Welcome!

CONTENTS

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 in our understanding of the bone-fat-brain connection. We believe that it is crucial to bring together both clinical and basic investigators from distinct scientific disciplines, including metabolism, bone physiology, and neurology, to discuss and disseminate the most up-to-date information available, to encourage open discussion, and to develop scientific approaches that will facilitate development of a reliable knowledge base. We believe that these discussions will facilitate translational research and transfer of information to the clinical arena.

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 in the study of bone, fat, and brain connections.

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 Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Arthritis, Musculoskeletal and Skin Diseases, Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institute of Arthritis and Musculoskeletal And Skin Diseases (NIAMS), National Institute of Neurological Disorders and Stroke (NINDS) and National Institute on Aging (NIA).

We are grateful for the co-sponsorship of the American Society for Nutrition (ASN), Foundation for Osteoporosis Research and Education (FORE), National Osteoporosis Foundation (NOF), Orthopaedic Research Society (ORS), The Endocrine Society (ENDO), The Paget Foundation for Paget's Disease of Bone and Related Disorders, 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,

Barbara E. Kream, Ph.D.
ASBMR Immediate Past-President

Gerard Karsenty, M.D., Ph.D.
Organizing Committee Co-Chair

Clifford Rosen, M.D.
Organizing Committee Co-Chair

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National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).
Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD)
National Institute of Arthritis and Musculoskeletal And Skin Diseases (NIAMS)
National Institute of Neurological Disorders and Stroke (NINDS)
National Institute on Aging (NIA)

American Society for Bone and Mineral Research
New Frontiers in Skeletal Research: Bone, Fat, and Brain Connections

Organizing Committee

Clifford Rosen, M.D., *Co-Chair*
Maine Medical Center Research Institute, Scarborough, Maine, USA

Gerard Karsenty, M.D., Ph.D., *Co-Chair*
Columbia University, New York, New York, USA

Barbara E. Kream, Ph.D., *Immediate Past-President*
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Joel K. Elmquist, D.V.M., Ph.D.
The University of Texas Southwestern Medical Center, Dallas, Texas, USA

Anne Klibanski, M.D.
Massachusetts General Hospital, Boston, Massachusetts, USA

Beata Lecka-Czernik, Ph.D.
University of Toledo College of Medicine, Toledo, Ohio, USA

ASBMR Young Investigator Award Recipients

Supported in part by an educational grant from Eli Lilly and Company.

Benjamin Adler
Natasha Case
Jessica Costa
Alexis Cutchins
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Exhibitors

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General Information

VENUE

This meeting will take place in the Grand Ballroom Salons A–D of the Bethesda North Marriott Hotel and Conference Center located at 5701 Marinelli Road, Bethesda, Maryland, USA.

REGISTRATION

All registration services will take place in the Grand Ballroom Foyer of Bethesda North Marriott Hotel and Conference Center.

Registration Hours

Sunday, April 26, 2009	4:00 p.m. – 7:00 p.m.
Monday, April 27, 2009	7:00 a.m. – 2:00 p.m.
Tuesday, April 28, 2009	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 Middlebrook Room of the Bethesda North Marriott Hotel and Conference Center.

Speaker Ready Room Hours

Sunday, April 26, 2009	4:30 p.m. – 6:00 p.m.
Monday, April 27, 2009	7:00 a.m. – 8:00 p.m.
Tuesday, April 28, 2009	7:00 a.m. – 4:00 p.m.

POSTER INFORMATION

Posters will be displayed in the Grand Ballroom Salon E of the Bethesda North Marriott Hotel and Conference Center. Poster presentation time is scheduled during Poster Session I on Monday, April 27 from 10:45 a.m. to 11:30 a.m. and Poster Session II on Tuesday, April 28 from 10:50 a.m. to 11:35 a.m. Presenters must be at their posters during this time and available to answer questions.

	Monday, April 27, 2009	**Tuesday, April 28, 2009**
Poster Set-Up	7:00 a.m. – 8:00 a.m.	
Presentation Time	Poster Session I (M1-M93) 10:45 a.m. – 11:30 a.m.	Poster Session II (T2-T92) 10:50 a.m. – 11:35 a.m.
Poster Dismantle		4:00 p.m. – 4:15 p.m.
Poster Viewing Schedule		
Morning Break	10:30 a.m. – 10:45 a.m.	10:35 a.m. – 10:50 a.m.
Lunch Break	11:30 a.m. – 12:15 p.m.	11:35 a.m. – 12:20 p.m.
Afternoon Break	2:35 p.m. – 2:50 p.m.	2:25 p.m. – 2:40 p.m.
Post-Meeting	5:20 p.m. – 5:45 p.m.	3:50 p.m. – 4:00 p.m.

MEETING MEALS

Your registration for the meeting includes a continental breakfast and lunch on Monday, April 27 and Tuesday, April 28. Breakfast will be available in the Grand Ballroom Foyer, and lunch will be served in Grand Ballroom Salon F–K at the Bethesda North Marriott Hotel and Conference Center.

MEETING OBJECTIVE

In less than one decade, there has been tremendous progress in our understanding of the bone-fat-brain connection. The networks involved in this regulatory loop are complex and under the control of hormones, cytokines, and neuropeptides. To further the repository of knowledge in this area, a multi-disciplinary approach must be undertaken. Now is an opportune time to bring together in a two-day meeting, basic investigators and clinicians from distinct scientific disciplines, to discuss and disseminate the most up-to-date information about the physiology and pathobiology of these networks.

The program objectives of this meeting include understanding the role of the central nervous system in the homeostatic regulation of bone turnover and in pathologic conditions; delineating specific genes and cell determinants of osteoblastogenesis and the bone marrow interactions that underlie marrow adipogenesis during states of malnutrition, glucose intolerance, and aging; clarifying how changes in body composition and the metabolic syndrome affect bone remodeling and fracture risk; and defining the endocrine functions of the skeleton.

TARGET AUDIENCE

We anticipate that this meeting will bring together worldwide thought leaders. It should have broad appeal to investigators from many different fields and to clinicians who are interested in the latest breakthroughs with therapeutic relevance. We expect that many attendees will be young investigators, NIH funded researchers, industry scientists, intra-mural scientists from NIH, and clinicians interested in osteoporosis, diabetes mellitus, obesity, and psychiatric disorders.

CONTINUING MEDICAL EDUCATION

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. FASEB designates this educational activity for a maximum of 12.75 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

You must submit your application for CME credits online at: https://secure.faseb.org/faseb/cmeapplication/. After entering the site, you will need to select the activity you attended and enter the number printed on your badge to verify attendance. There is a $45.00 processing fee payable by credit card.

Sessions that are eligible for CME are listed on page 6.

Please keep your badge. You will need to provide the number printed on your badge to complete the online application.

Questions regarding your continuing education credits should be directed to:

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Office of Scientific Meetings and Conferences
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CME Session Worksheet
(For online submission)

Day	Time	Session Number and Title	Number of Hours Attended	Evaluation
Monday	Morning	**Session One:** Novel Endocrine Regulators of Bone Remodeling (2 hours, 20 minutes)		
	Afternoon	**Session Two:** Central Control of Bone Remodeling (2 hours, 20 minutes)		
		Session Three: Bone, Fat and Brain in the Clinical World (2 hours, 30 minutes)		
Tuesday	Morning	**Session Four:** Osteoblast and Adipocyte Differentiation (2 hours, 20 minutes)		
	Afternoon	**Session Five:** Bone as an Endocrine Organ (2 hours, 45 minutes)		
		Meeting Wrap-Up: Overall Summary with Questions and Answer Panel (30 minutes)		

The maximum number of credits for the meeting is 12.75 hours. If your total hours exceed 12.75, you will only receive credit for 12.75.

