the osteoclast lineage develop osteopetrosis bone phenotype. OIP-1 c-peptide region is critical for osteoclast (OCL) inhibitory activity; however a cognate receptor/membrane protein which interacts with OIP-1 is unknown. Evidence suggests a functional physical association between TSA-1 and Fc gamma receptor II B (FcRγ) on the surface of activated B-cells. Immunoreceptor tyrosine-based activation motif (ITAM) bearing adapter proteins such as FcRγ and DAP12 play a critical role in OCL development. We therefore, hypothesized that OIP-1 binding to FcRγ on osteoclast precursor cells inhibit OCL differentiation. We examined binding of the OIP-1 c-peptide to RAW 264.7 osteoclast progenitor cells using FACS analysis. Fluorescein conjugated OIP-1 c-peptide (10 uM) binds to these cells indicating the presence of a surface receptor or membrane protein partner in these cells. Co-immune precipitation and subsequent mass spectrometric analysis identified OIP-1 associated to FcRγ expressed in RAW264.7 cells. Confocal microscopy analysis demonstrated co-localization of fluorescein conjugated OIP-1 c-peptide with FcRγ expressed on the cell membrane in osteoclasts formed in RAW 264.7 and OIP-1 transgenic mouse bone marrow cultures. An ELISA binding assay confirmed that the OIP-1 c-peptide forms a 1:1 complex with recombinant FcRγ protein characterized by an equilibrium dissociation constant of K_d = 5 ± 1 uM. We further examined if OIP-1 signals through FcRγ to inhibit OCL differentiation. siRNA suppression of FcRγ expression in RAW 264.7 cells rescued OIP-1 c-peptide inhibition of RANKL stimulated OCL differentiation in vitro. OIP-1 mouse derived preosteoclast cells demonstrated significant inhibition of ITAM phosphorylation of FcRγ (6-fold) but not DAP12 protein. Also, OIP-1 mice demonstrated significant inhibition of Syk tyrosine kinase (4.5-fold) activation compared to wild-type mice. These results indicate that OIP-1 is an important physiologic regulator of osteoclast development and may have therapeutic utility for bone diseases with high bone turnover such as osteoporosis and Paget's disease of bone.

***Disclosures: S. Shanmugarajan**, None.*

Pathways Controlling Bone Formation

P15

ASBMR YOUNG INVESTIGATOR AWARD
Ultrasound Stimulated Human Osteoblasts release ATP which may Play a Role in Bone Healing

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The aim of this study was to determine whether the effects of low intensity pulsatile ultrasound on bone healing are due to osteoblastic release of ATP (Adenosine Triphosphate).
Ethical committee approval was obtained prior to commencing the study (REC reference number 05/Q1410/13 on 21st March 2005). Bone shavings from the tibial and femoral cuts of 8 patients with osteoarthritis undergoing total knee replacement were collected. These were cultured in dulbecco's modified eagles medium and passaged using trypsin once confluent.
Samples from serum starved specimens were collected before ultrasound stimulation and then every 5 minutes on commencing stimulation (till the 20th and then 40 minute marks). For controls the ultrasound probe was applied without stimulation. ATP levels were determined using the Luceferin and Luciferase assay.
In order to assess whether ATP and ultrasound had similar effects on bone formation, test specimens were exposed to ultrasound stimulation or 100 μML of ATP (ultrasound controls had the probe applied without stimulation whilst ATP controls had serum free medium added). Total P1NP (procollagen type 1N-terminal propeptide) and OPG (osteoprotegerin) levels were determined after 16 hours incubation.
There was a significant increase in ATP release from the ultrasound stimulated cells compared to controls (p-value of 0.036).
The mean P1NP levels on ultrasound stimulation and ATP exposure were 203.3 ng/ml (176.6 ng/ml in controls) and 150.4 ng/ml (120 ng/ml in controls). These differences were statistically significant with p values of .024 and .042 respectively.
The mean OPG levels on ultrasound stimulation and ATP exposure were 34.6 pmol/L (11.7 pmol/L in controls) and 21.9 pmol/L (16.9 pmol/L in controls). These differences were statistically significant with p values of .020 and .041 respectively.
These results confirm that ultrasound stimulation of osteoblasts releases ATP. In addition ultrasound and ATP increase the levels of total P1NP and OPG which may reflect an increase in bone mass formation. We therefore suggest that low intensity pulsatile ultrasound might act through ATP release to promote bone healing.

Test Group	Mean OPG	Mean P1NP	Mean % ATP change
Ultrasound stimulation	34.6 pmol/L	203.3 ng/ml	31.6
Ultrasound Probe only	11.7 pmol/L	176.6 ng/ml	-14.14
ATP 100μML	21.9 pmol/L	150.4 ng/ml	
Serum free medium	16.9 pmol/L	120 ng/ml	

***Disclosures: B.K. Mwaura**, Wrightington Hospital Research and Development Department 2.*

P16

Wnt5a a Context Dependent Stimulator of the Canonical Signaling Pathway Through LRP5 and its High Bone Mass Variants

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Wnts can provide instructive cues for the recruitment, maintenance, and differentiation of precursor cells in the bone micro-environment. Though Wnt/LRP5-signaling has been recognized as a central player in bone mass accrual, the specific Wnt(s) responsible for signaling through LRP5 in bone remain elusive. Here we focus on Wnt5a; a prototypical non-canonical Wnt-ligand expressed in human bone, and we describe a canonical signaling mechanism involving LRP5 and its high-bone mass variants.

Expression of Wnt5a and Fzd4 in adult human bone was determined by qRT-PCR analyses. All *in-vitro* studies were performed in a HEK293 cell background using stable or transient over-expression of LRP5/6, Fzd4 and Topflash reporter measuring Lef/TCF activity. Interestingly, Wnt5a antagonized Wnt3a in HEK293 cells and blunted Wnt3a induced canonical signaling activity (Topflash). However, we found that transient transfection and overexpression of both Fzd4/LRP5 conferred Wnt5a ability to dose-dependently induce canonical Lef/TCF signaling activity as also reported elsewhere recently (PloS Biology: 2006, 4:570). This effect was specific for LRP5 as parallel Fzd4/LRP6 overexpression did not confer Wnt5a canonical signaling activity. As expected, the canonical Wnt signaling inhibitor and LRP5/6 ligand, Dickkopf-1 (DKK1) potently antagonized Wnt5a activity through LRP5/Fzd4. Importantly, over-expression of the LRP5-high bone mass mutants (LRP5-HBM; G171R or A242T) also conferred Wnt5a canonical signaling activity with impaired sensitive to the inhibitory activity of DKK1. Domain-swapping of LRP5 and LRP6 propeller domains was used to more closely define the functional receptor domains involved in Wnt5a signaling. β-propeller (I+II) domain of LRP5/6 was swapped onto β-propeller (III+IV+Intracellular) domain of LRP6/5, respectively. Overexpression of domain-swapped LRPs indicated that the Wnt5a induced canonical signal is mediated through LRP5 (I+II) domain as LRP6 (I+II) domain eradicated Wnt5a canonical signaling activity, whereas conversely the LRP6 chimera conferred Wnt5a signaling activity via the LRP5 (I+II) domain.

In conclusion we describe context specific canonical Wnt5a activities depending exclusively on LRP5 (I+II)/LRP5-HBM and Fzd4. In agreement with the expression of Wnt5a in human bone tissue this finding may point to a delicate role of the specific ligand/receptor complex and a potential relation to the LRP5-HBM/DKK1 signaling system.

***Disclosures: N. Wei**, None.*

P17

Osteocyte sclerostin expression differs from control in both hip fracture and OA: contrasting effects on osteoid mineralising activity.

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Osteocytic sclerostin down-regulates bone formation. We showed (Jordan, 2003) that osteoarthritis of the hip (OA) is associated with increased bone formation in the femoral neck while femoral neck fracture (FNF) is associated with a reduced osteoblast response to increased bone resorption (Bell, 2000). To investigate whether sclerostin (scl) might mediate these effects, we analysed osteocytic scl expression in osteonal bone multicellular units (BMUs) in the femoral neck of patients with hip OA (5M, 5F; 49-92y), or FNF (5M, 5F; 73-87y) and post-mortem (PM) controls (5M, 6F; 61-90y). Scl expression as evidenced by immuno-cytochemistry was assessed between the cement line and the canal surface in 623 whole BMUs. Adjacent sections were reacted for alkaline phosphatase activity (ALP) and each BMU classified as quiescent (no ALP), low or high ALP expression (threshold: 50% osteoid surface ALP positive). Osteocytic expression of sclerostin and distance from the canal surface were recorded for each osteocyte. Scl expression differed between BMUs categorised by bone formation (quiescent: 90.0 +/- 1.5% (SE); low: 77.2 +/- 2.5%; high: 51.7+/- 2.8%; $p<0.0001$) and between the 3 subject groups (OA: 67.5 +/- 2.2%; FNF: 76.5 +/- 2.1%, PM: 75.1 +/- 2.7%; $p=0.007$). The disease effect was most evident in the BMUs with high ALP (OA: 38.9 +/- 4.0%; FNF: 63.6 +/- 4.1%; PM:52.6 +/- 6.0%; $p=0.007$). Forming BMUs showed greater mean distances of scl+ osteocytes from canal surfaces (respectively +14%, +7% compared to PM controls for OA and FNF: Dunnett's test $P<0.0001$); but these differences were much greater (+56%, +42% $P<0.0001$) for scl- osteocytes. In logistic modelling, the presence in the BMU of strong ALP activity at BMU level was highly dependent on the fraction of osteocytes that were scl-. This effect was significantly larger in OA; but in FNF was smaller than in controls (P both<0.0001). Osteocytes were grouped into quartiles according to distance from their osteoid surface. Scl-osteocytes in all quartiles had similar statistical effects on ALP expression. In conclusion, sclerostin expression is an important statistical determinant of mineralizing activity at BMU level possessed by all the BMU's osteocytes. But the failure in FNF of osteoid to express ALP appropriate to the % expressing scl demands explanation. In hip OA scl suppression might modulate, in part at least, the increased turnover and changes in bone tissue balance we previously observed in the femoral neck.

***Disclosures: J. Reeve**, Amgen 5.*

P18

Impaired Leptin Action in Osteoporotic Human-Mesenchymal Stem Cells (MSCs) Enhances Adipogenesis

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The bone marrow contains mesenchymal stem cells (MSCs) that can differentiate to the osteogenic and adipogenic lineages. In age-related osteoporosis, decreased bone volume is accompanied by increased adipose tissue in marrow, implying the adipogenic process in bone loss. Previously, we showed that leptin action on h-MSCs inhibits cells differentiation to adipocytes. The aim of the present report was 1) to study the direct effect of leptin on adipogenic differentiation of control (c-) and osteoporotic (o-) MSCs; 2) to determine leptin and leptin soluble receptor (Ob-sR) availability in bone marrow samples, and 3) to measure leptin in serum samples. Postmenopausal women (65-75-years old), healthy (except for hip fracture) consented for a bone marrow sample obtained from the iliac crest during indicated surgical treatment. A blood sample was obtained and osteoporosis or normal status was defined by DEXA measurement. MSCs were isolated from the bone marrow samples and cultured in DMEM-10% fetal serum, at 37°C and 5% CO_2. Cells were subjected to adipogenic differentiation for specified period, in the presence or absence of 62.5 nM leptin. The number of adipocytes, the content of PPAR gamma protein and mRNA, and leptin mRNA were measured by flow cytometry, Western blot, and RT-PCR, respectively. Leptin and Ob-sR were directly measured in the bone marrow 990 x g supernatant and in serum samples using a commercial kit.

Results indicate that c-MSCs and o-MSCs differ in their adipogenic potentials as shown by the expression of active PPAR gamma protein. Leptin exerted an antiadipogenic effect only on c-MSCs, increasing the proportion of inactive phosphorylated PPAR gamma protein. Results suggest abnormal adipogenesis in o-MSCs not only because of increased adipocyte number, but of impaired leptin cells response. Moreover, leptin concentration both in serum and bone marrow was lower in osteoporotic samples than in control, while the Ob-sR level was similar in marrow samples from both types of donors. Results showed that serum leptin level positively correlates with t-score and body mass index (BMI). Soluble leptin receptor level showed positive correlation with BMI, but not with t-score.

Altogether, these results indicate that osteoporosis may be associated with decreased leptin sensitivity and add support to the proposition that leptin could mediate the protective effect of fat on bone tissue.

Disclosures: J.P. Rodriguez, *None.*

P19

Vitamin D3 induced antiapoptotic PI3K/Akt signaling in osteoblasts

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1 alpha,25 dihydroxyvitamin D3 (1,25D) actions in bone involve suppression of osteoblast apoptosis via rapid activation of PI3K kinase. However, the signaling remains only partially understood. We investigated here a nongenomic Akt survival cascade downstream of 1,25D stimulation of PI3K in ROS 17/2.8 and SaOS-2 cells, which express an osteoblast phenotype. We measured a dose and time-dependent 1,25D induction of Akt phosphorylation, with maximal response achieved with 10 nM 1,25D after 5 min. We found that staurosporine (STSP)-induced apoptosis was significantly reduced in 1,25D-pretreated osteoblasts. 1,25D-induced protection against apoptosis was abolished when cells were preincubated with inhibitors of PI3K activation. By means of siRNA silencing, we demonstrated that 1,25D induction of Akt phosphorylation requires a classic vitamin D receptor (VDR) located in the cell cytoplasm. Furthermore, non-osteoblastic CV-1 cells transfected with an EGFP-VDR construct responded to 1,25D treatment with a rapid Akt response associated with increased cell survival not detected in native, non transfected cells. We measured increased levels of the Akt substrates p-Bad and p-FKHR after 10 and 30 min of treatment with 10 nM 1,25D respectively, and significantly reduced activity of effector caspases 3/7 in osteoblasts pre-treated with hormone for 60 min. 1,25D-induced protection against apoptosis was abolished when osteoblasts were preincubated with Pertussis toxin. We conclude that nongenomic antiapoptotic effects of 1,25D occur via activation of a PI3K/Akt survival pathway that includes phosphorylation of multiple substrate proteins within one hour. The hormone appears to act via a cytoplasmic VDR that couples to a Pertussis toxin-sensitive G protein. Pro-survival effects of 1,25D in osteoblasts may explain in part its bone anabolic functions.

Disclosures: L.P. Zanello, *None.*

P20

ASBMR YOUNG INVESTIGATOR AWARD
Osteoclasts, independent of bone resorption, produce a factor activating wnt signalling and nodule formation by MC3T3-E1 preosteoblastic cells.

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Some osteopetrotic mutations (chloride channel ClC-7 and osteoclastic V-ATPase) lead to low bone resorption, increased numbers of osteoclasts and increased bone formation, findings which are not in line with the normal perception of bone formation being tightly coupled to bone resorption. In contrast, these findings indicate that the osteoclasts independent of their resorptive activity could be sources of anabolic signals for the osteoblasts.

We investigated whether osteoclasts secrete bone anabolic signals, and we elucidated the nature of the anabolic factor.

Conditioned media from mature human osteoclasts cultured on either bone slices or plastic were collected. Measuring TRACP and CTX-I validated osteoclast maturity and resorption. Conditioned media were applied to cultures of MC3T3-E1 preosteoblasts, followed by bone formation assessment by Alizarin red and Von Kossa staining after 20 days' culture. We assessed changes in transcription of osterix mRNA using a lightcycler. We assessed key osteoblast regulatory pathways by using UMR106.01 cells transiently transfected with reporter constructs. These were the TOPFlash vector, with 8 TCF/LEF response elements, the osteocalcin promoter with x6 tandem OSE repeats (6 x OSE), NFAT, AP-1 and NFkappaB.

Conditioned media from osteoclasts cultured on both bone and plastic stimulated nodule formation by the MC3T3-E1 cells to levels comparable to stimulation with 10ng/mL BMP-2, and it induced the expression of osterix mRNA. Conditioned media from osteoclasts cultured on both bone and plastic specifically induced a 4-7-fold activation of the TCF/LEF response system corresponding to induction by 20ng/mL of Wnt3A. The Wnt3A and conditioned medium signals were equally inhibited by addition of either 100ng/mL of DKK1 or 1mg/mL SOST, consistent with activation of the canonical Wnt signaling pathway. No activation of the OSE, NFAT, NFkappaB, or AP-1 reporters was detected, suggesting specific wnt activity.

In conclusion, we present evidence that osteoclasts, independent of their resorptive activity, secrete an activity that stimulates osteoblastic bone formation. Thereby novel anti-resorptive strategies that do not affects this signaling, may in contrast to traditional interventions assure inhibition of bone resorption without the secondary negative effects on bone formation, leading to a continuous positive calcium balance.

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

P21

Ccrn4l, a gene induced during adipose differentiation, impacts both cortical and trabecular bone compartments.

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Several studies have shown that marrow adiposity increases with age and is higher in patients with an osteoporotic fracture. While it is clear that osteoblasts and adipocytes arise from the same stem cell population, the factors involved in commitment of these Marrow Mesenchymal Stem cells (MSC) have not been completely elucidated. The CCR4 carbon catabolite repression 4-like gene (*Ccrn4l*), also known as *Noc*, is located on the third chromosome in mice and codes for the protein Nocturnin, a deadenylase thought contribute to circadian regulation. This gene is widely expressed including in bone marrow, liver and adipose tissue. Importantly, male *Ccrn4l*$^{-/-}$ knockout mice are resistant to diet induced obesity. On a high fat diet, these mice also exhibit an altered diurnal pattern of expression of Peroxisome Proliferator-activated Receptor Gamma (*Pparg*), a key regulator of MSC commitment, thus suggesting that *Ccrn4l* may have a role in skeletal turnover and marrow adiposity. Indeed, we have found by both Real Time PCR and by Western blot, that *Ccrn4l* is not expressed in undifferentiated 3T3-L1 cells, but when these cells are induced to differentiate into adipocytes, *Ccrn4l* expression is markedly enhanced. We have also found that female *Ccrn4l*$^{-/-}$ mice have increased femur length (control=14.7mm vs. null=15.2mm, p=0.0091) and increased body weight (control=19.22g vs. null=20.93g, p=0.0088) when fed a normal chow diet. In addition, these mice have greater femoral bone volume (control=18.2mm^3 vs. null=19.2mm^3, p=0.0118) and increased total femoral cortical bone density (control=1.062mg/mm^3 vs. null=1.077mg/mm^3, p=0.06) compared to age matched wildtype controls, even after correction for body weight and femur length. The BV/TV% of the distal femur in female *Ccrn4l*$^{-/-}$ mice is also increased, as compared to controls (control=3.43% vs. null=4.29%, p=0.06). These studies suggest that the *Ccrn4l* gene may play a key role in bone turnover, and that its relationship to metabolic factors underlies the important interaction between nutrient status and skeletal acquisition.

***Disclosures: C.L. Ackert-Bicknell**, None.*

P22

Static tension synergize rhBMP-2-induced differentiation of C2C12 myoblasts into osteoblast lineage

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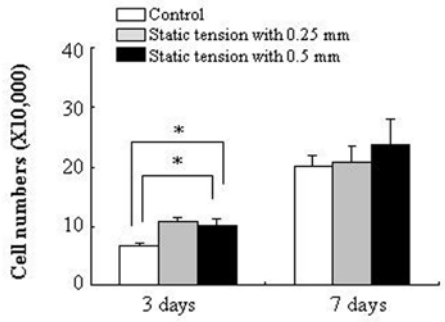
The purpose of this study was to evaluate the effect of static tension force on the differentiation of the mesenchymal cell line C2C12 into osteoblast lineage. C2C12 cells were cultured under continuous static tensile strain. To provide such strain to cells, BioFlex® culture plate was modified, where culture plate and base plate were fixed with by two distraction devices. The flexible membrane of each well in BioFlex® plate could be stretched as a reverse action of distractor (0.25 mm vertical movement/turn), and this tension was continuously applied during cell culure period. Cell proliferation was assessed by cell counting at 3 and 7 days (Fig. 1).