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 New Frontiers in Skeletal Research: Bone, Fat, and Brain Connections 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.

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
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6) Non-remunerative positions of influence such as officer, board member, trustee, spokesperson
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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 abridgment 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, audiotaping devices, and videotaping equipment is strictly prohibited within all Oral Scientific Sessions and the Poster Sessions without the express written permission of the ASBMR Convention Management. Unauthorized use of taping equipment may result in the confiscation of the equipment or the individual being asked to leave the Scientific Session. These rules will be strictly enforced.

ASBMR MEMBERSHIP

The ASBMR Membership Booth will be located in the Grand Ballroom Foyer of the Bethesda North Marriott Hotel and Conference Center. 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 31st Annual Meeting in Denver, Colorado, USA, September 11–15, 2009.

MEETING EVALUATION

An online evaluation form for the ASBMR Meeting on New Frontiers in Skeletal Research: Bone, Fat, and Brain Connections 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 New Frontiers in Skeletal Research: Bone, Fat, and Brain Connections may be released to the press before its official presentation date and time. Press releases must be embargoed until 1 hour after the presentation.

FUTURE ASBMR MEETING DATES

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

ASBMR 32nd Annual Meeting
October 8–12, 2010
Metro Toronto Convention Center
Toronto, Ontario, Canada

American Society for Bone and Mineral Research
New Frontiers in Skeletal Research: Bone, Fat and Brain Connections

Schedule-at-a-Glance

Monday, April 27, 2009

Time	Session	Location
7:00 a.m. – 8:00 a.m.	Breakfast	Grand Ballroom Foyer
8:00 a.m. – 8:10 a.m.	Opening Comments	Grand Ballroom Salon A–D
8:10 a.m. – 10:30 a.m.	Session 1: Novel Endocrine Regulators of Bone Modeling	
10:30 a.m. – 10:45 a.m.	Break/Exhibits Open	Grand Ballroom Foyer
10:45 a.m. – 11:30 a.m.	Poster Session I	Grand Ballroom Salon E
11:30 a.m. – 12:15 p.m.	Lunch and Poster Viewing	Grand Ballroom Salon F–H
12:15 p.m. – 2:35 p.m.	Session 2: Central Control of Bone Remodeling	Grand Ballroom Salon A–D
2:35 p.m. – 2:50 p.m.	Break/Exhibits Open	Grand Ballroom Foyer
2:50 p.m. – 5:20 p.m.	Session 3: Bone, Fat and Brain in the Clinical World	Grand Ballroom Salon A–D
5:20 p.m. – 5:45p.m.	Poster Viewing Open	Grand Ballroom Salon E
5:45 p.m. – 7:30 p.m.	Welcome and Networking Reception	Grand Ballroom Salon F-H

Tuesday, April 28, 2009

Time	Session	Location
7:00 a.m. – 8:00 a.m.	Breakfast/Exhibits Open	Grand Ballroom Foyer
8:00 a.m. – 10:35 a.m.	Session 4: Osteoblast and Adipocyte Differentiation	Grand Ballroom Salon A–D
10:35 a.m. – 10:50 a.m.	Break/Exhibits Open	Grand Ballroom Foyer
10:50 a.m. – 11:35 a.m.	Poster Session II	Grand Ballroom Salon E
11:35 a.m. – 12:20 p.m.	Lunch and Poster Viewing/Exhibits Open	Grand Ballroom Salon F-H
12:20 p.m. – 3:20 p.m.	Session 5: Bone as an Endocrine Organ	Grand Ballroom Salon A–D
2:25 p.m. – 2:40 p.m.	Break/Exhibits Open	Grand Ballroom Foyer
3:20 p.m. – 3:50 p.m.	Meeting Wrap Up	Grand Ballroom Salon A–D
3:50 p.m.	Meeting Adjourns	

EXHIBIT HOURS

Monday, April 27, 2009
10:30 a.m. – 12:15 p.m.
2:35 p.m. – 2:50 p.m.

Tuesday, April 2, 2009
7:00a.m. – 8:00 a.m.
10:35 a.m. – 12:20 p.m.
2:25 p.m. – 2:40 p.m.

American Society for Bone and Mineral Research
New Frontiers in Skeletal Research: Bone, Fat and Brain Connections

Monday, April 27, 2009

BREAKFAST
7:00 a.m. – 8:00 a.m.
Grand Ballroom Foyer

INTRODUCTION
8:00 a.m. – 8:10 a.m.

8:00 a.m. Opening Comments
Barbara Kream, Ph.D., Past-President, American Society for Bone and Mineral Research, University of Connecticut Health Center, Farmington, Connecticut, USA

Clifford Rosen, M.D., Organizing Committee Co-Chair, Maine Medical Center Research Institute, Scarborough, Maine, USA

Gerard Karsenty, M.D., Ph.D., Organizing Committee Co-Chair, Columbia University, New York, New York, USA

Ronald Margolis, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland, USA

SESSION 1:
8:10 am – 10:30 a.m.
Grand Ballroom Salon A–D

Novel Endocrine Regulators of Bone Remodeling
Moderator: Itai Bab, D.M.D., The Hebrew University, Jerusalem, Israel

Presentation Number

8:10 a.m. Keynote: Endocrine and Paracrine Regulation of the Skeleton **1**
Henry Kronenberg, M.D., Massachusetts General Hospital, Boston, Massachusetts, USA

9:00 a.m. An Update on the Pituitary-Bone Axis **2**
Mone Zaidi, M.D., Ph.D., Mount Sinai Medical Center, New York, New York, USA

9:25 a.m. Lrp5 Controls Bone Formation by Inhibiting Serotonin Synthesis in the Duodenum, an Entro Bone Endocrine Axis **3**
Gerard Karsenty, M.D., Ph.D., Columbia University, New York, New York, USA

9:50 a.m. Young Investigator Award Presentation
Neuro-anatomical Basis of Brain-derived Serotonin Regulation of Bone Remodeling **4**
Franck Oury, Columbia University, New York, New York, USA

10:05 a.m. TGFß1 and its Function as a Coupling Agent in Bone Turnover **5**
Xu Cao, Ph.D., University of Alabama at Birmingham, Birmingham, Alabama, USA

BREAK/EXHIBITS OPEN
10:30 a.m. – 10:45 a.m.
Grand Ballroom Foyer

POSTER SESSION I
10:45 a.m. – 11:30 a.m.
Grand Ballroom Salon E

LUNCH AND POSTER VIEWING
11:30 a.m. – 12:15 p.m.
Grand Ballroom Salon E–F

SESSION 2:
12:15 p.m. – 2:35 p.m.
Grand Ballroom Salon A–D

Central Control of Bone Remodeling
Moderator: Edith Gardner, Ph.D., Garvan Institute of Medical Research, Seattle, Washington, USA

		Presentation Number
12:15 p.m.	**Keynote: CNS Pathways Regulating Body Weight and Glucose Homeostasis** Joel Elmquist, D.V.M., Ph.D., The University of Texas Southwestern Medical Center, Dallas, Texas, USA	**6**
1:05 p.m.	**Molecular and Neural Mechanisms of Leptin Action** Martin Myers Jr., M.D., Ph.D., University of Michigan, Ann Arbor, Michigan, USA	**7**
1:30 p.m.	**Poster Oral Presentation** **Hypothalamic Expression of Truncated Activator Protein-1 Family Member Increases Bone Formation and Energy Expenditure, Leading to High Bone and Low Fat Mass** Glenn Rowe, Harvard Medical School, Boston, Massachusetts, USA	**8**
1:45 p.m.	**Genetic and Molecular Understanding of Serotonin Signaling in the Brain** Vijay Yadav, Ph.D., Columbia University, New York City, New York, USA	**9**
2:10 p.m.	**Neuromedin U and its Function in the Hypothalamus** Shu Takeda, M.D., Ph.D., Tokyo Medical and Dental University, Tokyo, Japan	**10**