Fig. 1



Osteoblast differentiation was measured by alkaline phosphatase (ALP) activity (Fig. 2) and real-time RT-PCR (fig. 3) of ALP, Osteopontin, and Osterix in the osteogenic medium supplemented with rhBMP-2 (50, 100 and 200 ng/ml).

Fig. 2

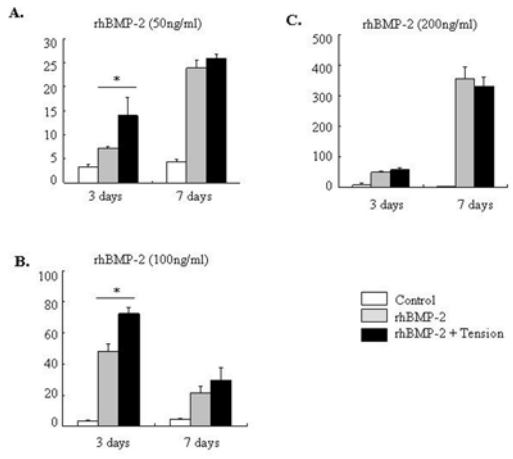
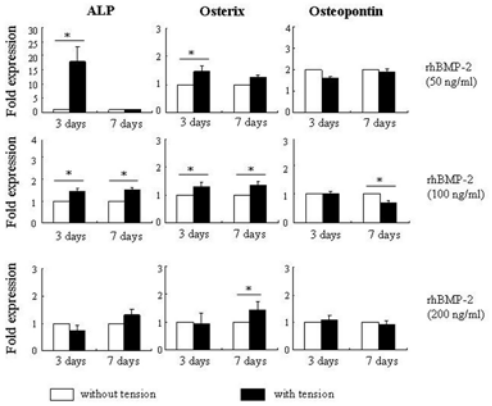


Fig. 3



Mechanical stress significantly stimulated cell proliferation at 3 days after continuous tension, but this effect was weakened at 7 days. It enhanced both ALP activity, and the mRNA expression of ALP and Osterix compared to the control group (without tension). Osteopontin mRNA expression was not induced by tension in any condition and even inhibited at 7 days. These results suggest that static tension stimulates cell proliferation, and synergizes the rhBMP-2-induced differentiation pathway of C2C12 cells into the osteoblasts. However, long-termed static tension is not as efficient as short- termed loading.

***Disclosures: S. Hwang**, The Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A060319) 2.*

P23

ASBMR YOUNG INVESTIGATOR AWARD Analysis of Gene Expression Profile in Rat Femoral Bone Marrow Ablation Model

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Bone is a dynamic organ that undergoes continuous remodeling by controlled reciprocal cycles of bone formation and bone resorption. Moreover, bone mass is preserved by a balance between the amount of bone formed and resorbed during the remodeling. Reduction in skeletal mass caused by an imbalance between bone resorption and bone formation would lead to common metabolic bone disorders such as osteoporosis. Bone marrow ablation in long bones induces intramembranous bone formation and subsequent bone resorption in order to regenerate normal bone marrow. This model has been used to study bone remodeling. Although the cascades of cellular events are well defined histologically, our understanding at the molecular level of bone formation and resorption in this model is limited. The purpose of this study was to assess the temporal gene expression profile in the rat femoral bone marrow ablation model using microarray analysis. This study was approved by the Institutional Animal Care and Use Committee. At each time point (1, 3, 5, 7, 10, 14, 28 and 56 days after marrow ablation),

3 animals were used for the microarray analysis and 2 animals were used for histological analysis. Intact animals were used as a baseline control (0 day). Total RNA was isolated from the bone marrow flushed out from the midshaft cavity contents exclusive of the epiphyses and growth plates. Expression profile of approximately 30,000 transcripts was analyzed using Rat Expression Array 230 2.0 (Affymetrix, Santa Clara, CA). Following preprocessing of raw expression data, we identified nearly 9,100 genes showing temporally changing expression patterns by using one-way analysis of variance with a moderated F statistic adjusting the p value for multiple testing with Benjamini-Hochberg FDR with the Bioconductor package limma. These 9,100 genes included genes that have been shown to regulate inflammation, angiogenesis, bone formation, and resorption. In order to identify temporal patterns of gene expression, we applied HOPAC clustering and identified 7 time course clusters. Our results indicate that numerous molecules are co-regulated during bone formation and resorption (remodeling) process in vivo. Further functional analyses are required to determine whether these molecules have a direct effect on bone remodeling. This knowledge may provide insight into the molecular bases of bone remodeling and therapeutic targets for metabolic bone disorders.

***Disclosures: K. Sena**, None.*

P24

ASBMR YOUNG INVESTIGATOR AWARD
Roles of SATB2 Overexpression in Regulating Tooth Development and Osteoblast Differentiation

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SATB2 plays an essential and critical role during osteogenic differentiation and skeletal patterning. It acts as a molecular node in a transcriptional network regulating skeletal development and osteoblast differentiation. SATB2 null mice demonstrated craniofacial abnormalities including shorter mandible, missing incisors, cleft palate, and alterations of various skeletal elements. Expressions of Osterix (Osx) and alkaline phosphatase (AP) were downregulated in long bones of Satb2-/- embryos and the formation of trabeculae and bone volume was reduced (Cell, 125:971-986, 2006).

To further investigate the expression pattern and functions of SATB2, we performed in situ hybridization of the head from wild type mice at E14.5 using an SATB2 cDNA probe (a gift from Dr. Grosschedl). We also transduced murine dental follicle cells (DFCs) isolated from mouse molar germs at the root forming stage, murine bone marrow stromal cells (BMSCs) and murine pre-osteoblastic cell line MC3T3 to achieve SATB2 overexpression in these cells. Cells transduced with empty vector serve as controls. RT-PCR analysis was performed using these cells. Cell migration assays were also performed to investigate the role of SATB2 in promoting engraftment of pre-osteoblasts or adult stem cells during wound regeneration. The results demonstrated that SATB2 was highly expressed in the dental mesenchymal components of incisors but could not be detected in epithelial components of the tooth germs. Moreover, SATB2 was highly expressed in developing palate and mandibular bone matrix and the expression of SATB2 in the edges of developing palatine processes was strong when these two processes were growing towards each other at the middle line. SATB2 overexpression in MC3T3 cells, BMSCs and DFCs induced increased expression levels of BSP, Osx, Runx2, and VEGFA in these cells. Migration rate was also increased in SATB2 ovexpressing MC3T3 cells and BMSCs.

In conclusion, the distribution of SATB2 expression in bones and teeth suggested an important role of SATB2 in osteogenesis and odontogenesis. Moreover, the expression of SATB2 in normal dental follicle cells of molar germs during root forming stage indicated a role of SATB2 in root formation. SATB2 overexpression enhanced the expressing levels of bone matrix proteins and osteogenic transcription factors. SATB2 also increases the expression level of VEGFA, which indicated its potential role in promoting angiogenesis during tissue regeneration.

***Disclosures: J. Zhang**, NIH grants DE11088 and DE16710 to Jake Chen 2.*

P25

Effects of Glucocorticoid Dose on the BMD Response to Teriparatide or Alendronate Therapy

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A randomized, double-blind, clinical trial studied the effect of glucocorticoid (GC) dose on BMD response to teriparatide (TPTD 20 μg/d) or alendronate (ALN 10 mg/d) in 428 patients with GC-induced osteoporosis (≥21 yr, having taken ≥5 mg/d prednisone equivalent for ≥3 mo prior to screening). Lumbar spine (LS), femoral neck (FN), and total hip (TH) BMD were measured by DXA at baseline and 18-mo endpoint. This post hoc analysis included 388 patients with baseline and ≥1 post-baseline BMD measurement, and baseline GC dose data. Baseline GC dose (mean over 30 d before randomization) was categorized as Low (≤5 mg/d), Medium (>5 and <15 mg/d), or High (≥15 mg/d). Data were analyzed using analysis of variance and covariance (continuous data) with no adjustment for multiple comparisons. Baseline LS, FN, and TH BMD were not significantly different between treatment groups, or between GC doses within each treatment group. In the overall population (n=428), the baseline to endpoint increases in BMD were different between treatment groups at the LS (TPTD, 8.2%; ALN, 3.9%; P<0.001), FN (TPTD, 4.4%; ALN, 2.8%; P<0.01), and TH (TPTD, 3.8%; ALN, 2.4%; P<0.05). In the GC dose subgroup (n=388), LS BMD increases differed across the 3 GC doses (P=0.052). The relative difference for LS BMD increases between the TPTD and ALN groups was not statistically different among the 3 GC dose groups (treatment-

by-GC dose interaction P=0.33). Both TPTD and ALN increased LS and TH BMD from baseline to endpoint for patients taking Low, Medium, or High GC doses. FN BMD was increased from baseline to endpoint in patients taking Low or Medium GC doses, but those taking High GC dose had no significant increase (TPTD, 1.4%; ALN, 1.8%). The changes in LS BMD at the Low, Medium, and High GC doses were 8.5%, 6.8%, and 4.9%, respectively in the TPTD group (P=0.028), and 3.8%, 3.0%, and 3.0%, respectively in the ALN group (P=0.7). When analyzed as a continuous variable, higher GC dose had a negative effect on percent increase in LS BMD (slope= -0.15, SE=0.07, P=0.049), regardless of treatment. The GC dose did not significantly affect the FN or TH BMD increases in either the TPTD or ALN groups. TPTD and ALN increased BMD at the LS and hip across a range of baseline GC doses, with the overall LS BMD increases being significantly greater with TPTD, an anabolic agent, compared with ALN, an antiresorptive. Higher GC doses appear to attenuate the LS BMD increase for TPTD and ALN, but seem to have less impact in ALN-treated subjects. Different mechanisms of action may contribute to these observed differences.