BREAK/EXHIBITS OPEN
2:35 p.m. – 2:50 p.m.
Grand Ballroom Foyer

SESSION 3:
2:50 p.m. – 5:20 p.m.
Grand Ballroom Salon A–D

Bone, Fat and Brain in the Clinical World
Moderator: Catherine Gordon, M.D., Children's Hospital Boston and Harvard Medical School, Boston, Massachusetts, USA

Presentation Number

2:50 p.m. **Keynote: Anorexia Nervosa: A Paradigm of Neuroendocrine Regulation of Body Composition** **11**
Anne Klibanski, M.D., Massachusetts General Hospital, Boston, Massachusetts, USA

3:40 p.m. **Actions of Locally Generated Glucocorticoids in Metabolism and Inflammation** **12**
Karen Chapman, Ph.D., The Queen's Medical Research Institute, Edinburgh, UK

4:05 p.m. **Imaging Marrow Fat in Humans** **13**
Miriam Bredella, M.D., Ph.D., Massachusetts General Hospital, Boston, Massachusetts, USA

4:30 p.m. **Bariatric Surgery - Benefits and Risks for the Skeleton** **14**
Shonni Silverberg, M.D., Columbia University, New York, New York, USA

4:55 p.m. **Depression and Osteoporosis** **15**
Philip Gold, M.D., National Institutes of Health, Bethesda, Maryland, USA

Poster Viewing Open
5:20 p.m. – 5:45 p.m.
Grand Ballroom E

Welcome and Networking Reception
5:45 p.m. - 7:30 p.m.
Grand Ballroom Salon F–H

Tuesday, April 28, 2009

BREAKFAST/EXHIBITS OPEN
7:00 a.m. – 8:00 a.m.
Grand Ballroom Foyer

SESSION 4:
8:00 a.m. – 10:35 a.m.
Grand Ballroom Salon A–D

Osteoblast and Adipocyte Differentiation
Moderator: Kassem Moustapha, M.D., Ph.D., Odense University Hospital, Odense, Denmark

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Presentation Number

8:15 a.m. **Keynote: PPARγ Regulates Osteoclastogenesis in Mice** **16**
Ronald Evans, Ph.D., The Salk Institute, San Diego, California, USA

9:05 a.m. **Regulation of Adipocyte Differentiation and Metabolism by Wnt Signaling** **17**
Ormond MacDougald, Ph.D., University of Michigan, Ann Arbor, Michigan, USA

9:30 a.m. **Estrogen Modulation of Marrow Osteoblastogenesis and Adipogenesis** **18**
Sundeep Khosla, M.D., Mayo Clinic College of Medicine, Rochester, Minnesota, USA

Presentation Number

9:55 a.m. **Young Investigator Award Presentation**
Histone Deacetylase 3 Depletion Decreases Bone Density and Increases Marrow Fat by Elevating the Expression of PPARγ2 and Wnt/Beta-catenin Antagonists — **19**
Tiffany Whitney, Mayo Clinic Rochester, Rochester, Minnesota, USA

10:10 a.m. **Progression of Mesenchymal Progenitor Cells into the Osteogenic and Adipogenic Lineages** — **20**
David Rowe, M.D., University of Connecticut Health Center, Farmington, Connecticut, USA

BREAK/EXHIBITS OPEN
10:35 a.m. – 10:50 a.m.
Grand Ballroom Foyer

POSTER SESSION II
10:50 a.m. – 11:35 a.m.
Grand Ballroom Salon E

LUNCH AND POSTER VIEWING/EXHBITS OPEN
11:35 a.m. – 12:20 p.m.
Grand Ballroom Salon F–H

SESSION 5:
12:20 p.m. – 3:20p.m.
Grand Ballroom Salon A–D

Bone as an Endocrine Organ
Moderator: Jennifer J. Westendorf, Ph.D., Mayo Clinic, Rochester, Minnesota, USA

Presentation Number

12:20 p.m. **Keynote: The Developmental Origins of Fat and its Impact on Disease** — **21**
Ronald Kahn, M.D., Joslin Diabetes Center, Boston, Massachusetts, USA

1:10 p.m. **Insulin Receptor Signaling in Osteoblasts Regulates Bone and Body Composition** — **22**
Thomas L. Clemens, Ph.D., University of Alabama at Birmingham, Birmingham, Alabama, USA

1:35 p.m. **FoxO1 Contributes Through its Osteoblastic Expression to the Endocrine Function of Bone** — **23**
Stavroula Kousteni, Ph.D., Columbia University Medical Center, New York, New York, USA

2:00 p.m. **Regulation of Beta-Cell Differentiation** — **24**
Klaus H. Kaestner, Ph.D., University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

BREAK/EXHIBITS OPEN
2:25 p.m. – 2:40 p.m.
Grand Ballroom Foyer

SESSION 5 (Continued)
2:40 p.m. – 3:20 p.m.
Grand Ballroom Salon A–D

Presentation Number

2:40 p.m. **Young Investigator Award Presentation** **25**
Serum Osteocalcin Concentrations, Variants in the Osteocalcin Gene, and their Relationship to Diabetes, Adiponectin Concentration and Visceral Adipose Tissue Volume in Adult Men and Women: The Framingham Study
Yi-Hsiang Hsu, Harvard University, Boston, Massachusetts, USA

2:55 p.m. **Research Perspective on Clinical Implications of Thiazolidinediones Effects on Bone and Bone Fat Metabolism** **26**
Beata Lecka-Czernik, Ph.D., University of Toledo College of Medicine, Toledo, Ohio, USA

MEETING WRAP-UP
3:20 p.m. – 3:50 p.m.
Grand Ballroom Salon A–D

3:20 p.m. **Overall Summary with a Question and Answer Panel from the Five Moderators**
Clifford Rosen, M.D., Organizing Committee Co-Chair, Maine Medical Center Research Institute, Scarborough, Maine, USA

Gerard Karsenty, M.D., Ph.D., Organizing Committee Co-Chair, Columbia University, New York, New York, USA

Meeting Adjourns
3:50 p.m.

Note: *"*" in the author block refers to presenting author*

Session 1: Novel Endocrine Regulators of Bone Remodeling

1

Endocrine and Paracrine Regulation of the Skeleton

H.M. Kronenberg*. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

The skeleton acts locally to protect vital organs and to act as lever arms for muscle movement. Local paracrine regulation is essential to assure that the shape, size, and strength of bones are optimal for these functions. The skeleton also is a major regulator of mineral metabolism and hematopoiesis; hormones regulate these systemic functions. The effective integration of local regulators and hormonal regulators is crucial for maintaining these multiple functions of bone. Some hormones, like growth hormone act partly through insulin-like growth factor-1 (IGF-1) produced in the liver and also by IGF-1 produced locally in bone as a paracrine factor. Other hormones, like parathyroid hormone (PTH), act through receptors that also respond to the paracrine factor, PTH-related protein (PTHrP). Actions of hormones such as PTH are modulated by those of other hormones, such as 1,25 dihydroxyvitamin D, made in the kidney in response to PTH, and by cells in the skeletal micro-environment, such as T cells. PTH modulates the synthesis of some paracrine factors, such as IGF-1 and fibroblast growth factor-2 and regulates the actions of actions of other paracrine factors, such as wnts, through multiple mechanisms.

How and whether the PTH/PTHrP receptor distinguishes activation by PTH vs. locally produced PTHrP remains uncertain. Recent studies suggest that PTHrP (1-36) exits the receptor considerably more quickly than does PTH, with implications for the cellular processing of these two ligands and for the kinetics of signaling. These differences may well reflect the differing teleologic constraints on hormonal vs. paracrine regulation of the receptor for these two ligands. Both ligands have complex actions on bone, increasing both bone formation and resorption by changing the cellular make-up of bones, as well as the activity of mature osteoblasts, osteocytes, and osteoclasts. Binding of PTH to the PTH/PTHrP receptor activates multiple G proteins. The phenotypes of genetically altered mice suggest the fundamental importance of Gs as a mediator of the actions of PTH and many other hormones and paracrine factors on osteoblasts. Absence of Gs α from cells of the osteoblast lineage leads to bones so weak that they fracture at birth. Mice with PTH/PTHrP receptors that cannot activate Gq/G11 also have osteoporosis. When such mice are given PTH infusions or are placed on a low calcium diet, the marrow stromal cell accumulation found in normal mice ("osteitis fibrosa") fails to occur. Thus, multiple G proteins are important for mediating actions of PTH and probably PTHrP on cells of the osteoblast lineage.

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

2

An Update on the Pituitary-Bone Axis

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We provide an update on our studies that began with our description, in 2003, of the skeletal phenotype of mice lacking the TSH receptor[1]. TSH receptor haploinsufficient mice showed a normal thyroid axis, but were osteopenic, suggesting that the effects of TSH signaling were independent of thyroid hormone[1]. In separate studies, we found that FSHβ haploinsufficiency in mice caused a reduction in bone resorption and a high bone mass in the face of normal estrogen[2]. With the demonstration *in vitro* that TSH and FSH were anti- and pro-resorptive, we predicted that the respective anterior pituitary hormones can bypass traditional endocrine organs to exert effects on the skeleton with remarkable sensitivity. Since then, we have demonstrated that TSH prevents bone loss in ovariectomized mice[3]; that over-expression of the TSH receptor reduces osteoclast formation[3]; that the absence of the osteoclastogenic cytokine TNF$\acute{\alpha}$ in double mutant, $TSHR^{-/-}/TNF\acute{\alpha}^{-/-}$, rescues the $TSHR^{-/-}$ phenotype[4]; and more recently, that TSH attenuates the formation of osteoclasts from embryonic stem cells *in vitro*. Furthermore, we recently showed that oxytocin, a neurohypophyseal hormone, *hitherto* thought solely to modulate lactation and social bonding, regulates bone mass directly. Deletion of OT or the OT receptor in male or female mice causes osteoporosis resulting from reduced bone formation. Consistent with this, oxytocin stimulates the differentiation of osteoblasts to a mineralizing phenotype by causing the up-regulation of BMP-2, Osterix and ATF-4. Oxytocin has dual effects on the osteoclast: it stimulates osteoclast formation both directly, by activating NF-□B and MAP kinase signaling, and indirectly, by up-regulating RANK-L. On the other hand, it inhibits bone resorption by mature osteoclasts by triggering cytosolic Ca^{2+} release and nitric oxide synthesis. The complementary genetic and pharmacologic studies reveal oxytocin as a novel anabolic regulator of bone mass.

References

[1]Abe, E., et al., (2003) *Cell* 175: 151-162; [2]Sun, L., et al., (2006) *Cell* 125: 247-260; [3]Sun. L., et al., (2008) *PNAS* 105: 4289-4294; [4]Hase, et al., (2006) *PNAS* 103: 12849-12854; [5]Tamma, et al., (2009) *PNAS.* In press.

***Disclosures**: **M. Zaidi**, Procter & Gamble 2; Sanofi-Aventis 8; Novartis 8; Roche 8; GlaxoSmithKline 8.*

3

Lrp5 Controls Bone Formation by Inhibiting Serotonin Synthesis in the Duodenum, an Entro Bone Endocrine Axis

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Loss- and gain-of-function mutations in the broadly expressed gene Lrp5 affect bone formation, causing osteoporosis and high bone mass, respectively. Although Lrp5 is viewed as a Wnt coreceptor, osteoblast-specific disruption of b-Catenin does not affect bone formation. Instead, we show here that Lrp5 inhibits expression of Tph1, the rate-limiting biosynthetic enzyme for serotonin in enterochromaffin cells of the duodenum. Accordingly, decreasing serotonin blood levels normalizes bone formation and bone mass in Lrp5-deficient mice, and gut- but not osteoblast-specific Lrp5 inactivation decreases bone formation in a b-Catenin-independent manner. Moreover, gut-specific activation of Lrp5, or inactivation of Tph1, increases bone mass and prevents ovariectomy-induced bone loss. Serotonin acts on osteoblasts through the Htr1b receptor and CREB to inhibit their proliferation. By identifying duodenumderived serotonin as a hormone inhibiting bone formation in an Lrp5-

dependent manner, this study broadens our understanding of bone remodeling and suggests potential therapies to increase bone mass.

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

4

ASBMR YOUNG INVESTIGATOR AWARD
Neuro-anatomical Basis of Brain-derived Serotonin Regulation of Bone Remodeling

G. Karsenty[1], D. Oury*[1], V. Yadav[1]. [1]Columbia University, New York, NY, USA

The demonstration that serotonin made in brainstem neurons favors bone mass accrual was surprising and prompted us to launch a neuro-anatomical study in order to understand how this function occurs. To that end we performed axon guidance experiment using as a tool the *Rosa26R-Ecfp* mice. In these latter mouse model the *Ecfp* (Enhanced Cyan fluorescent protein) reporter gene containing a floxed transcriptional blocker cassette inserted between the transcription start site and the ATG is placed downstream of the *Rosa26* promoter. Because of the presence of the floxed transcriptional blocker cassette, *Ecfp* can only be expressed after *Cre*-mediated deletion of this blocker. We crossed *Rosa26R-Ecfp* mice with *Sert-Cre* transgenic mice that express *Cre* only in serotonergic neurons. *Ecfp* immuno-histochemistry staining of *Sert-cre*; *Rosa26R-Ecfp* showed that the axons emanating from serotonergic neurons project to multiple locations in the hypothalamus as well as to the thalamus and cortex. Given the proposed role of the hypothalamus in the control of homeostatic functions we focused on this structure for the next set of studies. *In situ* hybridization performed on adjacent sections demonstrated that axonal projections reached *Sim-1*expressing PVH neurons, *Sf1*-expressing VMH neurons, and *Pomc-1* and *Npy*-expressing Arcuate neurons of the hypothalamus. These findings were confirmed by fluorescent dextran tracing. Indeed following anterograde and retrograde labelling in the *Tph2*+/- mice PVN, VMH and Arcuate neurons were targeted by neuronal projections emanating from *Tph2*-expressing neurons in the raphe nuclei. These morphological data indicating that serotonin signals to various hypothalamic neurons provide a neuro-anatomical basis for the osteogenic function of brain-derived serotonin.