***Disclosures: K. Krohn**, Kelly Krohn 1, 3; Robert A Adler 2, 5; JP Devogelaer 2, 5; Chris Recknor 2, 5.*

P26

Responses of Human Marrow Stromal Cells to PTH Decrease with Age

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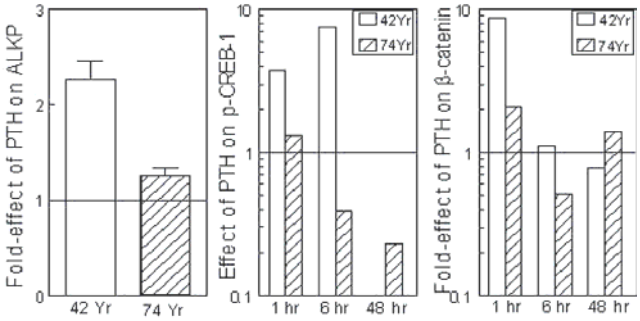
When applied intermittently *in vivo*, Parathyroid Hormone (PTH), has osteoanabolic effects. We had previously shown age-related decline in osteoblastogenesis by human marrow-derived stromal cells (hMSCs). In this study, we compared the age-related responses of hMSCs to PTH1-34 *in vitro*. Low-density, adherent hMSCs were isolated from tissue discarded during orthopedic surgery. Osteoblast differentiation was assayed after culturing confluent cells in αMEM, 1% FBS-HI with osteogenic supplements (10 nM dexamethasone, 5 mM ß-glycerophosphate, and 170 µM ascorbic phosphate). First, we assessed the effect of 10 nM of hPTH1-34 on the proliferation of hMSCs and surveyed the effects of PTH on expression of cell proliferation/cycle-related genes. Our pilot data showed that hPTH increased proliferation of hMSCs and decreased expression of p14 and p16 gene, with no detectable effects on p53, p21, E2F1, or Cyclin E. We found that REX, Bmi1, OCT4, and NANOG were expressed in hMSCs and that hPTH (6 hrs) stimulated REX and Bmi1 gene expression in hMSCs, with apparently greater magnitude in cells from the elder. Second, osteoblast differentiation was measured by alkaline phosphatase (AlkP) activity at day 7 and mineralization by quantitative Alizarin-Red assay at day 21. PTH1-34 (10nM) stimulated AlkP activity in cells from an adult to a magnitude twice that in cells from an elder (p=0.024), and significantly enhanced hMSCs mineralization (p<0.05). RT-PCR data showed that hPTH increased IGF-I, Osterix, and AlkP mRNA after 3 days treatment. Third, PTH1-34 activated p-CREB-1 signaling and increased ß-catenin (a key mediator of Wnt signaling) levels as shown by Western immunoblot. PTH activation of p-CREB and ß-catenin was dramatically diminished in hMSCs from the 74-year-old subject, as compared with the cells from younger subjects. In sum, PTH1-34 increased proliferation of hMSCs, downregulated intracellular inhibitors of proliferation, and stimulated stem cell self-renewal markers in hMSCs from elders. There were age-related decreases in hMSCs differentiation into osteoblasts and in the magnitude of stimulation of osteoblastogenesis by PTH. PTH signaling of CREB and ß-catenin was diminished with age. Intrinsic alterations in signaling responses to osteoanabolic agents may explain cellular and tissue aging.



***Disclosures: S. Zhou**, None.*

P27

Nitric Oxide: An Important Modulator of Bone Remodeling

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Nitric oxide (NO) is a ubiquitous molecule involved in cellular functions in most cells in the body. While osteocytes are involved in constant communication between bone cells, diffusible small molecules such as H^+ and NO are involved in short-term regulation of bone metabolism (Wimalawansa, 1987). *In vitro* and *in vivo* studies conducted over the past two decades have demonstrated regulatory roles of NO in bone metabolism and in particular inhibition of osteoclastic bone resorption. Furthermore, NO/cGMP is possibly the final common pathway of actions of some other agents including statins. Since menopause leads to NO deficiency, there is a plausible biological basis for the use of NO supplementation therapy in menopause. The beneficial effect of estrogen on bone is abolished in the presence of a NO-syntheses enzyme inhibitors suggesting at least in part, the beneficial skeletal effects are mediated via the NO/cGMP pathway (*Bone*, 18: 301-304, 1996). Since HRT has potential adverse effects (WHI study), it is sensible to supplement NO directly, rather than using HRT. The first human randomized clinical study demonstrated an equivalent efficacy of nitroglycerin to estrogen in prevention of early post-menopause bone loss *(JBMR, 15: 2240-2244, 2000)*. If the efficacy of NG in prevention of bone loss is confirmed in a larger clinical study, it may become a highly cost-effective, safe, alternative therapy

for osteoporosis and may supplant HRT and SERMs in preventing and treating postmenopausal osteoporosis and SARMs in men.

NO donor, nitroglycerin prevent both estrogen-deficiency and corticosteroid-induced bone losses as determined by improvements in bone mass, biochemical variables of bone turnover and bone histomorphometry in rats. NO donors increase ostaocalcin and the formation of a mineralized matrix by osteoblasts *in vitro* suggesting direct anabolic effects on bone. Albeit, these beneficial osteoblasts and osteoclasts effects are mild in comparison to bisphosphonates, devoid of adverse effects and the gentle nature, decoupling, and the histomorphometric improvements suggests that in the long term NO is likely to improve bone quality and decrease fractures. Data reported from cell culture, animal and human studies using NO donor nitroglycerine is promising. If the efficacy is proven from a large clinical study such as NOVEL study, this treatment is likely to become a preferred cost-effective therapy for osteoporosis prevention and treatment in the future.

Taken in to consideration the low cost, decoupling effect of NO on osteoblasts and osteoclasts, NO donor have potential as therapeutic agent to treat osteoporosis highly cost-effectively, improving skeletal health and decreasing fracture rates.

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

Anabolic Agents

P28

ASBMR YOUNG INVESTIGATOR AWARD
Osteotropic beta-Cyclodextrin as a Novel Bone Anabolic Agent

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An osteotropic alendronate-β-cyclodextrin conjugate (ALN-β-CD) was designed and synthesized for local delivery of therapeutic agents to bone and teeth. When evaluated as a delivery system for prostaglandin E_1 (PGE_1) in a bilateral rat mandible model, it is shown that ALN-β-CD/PGE_1 complex causes strong local bone anabolic reaction. Surprisingly, when tested as a control, ALN-β-CD itself was found to be bone anabolic. To probe its anabolic mechanism, control groups were added. The results show that ALN-β-CD could generate a large amount of new bone localized at the site of injection. In agreement with previous report, ALN was found to simulate new bone formation, but only peripheral to the site of injection (Fig 1).

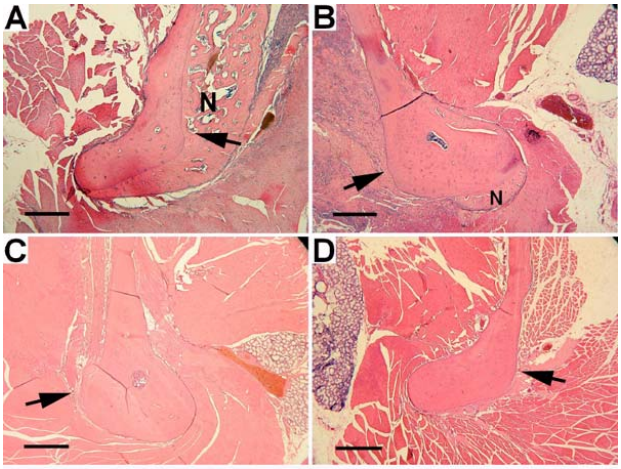

Figure 1. New bone formation on mandibles with different formulations.(A) ALN-ß-CD; (B) ALN;(C) PGE_1;(D) Saline. Bar size = 0.5 mm. Arrow indicates the approximate site of injection. N = New bone.

PGE_1 and saline injections do not induce bone formation. In addition to histology data, micro-CT analyses also confirm these findings (Fig 2).

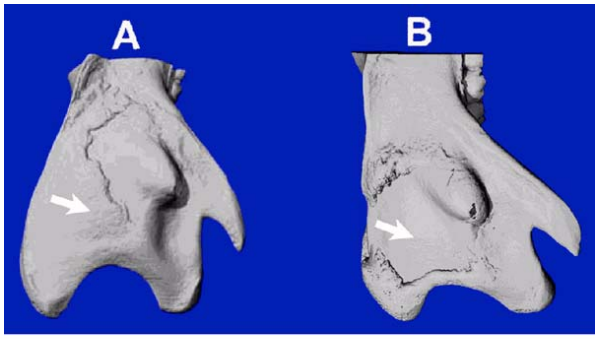

Figure 2. Representative µ-CT images of the lateral aspect of rat mandibular bones 24 days after treatments with ALN-ß-CD (A) and ALN (B). Arrow indicates the approximate site of injection.

While these data could not fully elucidate the bone anabolic mechanism of ALN-β-CD, they clearly indicate that it is independent of mechanical stimuli to the periosteum or treatment with ALN alone. Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides. The annulus of these doughnut-like molecules is hydrophobic, which enables the formation of inclusion complexes with many lipophilic compounds (e.g. PGEs, lipids, vitamin D, steroids). While CD derivatives are considered as biologically inert, ALN-β-CD may act as an immobilized molecular host in bone to attract, complex and augment local endogenous bone anabolic agents' level, which directly mediate the bone formation. An in vitro PGE_1 adsorption study with ALN-β-CD-bound hydroxyapatite powder supports this hypothesis.

***Disclosures: X. Liu**, None.*

P29

Characteristics of Subjects with Incident Vertebral Fractures During a Trial of Teriparatide versus Alendronate in the Treatment of Glucocorticoid-Induced Osteoporosis

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Characteristics are reported for subjects with glucocorticoid-induced osteoporosis (GIOP) who incurred new vertebral fractures during an active-controlled, double-blind clinical trial of teriparatide and alendronate, treatments with opposite effects on bone remodeling (McClung et al. 2005, Arch Intern Med).

We recently reported the results of the primary 18-month phase of a clinical trial in women and men with GIOP, having taken glucocorticoids (GC) for ≥3 months (prednisone equivalent of ≥5 mg/day) (Saag et al. 2007 EULAR). An 18-month continuation phase is in progress. The 428 subjects (76% women) were randomized to teriparatide, 20 µg/day (n=214) or alendronate, 10 mg/day (n=214). To identify potential risk factors associated with the incidence of radiographic vertebral fractures during the study, we applied a logistic regression model and compared proportions of patients without and with new vertebral fractures and the adjusted odds ratios (OR). New vertebral fractures occurred in 2.6% of the overall study cohort. We report here the characteristics of those without (nonfracture group) and with (fracture group) a new vertebral fracture during the primary 18-month phase.