***Disclosures: F. Oury**, None*

5

TGFß1 and its Function as a Coupling Agent in Bone Turnover

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In the adult skeleton, bone is continuously being formed and resorbed. This bone remodeling process is accomplished by a precise coordination of the activities of two cell types: osteoblasts, which deposit the calcified bone matrix, and osteoclasts, which resorb bone. Bone resorption and formation do not occur along the bone surface at random. Rather, they occur at specific anatomical sites and follow a well-defined sequence of events, which is known as the bone remodeling cycle. Disturbances of the bone remodeling process are often associated with skeletal diseases, including Camurati-Engelmann disease (CED), which is an inherited skeleton remodeling disorder, characterized by a fusiform thickening of the diaphyses of the long bones and skull.
It has been thought for some time that bone resorption and formation are coupled through release of factor(s), from the bone matrix during osteoclastic bone resorption that directs migration of bone marrow stromal cells (BMSCs) to the bone resorptive surfaces. We show that the active TGFβ1 released in response to osteoclastic bone resorption induces migration of BMSCs in vitro. Moreover, we also demonstrate that the active TGFβ1 released during bone resorption coordinates bone formation by inducing migration of BMSCs to the bone resorptive sites in different animal models. This process is mediated through the Smad signaling pathway. Analysis of a mouse model that we established carrying a CED-derived TGFβ1 mutation, which exhibits the typical progressive diaphyseal dysplasia seen in CED patients with over 50% tibial fractures, we found high levels of active TGFβ1 in the bone marrow. Treatment with a TGFβ type I receptor inhibitor partially rescued the uncoupled bone remodeling and prevented the tibial fractures. Thus, TGFβ1 functions as a primary factor for recruitment of BMSCs to the bone remodeling surfaces in the coupling process. Therapy based on modulation of the activity of the factor(s) coupling bone resorption and formation could be an effective treatment for the bone remodeling diseases.

***Disclosures: X. Cao,** None*

Session 2: Central Control of Bone Remodeling

6

CNS Pathways Regulating Body Weight and Glucose Homeostasis

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Metabolic cues such as leptin and insulin directly act on key collection of neurons both within and outside the hypothalamus to regulate food intake and body weight and glucose homeostasis. In addition, it is now established that classic neurotransmitters may also act on the same neuronal groups to regulate energy balance. However, the inherent complexity of these CNS circuits has made it extremely difficult to definitively identify the key neurons that are required to maintain glucose homeostasis and energy balance. Over the past several years the ability to manipulate gene expression in a neuron-specific fashion has become feasible. In this talk, we will describe some our recent findings using mouse models that allow neuron-specific manipulation of receptors regulating energy balance and glucose homeostasis. We will focus much of our attention on the role of insulin and leptin to regulate melanocortin neurons. We will also describe results using a novel mouse model to investigate the role of serotonin action on hypothalamic POMC neurons to regulate food intake, body weight and glucose homeostasis.

***Disclosures: J.K. Elmquist,** Sanofi-Aventis 2.*

7

Molecular and Neural Mechanisms of Leptin Action

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Purpose: To determine the molecular signals and neural populations by which leptin acts in the central nervous system to mediate specific aspects of physiologic regulation.
Methods: We generated and physiologically characterized multiple "knock-in" mouse lines in which the leptin receptor gene (Lepr) was replaced by the coding sequences for mutant receptors unable to mediate specific intracellular signals. We also generated additional mouse lines to permit the neuroanatomic analysis of specific populations of leptin receptor-expressing neurons in the brain.

Results and conclusions: Leptin receptors null for STAT3 signaling produced obese mice with db/db-like vertebral trabecular bone, but with some preserved neuroendocrine and other functions, suggesting an important role for this signal in energy intake and expenditure. Leptin receptors null for SHP2/SOCS3 binding were lean and leptin sensitive, suggesting a role for this signal in feedback inhibition of the receptor. Leptin receptor neurons in the PMV and LHA link leptin action to the reproductive and mesolimbic dopamine systems, respectively.

Disclosures: M.G. Myers, Jr., *Amylin Pharmaceuticals 5.*

8

ASBMR POSTER ORAL PRESENTATION

Hypothalamic Expression of Truncated Activator Protein-1 Family Member Increases Bone Formation and Energy Expenditure, Leading to High Bone and Low Fat Mass

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Central effectors have been shown to play a pivotal role in the regulation of both energy balance and bone homeostasis. Overexpression of ΔFosB in mice under the control of the enolase 2 promoter (ENO2), which drives expression in bone, fat and the brain, results in increased bone formation, increased energy expenditure and decreased adipose mass (Rowe et al., Endocrinology 2009). Furthermore, Δ2ΔFosB a further truncated form of ΔFosB with further reduced AP-1 transcriptional activity was able to recapitulate the bone and adipose phenotype observed in the ENO2-ΔFosB mice, suggesting that decreased AP-1 activity was involved in inducing both phenotypes (Sabatakos et al., JBMR 2008). Although targeted expression of ΔFosB to the osteoblasts (OBs) with the osteocalcin promoter (OG2) was able to recapitulate the bone phenotype observed in the ENO2-ΔFosB mice, it had no effect on adipose mass, indicating that the decrease in fat is not OB-dependent. Furthermore, targeted expression of ΔFosB to the adipocyte with the adipocyte protein 2 (aP2) promoter failed to recapitulate either the bone or the adipose phenotype. Taken together these observations suggested that the bone phenotype had a cell-autonomous component but the adipose phenotype did not. As the fat phenotype was not OB-dependent either, as shown in the OG2-ΔFosB mice, we then explored the possibility of a central effect of ΔFosB, as it is highly expressed in the brain, both endogenously and when driven by the ENO2 promoter. *In situ* hybridization of the brain revealed that ΔFosB is also overexpressed in the hypothalamus in ENO2-ΔFosB mice. Given the recent evidence of 1.) a central role of AP-1 in the regulation of energy (Hotsmisligil GS Nature 2006) and 2.) a hypothalamic relay in the regulation of bone mass (Takeda and Karsenty Bone 2008), we hypothesized that reduced AP-1 transcriptional activity in the brain could contribute to increase bone formation and/or energy expenditure, this latter leading to decreased adipose mass. To test this hypothesis, we used an artificial antagonist of AP-1 activity, DNJunD, which lacks the transactivation domain, but still retains JunD dimerization and DNA binding abilities. Stereotaxic injections of adeno-associated viruses (AAV) encoding DNJunD was targeted to the ventral portion of the hypothalamus in mice. Six weeks post-injection, the mice injected with DNJunD exhibited an >3-fold increase in mineral apposition rate (MAR) resulting in an >7-fold increase in bone formation rates and a higher bone mass. In addition, the DNJunD-injected mice also had an increase in energy expenditure and reduced adipocyte size, phenocopying the observations in the ENO2-ΔFosB mice. These results suggest that regulation of AP-1 activity in the hypothalamus can lead to profound increases in both bone formation and energy expenditure, leading to increased bone mass and decreased adipose mass respectively.

Disclosures: G. Rowe*, None*

9

Genetic and Molecular Understanding of Serotonin Signaling in the Brain

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Bone remodeling, a homeostatic process by which vertebrates renew their skeleton throughout adult life is regulated by peripheral and central signals. Serotonin, a bioamine synthesized from the essential amino acid tryptophan regulates several homeostatic functions in the body in a neuroendocrine or endocrine manner. In gut the rate-limiting step of serotonin biosynthesis is carried out by the enzyme Tryptophan hydroxylase 1 (Tph1), while in the brain it is carried out by the enzyme called Tryptophan hydroxylase 2 (Tph2). In the context of the recent demonstration of an endocrine pathway regulating bone remodeling through gut-derived serotonin, it is important to understand the effect of brain-derived serotonin in the regulation of bone mass. To address the role of brain-derived serotonin we generated mutant mice unable to synthesize serotonin in the brain (*Tph2*-/- mice). In contrast to the high bone mass in mice that lacked serotonin in the periphery (*Tph1*-/- mice), mice lacking serotonin in the brain (*Tph2*-/- mice) developed severe osteopenia due to a decrease in bone formation and a concomitant increase in bone resorption. Brain-derived serotonin acting through *Htr2c* receptor expressed in the ventromedial hypothalamus positively favors bone mass accrual by inhibiting sympathetic tone. These results of an opposite nature in mice lacking serotonin centrally versus peripherally made us analyze the relative importance of brain- versus gut-derived serotonin in the regulation of bone remodeling. Histomorphometric and μCT analyses of mice that lack serotonin everywhere in the body (*Tph1*-/-;*Tph2*-/- mice) revealed a low bone mass phenotype demonstrating that brain-derived serotonin dominates over gut-derived serotonin when it comes to regulation of bone remodeling. In summary, we have uncovered a molecular framework for the brain serotonergic system that acts through hypothalamus to positively regulate bone mass.

Disclosures: V.K. Yadav, *None*

10

Neuromedin U and its Function in the Hypothalamus

S. Takeda*. Keio University, Tokyo, Japan

We previously demonstrated that leptin inhibits bone formation through the sympathetic nervous system via hypothalamus. In addition, many epidemiological studies confirmed the effect of beta blockers on bone mass or fracture. In search of neuropeptides regulating bone remodeling centrally, we focused on neuromedin U (NMU); NMU is an anorexigenic neuropeptide whose expression is abolished in ob/ob mice and induced by leptin treatment. We found that NMU-deficient (NMU-/-) mice have high bone mass due to an increase in bone formation and osteoblast proliferation.
However, treatment of osteoblasts with NMU failed to demonstrate any effect on osteoblast proliferation. Instead, intracerebroventricular infusion of NMU to NMU-/- mice rescued bone abnormality, suggesting NMU acts in the central nervous system not directly on bone cells to regulate bone remodelling. Surprisingly, inhibition of bone formation by leptin or sympathetic nervous system activation was abolished in NMU-/- mice and, moreover, NMU-/- bone

demonstrated an altered expression of molecular clock genes, a mediator of the inhibition of bone formation by leptin. These results suggest that NMU is the novel central mediator of leptin-dependent

regulation of bone mass. I will summarize the genetic, molecular and physiological bases for the central control of bone remodeling and discusses the future directions of neuroskeletal biology.

Disclosures: S. Takeda, *None*

Session 3: Bone, Fat and Brain in the Clinical World

11

Anorexia Nervosa: A Paradigm of Neuroendocrine Regulation of Body Composition

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In humans, states of extreme undernutrition provide a model of disrupted neuroendocrine regulatory pathways linking brain, appetite regulatory hormones, adipogenesis, and skeletal remodeling. One such disorder is anorexia nervosa (AN), a primary psychiatric disorder affecting up to 1 percent of young women characterized by self-induced starvation, decreased body fat, and rapid, often irreversible bone loss. Inadequate peri-pubertal bone acquisition is a hallmark of this disorder. Approximately 90% of young women with AN are osteopenic and 40% meet criteria for osteoporosis. Bone formation is markedly reduced and bone resorption increased. Fat mass independently predicts bone density. Paradoxically, bone marrow fat content assessed by proton magnetic resonance spectroscopy (1H-MRS), is significantly higher than normal and inversely related to bone mass, suggesting a shift in mesenchymal stem cell differentiation. In AN, a number of neuroendocrine abnormalities include suppressed GnRH pulsatility resulting in deficiencies in ovarian hormones, acquired GH resistance with elevated GH levels and decreased synthesis of IGF-I all contributing to bone loss. Hypercortisolemia, attributable to CRH-ACTH-adrenal axis activation, is determined by decreased fat mass and may suppress bone formation and increase marrow adiposity. A new and important link between fat mass and bone density in AN has emerged from investigation of anorexigenic and orexigenic hormones that impact food regulation, energy expenditure and bone mass and importantly, vary with body fat. Decreased adipokine secretion, specifically leptin, may affect both reproductive function and bone mass. Increased secretion of **a**ppetite regulating hormones which may mediate bone loss in AN include Peptide YY (PYY) an anorexigenic hormone and ghrelin, which is orexigenic. Mean overnight PYY levels strongly and inversely correlate with BMD at multiple sites and are the primary determinant of spine BMD in AN. The orexigenic peptide ghrelin, secreted during fasting, links bone and adipogenesis and predicts changes in spine and whole- body bone mass in adolescents with AN. The marked increases in appetite regulatory hormones and other neuroendocrine abnormalities pose the question as to whether these findings are functional adaptations to starvation and/or primary drivers of impaired adipogenesis, energy expenditure and low skeletal mass.

Disclosures: A. Klibanski, *Tercica 5; Ipsen 5.*

12

Actions of Locally Generated Glucocorticoids in Metabolism and Inflammation

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Purpose: Glucocorticoids exert potent anti-inflammatory effects and are widely used to treat chronic inflammatory conditions such as arthritis. However, excessive glucocorticoid action (eg Cushing's disease) causes obesity, insulin resistance, type 2 diabetes, dyslipidemia and hypertension. Recent work has shown that in the highly prevalent Metabolic Syndrome, which phenotypically resembles Cushing's disease but with normal plasma glucocorticoid levels, *local* glucocorticoid action - within adipose tissue - is increased due to elevated expression of the glucocorticoid amplifying enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). In intact cells, 11β-HSD111β-HSD1 converts intrinsically inert glucocorticoids (cortisone, 11-dehydrocorticosterone) into active forms (cortisol, corticosterone). Inhibition of (or lack of) 11β-HSD1 improves insulin sensitivity in both humans and rodents and shows promise for treatment of type 2 diabetes. This raises the obvious question as to whether the normal action of 11β-HSD1 is anti- or pro-inflammatory?
Methods*:* Inflammation was induced in C57BL/6 (WT) or congenic 11β-HSD1-deficient (KO) mice by intra-peritoneal (i.p.) injection of thioglycollate (sterile peritonitis), intra-pleural injection of carrageenan (pleurisy) or i.p. injection of arthritogenic K/BxN serum (arthritis). Mice were killed and inflammatory response evaluated.
Results: In all 3 experimental models, 11β-HSD1-deficient mice showed earlier and/or more severe inflammation. More cells were elicited in the pleural cavity of KO mice than WT following injection of carrageenan. Similarly, 4h and 24h following injection of thioglycollate, more cells were elicited in the peritoneum of KO mice. Moreover, in WT, 11β-HSD1 activity was highly expressed in cells elicited to the peritoneum (<12-fold higher expression than in resident peritoneal cells), remaining high until the inflammation resolved at 4d. In the arthritis model, onset of inflammation occurred ~1d earlier in KO than in WT mice, and was slower to resolve in KO. At resolution (21d), histology showed the presence of ganglion cysts uniquely in KO, as well as greater exostosis of the bone in KO compared to WT. It is unlikely that altered HPA activation contributed to the arthritis phenotype as plasma corticosterone levels were similar between genotypes 2d following injection of K/BxN serum.
Conclusions: These data demonstrate early and high expression of 11β-HSD1 in pro-inflammatory cells and suggest that 11β-HSD1 is induced early during an inflammatory response to limit acute inflammation.