Baseline characteristics of the nonfracture (n=417) and fracture (n=11) groups were age (years), 57±1 and 61±4 (p=0.27); lumbar spine bone mineral density (BMD) (g/cm^2), 0.85±0.01 and 0.80±0.04 (p=0.26); GC dose (mg/d), 9.8±0.4 and 12.2±2.6 (p=0.36); prevalent vertebral fractures, 0.7±0.1 and 2.0±0.9 (p=0.01); spinal deformity index (SDI), 1.0±0.1 and 2.7±1.2 (p=0.02); prior bisphosphonate therapy, 8.6% and 36.4% (p=0.01). The percent change in BMD was 5.3±0.3 and 9.2±3.5 (p=0.07) and the average GC dose (mg/d) was 10.2±0.8 and 14.5±2.9 (p=0.42) for the nonfracture and fracture groups, respectively. There were more people with incident vertebral fractures in the alendronate group (n=10) than in the teriparatide group (n=1) (p=0.004).

Adjusting for treatment group, we identified these risk factors (OR, 95% confidence interval [CI], and p-value): prior bisphosphonate therapy (OR=6.45, [1.74, 23.81], p=0.01), prevalent vertebral fractures (OR=1.30, [1.05, 1.62], p=0.02), and SDI (OR=1.16, [1.00, 1.35], p=0.04). The results from the 18-month continuation phase may identify additional risk factors for vertebral fractures that could be incorporated into the assessment of patients treated with glucocorticoids.

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

P30

Bone Remodelling Balance Index and Bone Turnover Index in Patients Treated with Teriparatide

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Bone volumetric density increases substantively in cancellous bone in patients who are treated with parathyroid hormone. In pivotal trials of parathyroid hormone therapy, visual analysis of bone turnover markers (BTM) suggests a triphasic pattern - namely, an immediate increase in bone formation; a contemporaneous increase in activation of new bone remodelling units (BRUs) with consequent increase in both resorption and formation activity; culminating in a gradual reduction in remodelling activity back towards baseline over 18 months.

In a pilot study of patients receiving Teriparatide 20 mcg daily, we sought to measure BTM on a serial basis and apply our derivative indices of bone remodelling activity. Fasting early morning blood and urine were collected from 6 post-menopausal women with severe osteoporosis (lumbar spine BMD T-score ≤-4.0) pre-treatment and 3, 6, 12 months following initiation of Teriparatide. Bone formation markers, bone alkaline phosphatase (BAP), procollagen type 1 N pro-peptide (P1NP) and intact osteocalcin (OCI) and the bone resorption marker C-terminal telopeptide (β-CTx) were measured in serum. Bone resorption markers deoxypryidinoline crosslinks (DPD/Cr) and N-terminal telopeptide of type 1 collagen (NTx/Cr) were measured in urine.

After 3 months treatment BAP, P1NP and OCI increased by 23%, 174% and 79% respectively; and NTX/Cr, DPD/Cr and CTx increased by 119%, 23% and 110% respectively. At 12 months all markers remained high. The bone remodelling balance index (BRBI, formation minus resorption) and bone turnover index (BTI, [formation plus resorption]/2) were calculated using P1NP T-scores and DPD/Cr T-scores. Results are given in the table. At baseline, bone balance was neutral and bone turnover was mid-normal. Bone balance increased markedly and reached a plateau at 6 months. Bone turnover also increased markedly, but began to decline after 6 months.

The high bone turnover with a positive remodelling balance agree with the concept that teriparatide enhances bone formation as well as increasing activation frequency of BRUs that may account for the striking increase in cancellous bone mass. We recommend more detailed analysis of BTM and derivative indices including their relationship to alterations bone mineral density.

Months	PINP T-score	DPD T-score	BRBI	BTI
0	0.09±1.71	0.48±1.97	-0.39±2.35	0.29±1.42
3	5.72±4.13	1.58±3.04	4.14±3.36	3.65±3.21
6	11.16±10.02	4.23±4.36	6.93±6.21	7.70±7.07
12	9.06±6.99	2.31±3.40	6.75±6.24	5.68±4.53

***Disclosures: J.J. Brady**, None.*

P31

Safety and Efficacy of 36 Months Treatment of Postmenopausal Osteoporotic Women with Parathyroid Hormone 1-84

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Daily treatment of postmenopausal osteoporotic women with 100 μg parathyroid hormone 1-84 (PTH) in the TOP study increased areal BMD by DXA and trabecular BMD by QCT at lumbar spine and hip. Bone turnover markers and iliac crest biopsies showed high rates of bone formation and higher trabecular bone volume in PTH-treated subjects at 18 months. However, little is known about the long term safety and efficacy of PTH treatment. We report here the results of the Treatment Extension Study (TRES) which evaluated the safety and efficacy of 36 months of PTH treatment. 99 subjects who completed 18 months of daily PTH treatment with vitamin D (400 IU) and with or without supplemental calcium (700 mg/day) in the Open Label Extension Study (OLES) were enrolled and continued on the same open label regimen for another 18 months. There was a median ~60 day interruption (range 0-112 days) in PTH treatment between OLES and TRES. The TRES population was similar to that of TOP where they began except that the most were from Argentina. The ITT population for TRES comprised 91 subjects and 62 completed the study; discontinuations were primarily for development of exclusion criteria and adverse events (AE). Lumbar spine BMD had increased by 8.0% at the end of OLES, was 7.0% higher at the TRES baseline (the decrease reflecting treatment interruption) and was 8.5% above the OLES baseline after 36 months, the increase from 7.0% occurring between 18 and 24 months. Regarding safety, analysis of 14 iliac crest biopsies collected after 36 months of treatment showed normal trabecular and cortical bone architecture with no abnormal histological features. Most subjects experienced at least one AE during TRES, 96% of which were mild or moderate in severity and 68% were considered related to study drug or of unknown etiology. There were 9 serious AEs but no deaths during TRES. Serum Ca increased by ~0.3 mg/dL during OLES but decreased during TRES; mean serum Ca at 36 months averaged 0.3 mg/dL below the OLES baseline. 24-hour urine Ca changed little during OLES, but decreased to below the OLES baseline after 36 months, reflecting the decrease in serum Ca. In summary, daily treatment of postmenopausal osteoporotic women with PTH(1-84) for 36 months resulted in a small additional increase in lumbar spine BMD over that observed after 18 months. Treatment with PTH for 36 months was generally safe and biopsies showed that bone was entirely normal. Moreover, modest elevations in serum and urine Ca were reversed with prolonged treatment.

Disclosures: R.R. Recker, *NPS Pharmaceuticals 5; Roche 2, 5; GSK 2, 5; Proctor & Gamble 5.*

P32

The Use of RAP-011, a Novel Bone Anabolic Agent, in Combination with Bisphosphonate Using a Mouse Model of Established Bone Loss

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RAP-011, a novel soluble receptor fusion protein based on the activin receptor type IIA has been shown to increase bone density *in vivo*. Here, we investigate the efficacy of RAP-011 compared with parathyroid hormone (PTH) over a 6 week treatment period followed by zoledronic acid (ZOL) as maintenance therapy with a follow up period of 12 weeks.

Eight week old C57BL/6 mice were ovariectomized (OVX) and allowed to lose bone for 8 weeks. All mice were analyzed by dual-energy x-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT) prior to treatment and at 6, 10, 14 and 18 weeks after treatment began. Mice were administered RAP-011 (IP, 10 mg/kg, biw), PTH (SC, 80 μg/kg/day) or VEH for a total of 6 weeks. At the end of the 6 week treatment period each group was divided in half with one group receiving a single dose of ZOL (20 μg/kg, IP) and the other receiving VEH. Changes in BMD were analyzed by DEXA and pQCT at weeks 10, 14 and 18 after the initial dosing.

At the 6 week time point, total bone mineral density (BMD) assessed by DEXA was increased 8.3% in RAP-011 treated mice, and 11% in PTH treated mice compared to baseline measurements ($p \leq 0.01$). pQCT analysis of the proximal tibia demonstrated that OVX VEH treated mice had a 7% decrease in trabecular bone mineral density (TbBMD), PTH treated mice had a decrease of 1.1% and RAP-011 treated mice had an increase of 3% relative to baseline ($P \leq 0.05$).

Total BMD and TbBMD were assessed at 4, 8 and 12 weeks after ZOL treatment began. Four weeks after the initial treatment was stopped (week 10), mice treated with RAP-011 with no further therapy increased total BMD by 1.8% and TbBMD by 5% ($P<0.05$ vs controls). Treatment with the ZOL after RAP-011 led to an increase of 4% of total BMD and a 10% increase in TbBMD ($P<0.05$ for both). Four weeks after PTH administration was halted the VEH treated mice lost 4% of their total BMD and 1.1 % of their TbBMD ($p<0.05$). Mice receiving maintenance therapy with ZOL after the cessation of PTH treatment had an increase in total BMD of 1.5% and an increase of 2.1% in TbBMD ($P<0.05$).

Consistent with our previously reported results, these data show that 6 weeks of treatment with RAP-011 or PTH increased bone mineral density in a model of established bone loss. Once treatment with PTH is stopped there is a rapid decrease in BMD to control levels, whereas RAP-011 treated mice maintain the increased BMD after treatment is withdrawn. Administration of ZOL subsequent to RAP-011 helps maintain the newly formed bone. These results indicate that RAP-011 is an anabolic therapy that does not alter the bone microenvironment in such a way as to preclude the use of a maintenance anitresorptive therapy.

Disclosures: R. Pearsall, *Acceleron Pharma 3.*

Targets/Agents Affecting Both Bone Resorption and Formation

P33

ASBMR YOUNG INVESTIGATOR AWARD
Geometrical and Biomechanical Properties of Rat Femurs with Tumor-induced Osteolysis after Anti-Resorptive or Anti-Cancer Treatments

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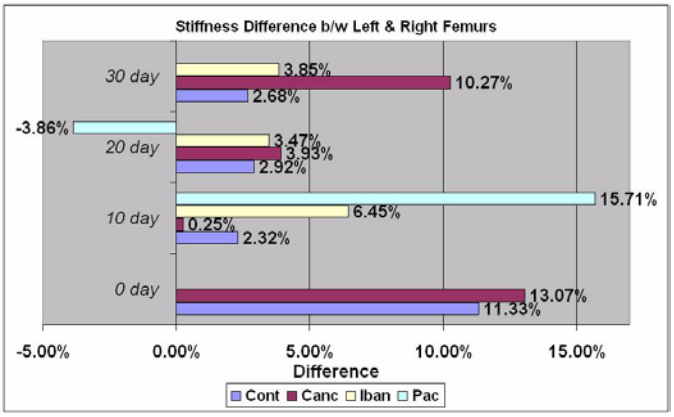
The study investigates the efficacy of Ibandronate (anti-resorptive) and Paclitaxel (anti-cancer) in treating tumor-induced osteolysis by assessing changes in geometrical and mechanical properties of the affected bone. A rat femur model for tumor-induced osteolysis using W256 malignant breast cancer cells was used. Of the 30 rats implanted with cancer cells, 12 were injected cancer cells only (Canc), 9 received ibandronate (Iban), and 9 received paclitaxel (Pac). Another 12 rats underwent a sham operation (Cont). At 10-days intervals up to 30-days, the paired-femurs were assessed by μCT scans to quantify the geometrical parameters. The paired-bones were also assessed by a 3-point bending test for their mechanical property.

The total BV was significantly smaller in the tumor-induced compared to the contra-lateral femur in the Canc group. While the geometric parameters were markedly lower for both Iban and Pac groups, The Iban group seems more successful, with a further 5.5% reduction in total BV difference, in comparison to Pac group. This pattern is repeated in CSA of cortical bones at 25% of the distal femur length.

Higher Tb.Th, Tb.N, BV/TV, and BS/BV of the tumor-implanted femurs were noted in both Iban or Pac groups compared to those in Canc group. With the exception in Tb.N, Iban group was better than Pac group in maintaining the trabecular properties. The paired-difference in stiffness in the Iban group was lowered to 3.5-4.0% at 30-days, whereas the Pac group had a stiffness lowered to -3.86% at 20-days. The results suggest that both Iban and Pac treatments in tumor osteolysis have the potential to preserve the structural integrity of the affected bones, and improve the mechanical property. While ibandronate results in less reduction imprint the in bone geometry, paclitaxel seems more effective in maintaining its stiffness.

Paired-differences in Geometrical Parameters Groups at 30-days (20-days for Pac)				
Paired-differences between the Intact and Operated Femurs (%)	Cont	Canc	Iban	Pac
Total Bone Volume, BV	0.08	10.80	-0.13	5.35
Cross-sectional Area, CSA	10.19	16.18	3.63	12.54
Trabecular thickness, Tb.Th	-20.66	0.13	-15.82	-3.20
Trabecular number, Tb.N	7.41	20.75	1.96	-16.22
Trabecular separation, Tb.Sp	-2.73	-5.10	28.85	15.89
Bone Volume Density, BV/TV	-0.89	14.87	-16.69	-11.16



***Disclosures: T. Lee**, None.*

P34

A Bone-Targeted, Macromolecular Delivery System Sustains the Anabolic Effects of Prostaglandin E1 on Indices of Bone Formation

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Macromolecular delivery systems have therapeutic uses in certain diseases, such as cancers, because of their ability to deliver and release drugs to specific organs and tissues. Aspartic acid octapeptides (Asp8) are known to seek bone surfaces and when attached to N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers exhibit preferential uptake on resorption surfaces. We report here the first use of a bone-targeted macromolecular delivery system conjugated with a known bone-active drug in an in vivo model of skeletal disease. The first objective was to confirm the uptake and localization of Asp8-HPMA-FITC copolymers on bone surfaces in ovariectomized (ovx) rats. The second objective was to determine if Asp8-HPMA-FITC conjugated with a

prototype anabolic drug, PGE1, attached via a cathepsin-K-sensitive linkage, would sustain stimulatory effects on bone formation in the aged, ovx rat model. Using tetracycline-labeling of bone formation surfaces as a comparison, Asp8-HPMA-FITC was found to deposit on bone surfaces with preferential uptake on resorption surfaces. Companion histological evaluation showed Asp8-HPMA-FITC uptake on active osteoclast surfaces. In an initial study conducted for 10 days in aged, ovx rats, a single iv injection of Asp8-HPMA-FITC-PGE1 resulted in significant increases in indices of bone formation. In the second in vivo study, a single iv injection of the Asp8-HPMA-FITC-PGE1 conjugate resulted in significantly greater bone formation indices measured 4 weeks after injection. In conclusion, these initial studies show that HPMA copolymers can be targeted to bone surfaces using Asp8 as the targeting moiety, with preferential uptake on resorption surfaces. This study also demonstrates that a well known, but labile anabolic agent (PGE1), attached to the HPMA copolymers and given by a single injection to ovx rats will result in sustained increases in bone formation indices from 10 days to 4 weeks. Macromolecular drug delivery systems are exhibiting considerable versatility and when targeted to bone may offer a number of therapeutic opportunities and advantages for the treatment of certain skeletal diseases.

Disclosures: S.C. Miller, *None.*

P35

ASBMR YOUNG INVESTIGATOR AWARD
Supplementation with Different Sources of the Antioxidant Lycopene Significantly Decreases Bone Resorption and Oxidative Stress Parameters in Postmenopausal Women

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We conducted a randomized, intervention study to determine whether supplementation with different sources of the antioxidant lycopene would decrease the risk of osteoporosis in postmenopausal women. 42 postmenopausal women aged 50-60 have been recruited in this study (Research Ethics Board approved). Following a washout period during which no lycopene-containing foods were consumed, participants were randomly assigned to consume (1) regular tomato juice, (2) lycopene-enriched tomato juice, (3) lycopene capsules or (4) placebo capsules, for a period of 4 months. Serum samples collected at baseline and after 4 months were assayed for bone alkaline phosphate (BAP), cross-linked aminoterminal N-telopeptide (NTx), total antioxidant capacity (TAC), lipid peroxidation and protein oxidation (where increased protein thiols indicates decreased oxidation). An unpaired t-test was used to analyze results presented in the table.

Parameters	Mean % change after 4 months intervention, supplement group (p value vs. placebo)			
	1	2	3	4
Protein Thiols	16.92 ± 6.73 (p<0.01)	10.35 ± 7.48 (p<0.08)	16.45 ± 7.2 (p<0.05)	-6.05 ± 3.76
TAC	14.69 ± 9.42 (p<0.05)	20.58 ± 4.48 (p<0.0001)	-1.37 ± 3.24 (p<0.08)	-10.39 ± 4.38
NTx	-13.33 ± 7.06 (p<0.10)	-12.22 ± 4.63 (p<0.05)	-16.81 ± 7.42 (p<0.05)	14.35 ± 18.54

As shown, we have demonstrated that supplementation with lycopene (juice or capsule form) increases antioxidant capacity, resulting in decreased protein oxidation in postmenopausal women. This decrease in oxidative stress may be responsible for the corresponding decrease in bone resorption. No significant changes were seen in BAP or lipid peroxidation (data not shown), which is consistent with previous results from our cross-sectional study (Osteoporos Int., 2007;18(1):109-15) and is similar to intervention studies on other agents. Different magnitudes of change among supplement groups could be accounted for by differences in the absorption of lycopene. In conclusion, our results suggest that lycopene, which can be obtained through the daily diet, may decrease the risk of osteoporosis in postmenopausal women. Therefore, lycopene may be used as a natural complementary or alternative supplement for the prevention of osteoporosis.

Disclosures: E.S. Mackinnon, *None.*

P36

ASBMR YOUNG INVESTIGATOR AWARD
Effects of Intermittent Administration of Parathyroid Hormone (1-34 hPTH) on Distraction Osteogenesis in Rabbits

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<Objective> Our study examined the effects of intermittent administration of parathyroid hormone (1-34 hPTH) on distraction osteogenesis.<Materials and Methods> An external fixator was applied to 15 immatured white Japanese rabbits and an osteotomy was performed on the right tibia. After a delay of 1-week, the distraction was started at a rate of 0.375 mm twice a day for 2 weeks. Beginning on distraction, hPTH was subcutaneously administered once a day, four days a week for a total of 4 weeks at a dose of 10 μg/kg (group P10) or 30 μg/kg (group P30) to five rabbits per group. Five rabbits received only the vehicle (group C) in the same manner. Seven weeks after osteotomy, the external fixator was removed, and rabbits were sacrificed 1-week later. We analysed the distracted callus by X-ray, DXA, pQCT, and μCT, to evaluated the bone union, BMD, cross section area and the morphology of callus. Three-point bending test was performed to assess biomechanical parameters. <Results> Bone union was found in all rabbits. The average BMD of the distracted callus (mg/mm^2) was 321.0, 330.8 and 354.4 for group C, group P10 and group P30, respectively. The total cross section area (mm^2) was 63.5, 53.6 and 81.7 and the value of group P30 was significantly larger than those of the other two groups. The cortical cross section

area (mm^2) of group P30 was largest and was significantly larger than that of group P10. μCT of group C showed the immature trabecular bone in the medullary cavity of the callus and the undefined lamellar formation of cortical bone. On the other hand, in PTH-administered groups, there was less immature trabecular bone in the medullary cavity, and the formation of lamellar structure in cortical bone was accelerated. Three-point bending analysis showed that fracture energy (N•mm) was 168.9, 240.1 and 583.0, respectively, and group P30 demonstrated a significantly larger value than the other two groups. <Discussion> PTH have a remodeling acceleration effect in bone fracture experiments. In relation to distraction osteogenesis, Seebach found an increase in bone mineral content and a stronger callus using rat femur models. The results in this study did not find any significant difference in bone density, but the cross section area and mechanical strength of the distracted callus of group P30 was significantly larger and higher. The morphology of the distracted callus in groups P10 and P30 also suggested a remodeling acceleration effect.We concluded that intermittent administration of PTH could be benefical to shorten the consolidation period of callus formation in distraction osteogenesis.