Disclosures: K. Chapman, *None*

13

Imaging Marrow Fat in Humans

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Purpose: Recent studies have demonstrated an important physiologic link between bone and fat. Bone and fat cells arise from the same mesenchymal precursor stem cells within the bone marrow, capable of differentiation into osteoblasts and adipocytes. Increased bone marrow fat affects biomechanical strength of bone, and studies have suggested that alterations in bone marrow fat content could explain bone fragility and fracture risk independent of bone mineral density

(BMD). Until recently, assessment of bone marrow fat had to be performed with invasive methods. New imaging techniques are available that allow assessment of bone marrow fat non-invasively and can be performed longitudinally.
Methods*:* The following imaging methods will be discussed: dual-energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS).
Results*:* DXA is frequently used to assess BMD in subjects at risk for, or with established osteoporosis. Although DXA does not allow quantification of bone marrow fat, studies have shown an inverse relationship between BMD and bone marrow fat content. QCT can be used to assess bone density; however, there is a high precision error for marrow fat quantification due to volume averaging of bone marrow fat, hematopoietic marrow, and trabecular bone. MRI and MRS have proven to be most useful for the assessment of bone marrow fat in humans. MRI and MRS are preformed on routine clinical scanners, allow evaluation of the entire skeleton, and do not require radiation or administration of contrast agents. MRI allows qualitative analysis of bone marrow. MRS has been used to quantify bone marrow fat content by assessing the lipid to water ratio of marrow. Several studies have shown an inverse correlation between bone marrow fat content assessed by MRS and BMD assessed by DXA. Increased vertebral bone marrow fat content by MRS has been found in postmenopausal women and in subjects with osteoporosis. In a recent study using MRS in young women with anorexia nervosa (AN), we found significantly greater vertebral and femoral bone marrow fat compared to normal-weight controls which was inversely correlated with BMD and other fat depots. This paradoxical increase in marrow fat when subcutaneous and visceral fat were markedly reduced raises important questions as to whether these changes represent a compensatory mechanism, or a default into the fat lineage as a result of impaired osteoblastogenesis.
Conclusion: Bone marrow fat content can be assessed non-invasively with several imaging techniques; most importantly MRI and MRS. Bone marrow fat content is anticipated to be a valuable surrogate marker for osteoporosis and fracture risk and allows new insights into the bone-fat connection.

Disclosures: M.A. Bredella, *None*

14

Bariatric Surgery - Benefits and Risks for the Skeleton

S.J. Silverberg*. Columbia University, New York, NY, USA

Bariatric surgery reverses many of the complications of obesity, and is associated with decreased mortality in obese individuals. This has led to a dramatic increase in the number of such procedures performed worldwide. In the face of this, there is an increasing body of data suggesting that bariatric surgery may have deleterious effects on bone and mineral metabolism.
The preoperative skeletal and mineral metabolism profile of those who undergo bariatric procedures is characterized by high bone density, although the vast majority are vitamin D deficient. Preoperative 25-hydroxyvitamin D levels are indeed best predicted by BMI. Those who are most overweight, African American and those who have limited sun exposure are most likely to be vitamin D deficient. These factors can be used to identify patients at highest risk for vitamin D deficiency, who may benefit from repletion prior to bariatric surgery. The effect of bariatric surgery itself on mineral metabolism and the skeleton depends on the specific procedure undertaken. Data on bilio-pancreatic diversion, jejuno-ileal bypass, gastric bypass and gastric banding procedures will be reviewed. The evolution of bariatric surgery has favored procedures that tend to diminish severe malabsorption, with its attendant marked vitamin D deficiency and secondary hyperparathyroid state. However, malabsorption of calcium and vitamin D are still seen after Roux-en-Y gastric bypass, which remains the gold standard procedure today. Bone loss in both malabsorptive and non-malabsorptive (banding) procedures may be most closely associated with the extent of weight loss itself. Bone loss is most commonly seen at the hip site on bone mineral density testing.
In summary, it is important to recognize and replete preoperative vitamin D deficiency in those who are about to undergo bariatric surgery. Bariatric procedures are associated with bone loss, particularly at weight bearing sites. The effect may be worse in those who have malabsorptive procedures, in which vitamin D deficiency and hyperparathyroidism may contribute to the decline. Future directions for research include: 1) determine the clinical significance of weight loss induced reductions in BMD in patients whose baseline BMD's are frequently high; 2) what regimens best prevent vitamin D deficiency after gastric bypass surgery; and 3) can the reduction in BMD after bariatric surgery be prevented.

Disclosures: S.J. Silverberg, *Merck 2.*

15

Depression and Osteoporosis

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Disclosures: P. Gold, *None*

Session 4: Osteoblast and Adipocyte Differentiation

16

PPARγ Regulates Osteoclastogenesis in Mice

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The nuclear receptor PPARγ is an activator of adipogenesis and a repressor of osteoblastogenesis. PARRg agonists such as Thiazolidinediones (TZD), have been shown to cause bone loss in both mice and rats, in part owing to increased bone resorption. In light of the increased rate of fracture reported in diabetic individuals treated with TZD's in clinical studies including the recent "A Diabetes Outcome Progression Trial (ADOPT) the potential role of PPARγ in osteoclast function and bone resorption remains a clinically important issue.

A recent study reveals an unexpected role for PPARγ and its ligand in promoting osteoclast differentiation in bone resorption. Loss of function by targeted PPARγ deletion impairs osteoclast differentiation in bone resorption, resulting in osteopetrosis and extramedullary hematopoesis. In contrast, gain of function by ligand activation of PPARγ accelerates osteoclast differentiation and bone resorption in a receptor-dependent manner. These findings have potential clinical implications, as they suggest that long-term rosiglitazone usage in the treatment of type II diabetes and insulin resistance may cause osteopetrosis, owing to a combination of decreased bone formation and increased bone resorption. They also suggest that selective PPARγ modulators may provide a new strategy for the treatment of bone diseases associated with increased osteoclast activation, such as osteoporosis and rheumatoid arthritis.

Disclosures: R. Evans, *None*

17

Regulation of Adipocyte Differentiation and Metabolism by Wnt Signaling

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Previous studies have established the Wnt/β-catenin signaling pathway as an important regulator of adipocyte differentiation. Specifically, Wnt10b inhibits preadipocyte differentiation *in vitro* and *in vivo*, and in mesenchymal precursors, Wnt10b shifts the balance of transcription factors in favor of osteoblastogenesis and bone formation. However, the potential effect of Wnts on mature adipocytes has not been investigated. Two members of the Wnt signaling pathway, frizzled 4 (Fz4) and secreted frizzled-related protein 5 (sFRP5), are induced during adipogenesis and expression is further increased with obesity. Consistent with a role for Wnts in regulating adipocyte metabolism, we found that addition of Wnt3a to differentiated 3T3-L1 adipocytes increased intracellular cAMP and extracellular release of glycerol, and these effects were not observed in adipocytes deficient for the Wnt receptor Fz4. Although adipogenesis and expression of most adipocyte genes does not appear to be influenced by a loss of Fz4, lipid accumulation in 3T3-L1 cells is reduced due to impaired *de novo* triacylglycerol synthesis. While the resistance of sFRP5 -/- mice to diet-induced obesity was consistent with our hypothesis that induction of sFRP5 with increasing adipocyte size was a signal to recruit new adipocytes, histological analysis reveals that decreased adipose tissue weight is due to reduced adipocyte size rather than number. Implantation of adipose tissues into genetically obese mice caused hypertrophy of adipocytes from both wild type and sFRP5 -/- mice, but also revealed that sFRP5, perhaps through altered mitochondrial metabolism, is required for development of very large adipocytes. Taken together, these findings suggest that Wnt signaling may be important, not only as a regulator of mesenchymal cell fate, but also as a regulator of adipocyte metabolism.