***Disclosures: H. Maruno**, None.*

P37

Osteoporosis in Postmenopausal Women: Development of a Cost-effective Novel Therapy-NOVEL Clinical Study

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The significant bone mineral density (BMD) loss in osteoporosis is responsible for the nearly approximately 1.8 million fractures a year, and for medical expenses of $20 billion annually in the U.S. Osteoporosis afflicts one out of two women and one out of five men over age 65, but is largely an under-diagnosed condition that is often diagnosed after a fracture. In the past, many women relied on hormone replacement therapy (HRT) after menopause to reduce the risk of heart disease and osteoporosis, but the utility of HRT has declined following the WHI study. The clinical trial we will describe here employed nitroglycerine ointment [as a source of providing nitric oxide, (NO)] to control bone destruction while improving bone formation. Nitroglycerine seems to be the only reliable agent that favorably affects both osteoblasts and osteoclasts simultaneously (i.e., functionally uncoupling these two cell types). The aim of the study is to prevent loss of BMD in a representative sample of post-menopausal women in central New Jersey.
Since no cure exists and a mere 10% decrease in bone mineral density (BMD) is associated with a two- to three-fold increase in fracture risk, optimizing peak bone mass and identifying the amount and rate of bone loss is helpful. In addition, cost-effective, affordable and safe alternative therapies are necessary to combat osteoporosis. We performed a prospective, controlled clinical study of early post-menopausal women with low lumbar and hip BMD T-scores who were screened for an osteoporosis prevention trial - Nitroglycerin as an Option: Value in Early Bone Loss (NOVEL), funded by the NIAMS. Of the 1,400 women interviewed by phone, 215 were screened and 186 were recruited for this three-year randomized double-blind, controlled clinical study. The subjects were characterized by demographic information, medical conditions, menopausal status, lumbar and hip T-score, Body Mass Index, smoking status, degree of physical activity as well as several other parameters. The analysis' primary outcome is detecting changes in BMD after three years of treatment as compared to a placebo. Secondary end points, including compliance, will be analyzed by Student's t-test and ANOVA, as applicable. During this study, we learned several lessons, including the value of the baseline safety labs; this led to the diagnosis of (unrelated to the study) serious medical conditions in two women. In addition, we developed effective strategies to recruit and retain study subjects in this primary prevention study. Findings from this NOVEL study could expand highly cost-effective and affordable therapeutic choices for women to combat osteoporosis.

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

Other

P38

Bisphosphonate-Induced Osteopetrosis: Metaphyseal Osteopenia, Osteosclerosis Fragility, and Novel Bone Modeling Defects After Drug Administration Ceases

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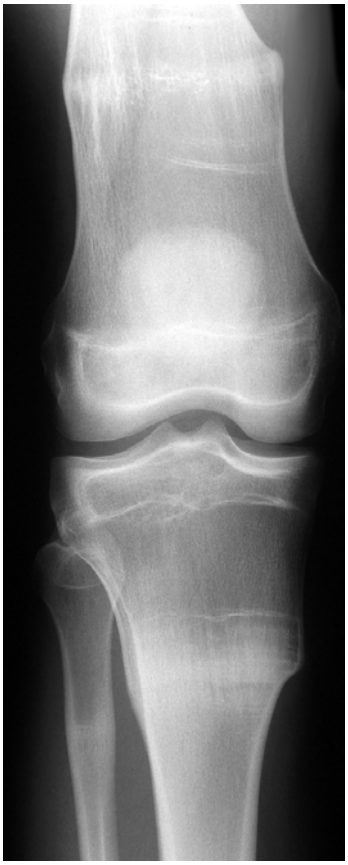
The first reported individual with drug-induced osteopetrosis (NEJM 349: 455, '03) agreed to re-evaluation 5 years after diagnosis (6 years after pamidronate (PMD) exposure ceased). At age 17 years, biochemical, radiological, and histopathologic parameters of skeletal homeostasis were reassessed. Idiopathic bone pain and hyperphosphatasemia of skeletal origin [that had led others to administer PMD] were still present, but serum alkaline phosphatase was lower in keeping with his now mature skeleton. Radiographs showed persistent modeling defects of osteopetrosis, especially in metaphyses of long bones, but with some unique features. Metaphyseal osteosclerosis had modeled into areas of increased diaphyseal density. Newer metaphyseal bone was not osteosclerotic, but unexpectedly osteopenic with thin cortices (*Figure*) and with cystic areas in trabecular bone shown by computed tomography. Metaphyses remained

widened, yet their surfaces were no longer abnormally straight or convex, but once again concave. A "bone-in-bone" appearance was now present in both the axial and appendicular skeleton (*Figure*).



Although DXA recorded normal spine BMD, radiographs suggested vertebral osteopenia surrounding the bands of osteosclerosis, but no collapses. L_4 spondylolysis persisted, and spondylolisthesis had developed. Claims of interval fractures included a Salter II break of an osteosclerotic distal radius, and subsequently a contralateral "chalkstick" fracture across an osteosclerotic ulnar diaphysis that was incompletely healed 2 years later. Repeat iliac crest biopsy showed that nascent endochondral bone contained an excess of unresorbed primary spongiosa ("cartilage bars"), but much less so than during PMD exposure. Osteoclasts -- abnormal during PMD administration (round cells without polarized nuclei, off of bone surfaces) -- were normal in number, location, and appearance.

BP toxicity during childhood can cause aberrations of skeletal modeling and remodeling that evolve years after drug withdrawal and carry into adult life.

***Disclosures: M.P. Whyte**, None.*

P39

ASBMR YOUNG INVESTIGATOR AWARD
The Effects of Estrogen Deficiency and Energy Deficiency on Osteoprotegerin and RANKL Levels in Premenopausal Exercising Women

S. L. West*, J. L. Scheid*, S. Awdishu*, J. D. Vescovi, M. J. De Souza. University of Toronto, Toronto, ON, Canada.

Physically active women can develop exercise associated menstrual cycle disturbances such as amenorrhea (i.e. estrogen deficiency) secondary to a chronic energy deficiency. The purpose of this cross-sectional observational study was to assess the effects of energy and estrogen deficiency on osteoprotegerin (OPG) and receptor activator of NF-κß ligand (RANKL) and their relationship to bone turnover in premenopausal exercising women. Serum PINP, NTX, OPG, RANKL, triiodothyronine (TT_3), urinary estrone 3-glucuronide (E1G), urinary pregnanediol 3-glucuronide (PdG), resting energy expenditure (REE), and BMD (by DXA) were measured repeatedly in 64 exercising women. Volunteers were retrospectively grouped: 1) Energy Replete+Estrogen Replete, 2) Energy Replete+Estrogen Deficient, 3) Energy Deficient+Estrogen Replete, and 4) Energy Deficient+Estrogen Deficient. 2X2 ANOVA were performed. No interactions of estrogen and energy status were observed across all variables. Subjects were similar with respect to age (23.3±1.1), weight (58.1±1.8 kg), height (165.9±1.4 cm), and BMI (21.1±0.6 kg/m^2) ($p>0.05$). A main effect of estrogen status was observed such that the estrogen deficient group demonstratedand suppressed TT_3 ($p=0.002$), REE ($p=0.052$), REE/FFM ($p=0.010$), REE:pREE ($p=0.026$), E1G AUC ($p<0.001$) and PdG AUC ($p=0.043$) vs. the estrogen replete group. Compared to the estrogen replete women, the estrogen deficient women demonstrated elevated NTX ($p=0.024$), which occurred in the presence of suppressed OPG ($p=0.054$). Total body BMD ($p=0.025$) and L1-L4 BMD ($p=0.012$) were also suppressed in the estrogen deficient group vs. the estrogen replete group. There was a main effect of energy status: the energy deficient group demonstrated suppressed PINP ($p=0.013$), REE/FFM ($p=0.007$), REE and REE:pREE (both $p<0.001$) compared to the energy replete group. RANKL was similar ($p>0.05$) across all comparisons. Our results confirm previous results that indicate energy status primarily impacts bone formation, while estrogen status impacts bone resorption. Our results also suggest that OPG may play a role in the etiology of increased bone resorption observed in exercising women with hypothalamic amenorrhea.

***Disclosures: S.L. West**, None.*

P40

Insufficiency Bilateral Femoral Shaft Fractures in Patient Taking Imatinib Mesylate for CML

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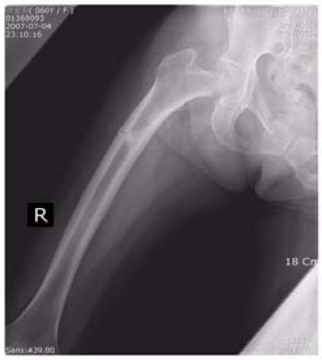
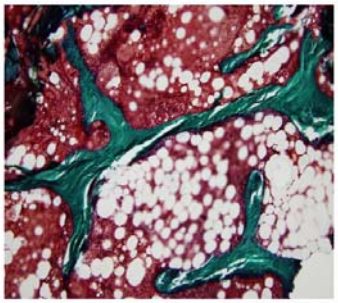
Imatinib mesylate (Gleevec, Norvatis) inhibits several tyrosine kinases associated with disease. These enzymes include BCR-ABL in patients with chronic myelogenous leukemia, the C-KIT in patients with gastrointestinal stromal tumors, and the platelet-derived growth factor receptors a and b in patients with certain myeloproliferative disorders and dermatofibrosarcoma protuberans. In general, most patients tolerate this drug well. We report insufficiency bilateral femoral shaft fractures in one patient taking imatinib mesylate for 10 months due to chronic myelogenous leukemia.

A 60-year-old woman had suffered from both thigh pain for 6 months. Bilateral progressive insufficiency fractures on femoral shaft were shown on plain radiograph (Fig. 1). Femur MRI revealed incomplete fractures and no evidence of bone metastasis on both femurs. Bone densitometry showed normal T-score around hip joint and spine. The levels of calcium, phosphorous, 25-hydroxyvitamin D and 1,25-dihydroxy-vitamin D in serum were in normal range. Parathyroid hormone level was slightly increased. The levels of osteocalcin and urinary n-telopeptide of collagen cross-links (NTx) were decreased. Iliac bone biopsy revealed normocelluar marrow without leukemic cells. The histomorphometric evaluation of bone revealed reduced bone turnover in spite of secondary hyperparathyroidism (Fig. 2).





The biochemical markers and histomorphometric evaluation suggest that this drug may affect bone metabolism, both bone formation and resorption. Long term administration of bisphophonate causes insufficiency fracture in sacrum, femur and pubic bones due to suppressed bone remodeling and osteonecrosis of jaw. To the best of our knowledge, it is the first case of insufficiency bilateral femoral shaft fractures in CML patients who was taking imatinib mesylate. More careful evaluation on bone metabolism and skeletal system should be taken in CML patients because imatinib mesylate should be taken for the rest of their lives.

***Disclosures: K. Yang**, None.*

P41

ASBMR YOUNG INVESTIGATOR AWARD
Efficacy of cholecalciferol 800 IU supplementation with or without calcium supplements in preventing falls of the elderly people: A Bayesian meta-analysis

T. Fan*[1], G. Bergman*[2], S. S. Sen*[1], P. Drost*[2], J. Jansen*[2]. [1]Global Outcomes Research, Reimbursement and HTA, Merck & Co., Inc, Whitehouse Station, NJ, USA, [2]Mapi Values, 3995 AX Houten, Netherlands Antilles.

To estimate the effect of cholecalciferol 800 IU supplementation with or without calcium supplements in preventing non-vertebral and hip fractures in postmenopausal women with recently published evidence.

A systematic review of studies in MEDLINE and EMBASE up to June 2007 was performed. Randomized controlled trials that assessed the vitamin D supplementation of 800 IU (cholecalciferol) oral daily in women aged 60 years and older were included. Findings from various studies were synthesized using Bayesian fixed and random-effects meta-analysis.

A total of 3,510 patients from 4 trials that compared vitamin D to placebo without calcium supplements were included in the assessment for non-vertebral fracture risk. For cholecalciferol 800 IU plus calcium supplementation versus placebo using a fixed effects model, the pooled odds ratio (OR) was for preventing non-vertebral fractures was 0.77 [CrL: 0.63 - 0.93]. This analysis also yielded a 99% probability that cholecalciferol 800 IU plus calcium is a better treatment than placebo. Using a random effect model for the prevention of non-vertebral endpoint, the pooled OR was less favourable 0.95 [CrL: 0.28 - 2.54].

A total of 7,473 elderly women from 5 randomized trials were included in the meta-analysis for hip fractures. In the fixed effects model, the pooled OR for preventing hip fractures was 0.72 (CrL: 0.55 - 0.91; probability of being the better treatment is 100%). The random effect model showed a more conservative estimate (OR: 0.73; CrL = 0.42-1.19), and the probability that Vitamin D being a better treatment relative to placebo was slightly lower (P=94%).

A sub-analysis was done to evaluate the independent effect of vitamin D over and above the effect of calcium supplementations, in preventing non-vertebral fractures. Using a fixed effects model, for cholecalciferol 800 IU versus placebo given calcium supplementation as background treatment, the pooled OR for preventing non-vertebral fractures was 0.81 [CrL: 0.11 -2.89]. Using a random effect model, the pooled OR was for preventing non-vertebral fractures was 0.73 [CrL: 0.17 - 1.93]. There is 87% probability that cholecalciferol 800 IU plus calcium is a better treatment than calcium supplementation alone in preventing non-vertebral fractures

Cholecalciferol 800 IU per is effective in preventing hip fractures in elderly or postmenopausal women. Furthermore, Cholecalciferol 800 IU per day provides additional benefits than calcium supplements in preventing non-vertebral fractures of elderly women.

***Disclosures: T. Fan**, Merck & Co., Inc 1, 3.*

P42

ASBMR YOUNG INVESTIGATOR AWARD
Efficacy of cholecalciferol 800 IU supplementation with or without calcium supplements in preventing falls of the elderly people: A Bayesian meta-analysis

T. Fan*[1], G. Bergman*[2], S. S. Sen*[1], P. Drost*[2], J. Jansen*[2]. [1]Merck & Co., Inc, Whitehouse Station, NJ, USA, [2]Mapi Values, 3995 AX Houten, Netherlands Antilles.

To estimate the effect of cholecalciferol 800 IU supplementation with or without calcium supplements in preventing falls in postmenopausal women with evidence from randomized clinical trials. A systematic review of studies in MEDLINE and EMBASE up to June 2007 was performed. Clinical trials that assessed the vitamin D supplementation of 800 IU (cholecalciferol) oral daily with or without calcium supplementation in men and women aged 60 years and older were included.

Findings from various studies were synthesized using Bayesian fixed and random-effects meta-analysis. The odds ratios and credibility limits (CrL) in preventing falls and the probability of being the best treatment given the level of uncertainty were estimated.

Four trials were included in the meta-analysis. The additional effects of cholecalciferol of 800 IU daily in preventing falls with calcium supplementations as background treatment were evaluated. In the fixed effect model, the pooled odds ratio (OR) in preventing falls was 0.59 [credential limits (CrL): 0.31 - 1.00, the probably of having better efficacy than placebo P = 97%]. In the random effect model, OR = 0.61 (CrL: 0.26 - 1.20; P = 94%).

Two trials with 720 women 60 years and older were included in a sub-analysis for female subjects. When calcium supplementations were not used as background treatment, cholecalciferol 800 IU daily had a 75% (OR: 0.91; CrL: 0.66-1.22) chance of being better than placebo in preventing falls in the fixed effect model and a 77% (OR: 0.96; CrL: 0.23- 2.14) chance of being better than placebo in random effect model. When calcium supplements were used in both arms, the chance of being better than placebo for vitamin D is 97% and 85% in the fixed effect model and random effect model, respectively.

Cholecalciferol 800 IU per day together with calcium supplemental is effective in preventing falls of elderly people. Cholecalciferol 800 IU even can provide additional benefits to calcium supplements in preventing falls for both male and female elderly people. More studies should be done to estimate the benefits of vitamin D3 in falls preventing.

***Disclosures: T. Fan**, Merck & Co., Inc 1, 3.*

P43

Phossy jaw and inorganic phosphorus chemistry: a timely hypothesis

W. B. Hinshaw. Markle & Hinshaw Gynecology, Franklin, NC, USA.

Numerous cases of osteonecrosis of the jaw (ONJ) and more than 20 cases of cortical long bone fractures, chiefly of the subtrochanteric femur, have been reported recently in association with bisphosphonate (BP) therapy. It has been pointed out that ONJ strongly resembles the "phossy" jaw disease described in workers in 19th century white phosphorus (P) match factories. There is a striking resemblance as well between the clinical presentations of the modern long-bone fracture patients and fracture cases reported in 1899 in Manchester white P match workers. Four low-impact femoral fractures were described in 2 such workers in addition to 44 other cases summarized. Some of the cases included concomitant phossy jaw. Delayed healing resembling the modern cases was also documented. The author stated (in 1899) "…we have sufficient presumptive evidence to show that the osseous tissues of the body generally can be so altered by the prolonged action of phosphorus or its compounds as to render them less resistant to the application of external violence."

The match-industry disease vanished with the substitution of red P for white. Elemental P occurs in several allotropes. The white, but not the red, spontaneously oxidizes in air to form P_2O_5, which may react with water to form several acids including pyrophosphoric acid (PPi). PPi would be present in inhaled white P smoke. Also, inhaled P_2O_5 could be hydrolyzed to PPi under physiologic conditions. Either thus could raise the serum concentration of PPi in the chronically-exposed individual.

PPi is elevated in children with congenital hypophosphatasia, which is associated with disorders in the function of the cortical alveolar bone and fragility fractures of the femur and metatarsals. The cortical alveolar bone has been suggested to be the originating locus in bisphosphonate-associated osteonecrosis of the jaw.

BPs were originally chosen as potential pharmacologic analogs of PPi, because the latter had been found to decrease hydroxyapatite (HAP) solubility. The central P-O-P group was replaced by P-$C(R_2)$-P, conveying resistance to hydrolysis. After binding to bone under active osteoclasts, the commonly-prescribed nitrogen-containing BPs are ingested and inhibit osteoclast farnesyl pyrophosphate synthase. The ultimately resulting cellular changes lead to reduced bone remodeling activation.

The hypothesis advanced is that the etiological agent of the match industry diseases of jaw necrosis and fragility fractures was PPi. This proposition is based upon the remarkable analogy between the clinical presentations in the problems associated with the match industry and the modern BP drugs and upon the fact that the two putative etiological agents share similar structural and HAP binding properties.

***Disclosures: W.B. Hinshaw**, Eli Lilly & Company 8; MDL-1760 Committee 5.*

AUTHOR INDEX

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The American Society for Bone and Mineral Research

Targeting Bone Remodeling for the Treatment of Osteoporosis

December 6-7, 2007
Washington, DC, USA

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		SESSION TITLE	NO. HOURS ATTENDED	EVALUATION
Thursday, December 6, 2007	8:10 am – 11:50 am	Overview of Bone Remodeling and Bone Modeling (3Hours, 20 Minutes)		
	1:00 pm – 5:20 am	Bone Resorption (4 Hours, 20 Minutes)		
Friday, December 7, 2007	8:00 am – 12:40 pm	Bone Formation (4 Hours, 20 Minutes)		
	2:00 pm – 3:00 pm	Targets Affecting Both Resorption and Formation of Bone (1 Hour)		
	3:00 pm – 3:20 pm	Wrap Up/Summary and Conclusions (20 Minutes)		

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This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the Federation of American Societies for Experimental Biology (FASEB) and The American Society for Bone and Mineral Research (ASBMR). FASEB is accredited by the ACCME to provide continuing medical education for physicians. The Federation designates this educational activity for up to 13.25 credit hours in Category I credit toward the AMA Physician's Recognition Award.

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