***Disclosures: O.A. MacDougald**, None*

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Estrogen Modulation of Marrow Osteoblastogenesis and Adipogenesis

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It is now generally accepted that age-related bone loss is due principally to an imbalance between bone resorption and bone formation. Moreover, between the ages of 20 and 90 years, trabecular bone volume decreases by 40% and marrow adipose volume increases by 300%. Further, mean wall thickness, which is an index of the work done by the team of osteoblasts in each basic multicellular unit, decreases by 43% over life. Since osteoblasts and adipocytes are derived from a common progenitor cell, these collective findings have led to the plausible hypothesis that age-related bone loss is due, at least in part, to a relative deficit in osteoblastogenesis and increase in adipogenesis. However, there is also increasing evidence, both *in vitro* and *in vivo*, that estrogen suppresses adipocyte and promotes osteoblast differentiation. Thus, in recent human studies in postmenopausal women, we tested the hypothesis that the apparent "age-related" increase in bone marrow adipocytes is due, at least in part, to estrogen deficiency. We evaluated paired iliac crest bone biopsies in 56 postmenopausal osteoporotic women (mean age, 64 years) treated either with placebo (n = 27) or transdermal estradiol (0.1 mg/d, n = 29) for 1 year. We found that while bone marrow adipocyte volume and number increased (by ~20% each), these parameters were either unchanged or decreased in the estrogen-treated women. These findings thus represent the first *in vivo* demonstration in humans that not only ongoing bone loss, but also the increase in bone marrow adipocyte number and size in postmenopausal women may be due to estrogen deficiency.
In further studies using ERα knock out mice and mice with point mutations in the DNA-binding domain of ERα, we have demonstrated that estrogen suppression of bone marrow adipogenesis requires ERα but does not require the direct DNA binding activity of the receptor. Moreover, since steroid receptor coactivator (SRC)-2 (TIF-2) is an important coactivator for ERα action, we have examined the bone and marrow adipocyte phenotype of SRC-2 knock out mice. These mice do have *in vivo* resistance to estrogen action, but despite this, they demonstrate increased bone mass secondary to increased bone formation. In addition, they have a marked decrease in bone marrow adipocytes and decreased adipogenesis and responses to the PPARγ ligand, rosiglitazone, consistent with concomitant resistance to PPARγ action. These data thus indicate that in this model, the skeletal consequences of resistance to PPARγ action are dominant over estrogen resistance, highlighting the key role played by PPARγ in skeletal metabolism.

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

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ASBMR YOUNG INVESTIGATOR AWARD
Histone Deacetylase 3 Depletion Decreases Bone Density and Increases Marrow Fat by Elevating the Expression of PPARγ2 and Wnt/Beta-catenin Antagonists

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Histone deacetylases (Hdac) remove acetyl groups from lysine residues in histone and non-histone substrates to regulate protein function, chromatin structure and gene expression. We previously demonstrated that Hdac3 represses Runx2 (Cbfa1) activity and blocks osteocalcingene expression in osteoblasts. Hdac3-deficient mice die during early embryogenesis. To determine the role of Hdac3 in bone formation, we crossed mice containing floxed (fl) Hdac3 alleles with transgenic mice expressing Cre recombinase under the control of the osterix (Osx) orosteocalcin (Ocn) promoters. The effects of osteoblast-directed depletion of Hdac3 on bone formation and density were assessed using micro-computed tomography and histomorphometry. Osx-Cre: Hdac3fl/-mice were runted, had dental malocclusions, dome-shaped skulls and severe deficits in intramembranous and endochondral bone formation, and died prematurely. Calvarial bones were significantly thinner and sutures inadequately fused. Bone volume density of the distal femur of 5.6 week male mice was decreased approximately 75% (from 19.5±7.83% to 4.85±2.18%; p=0.004). Trabecular number was significantly decreased approximately 50% (from 6.9±1.1/mm to 3.6±0.63/mm; p=0.0005). Trabecular separation was accordingly increased approximately 50% (from 0.15±0.02 mm to 0.29±0.06 mm; p=0.0007). Trabecular thickness was unchanged. Femurs from the Osx-Cre: Hdac3fl/- null animals had abundant vacuoles resembling adipocyte ghosts. Adipocyte density (AV/TV%) was significantly increased in the Osx-Cre: Hdac3fl/- mice by 85% (from 0.28± 0.16% to 1.94±0.22%; p=0.002). Adipocyte perimeter was elevated by 87% (from 1.78± 0.82/mm to 13.86±5.57/mm; p=0.0275). PPARγ2 and Wnt antagonists, including Axin2, were upregulated in osteoblasts from the Osx-Cre: Hdac3fl/-animals. The Ocn-Cre: Hdac3fl/- male mice had a 44% decrease in bone volume density and 32% decrease

in trabecular number. These results indicate that Hdac3 suppression in osteoblast-lineage cells suppresses canonical Wnt signaling, promotes adipocyte differentiation and is detrimental to skeletal health.

Disclosures: T. Whitney, *None*

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Progression of Mesenchymal Progenitor Cells into the Osteogenic and Adipogenic Lineages

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Purpose of the Study: Develop a series of GFP reporter mice, fluorescent imaging and cell isolation techniques to study lineage progression of mesenchymal progenitor cells.
Method: Candidate reporter transgenic lines were screened for a GFP expression in primary culture and in intact tissues. A method for assessing progenitor cell progression in real time in primary culture that included supravital stains for mineral and neutral fat was implemented. Subpopulations of GFP^+ cells were isolated by FAC for microarray.
Results: The bone lineage can be recognized by the onset of low Col3.6GFP expression in coalescing AP^+ nodules. Their expression intensifies as the nodule become multi-layered and begins to mineralize. Once mineralization is established Oc-GFP activate within the nodule. Multiplexing of GFP colors show that expression of one reporter will extend into an adjacent level of maturation so a single color cell can become double colored. In vitro adipogenesis was assessed in stromal cell cultures expanded for 10 days in non-differentiation medium followed by treatment with rosiglitazone and insulin. Within two days, AP2-GFP activates in a fibroblastic like cell that progresses to a neutral fat+ cells that progresses further to a neutral fat+ cell that loses GFP expression. Up to half of the AP2-GFP/neutral fat+ cells in a stromal cell culture either from bone marrow or fat resided in the myeloid lineage. No obvious morphological feature distinguished the two cell types. Microarray of the two populations show a remarkable similarity of gene activity associated with lipid metabolism.
A multipotential progenitor cell expressing SMAA-GFP can be expanded from bone marrow stromal (BMSC), bone chip outgrowth (BCOC) and adipocyte stromal (AdSC) cultures. BMSC will generate bone and fat nodules while AdSC only make fat under our in vitro induction conditions. Nodules of bone or fat differentiation that develop in BMSC cultures arise in distinctly different regions and show no evidence of transdifferentiation. Microarray of the SMAA positive cells prior to induction have a distinctly different expression pattern with BMSC and BCOC showing a bone/cartilage/tendon signature while AdSC have a response to wound signature.
Conclusion: Despite the similarity in GFP expression, $SMAA^+$ cells appear to represent a heterogeneous population of committed cells. These studies suggest that lineage commitment from multipotential progenitor cells toward the bone or fat lineage occurs very early in the culture or potentially even before the culture is initiated. Differentiation protocols reveal this prior commitment by promoting the development of the mature cellular phenotype. More attention in defining the cellular branch point will be required to fully understand the molecular and cellular events that control this lineage decision.

Disclosures: D. Rowe, *None*

Session 5: Bone as an Endocrine Organ

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The Developmental Origins of Fat and its Impact on Disease

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We are in the midst of a worldwide epidemic of obesity, which is driving in large part parallel epidemics of type 2 diabetes, metabolic syndrome, and other medical complications, including dyslipidemia, increased risk of atherosclerosis, hypertension, non-alcoholic fatty liver disease, reproductive dysfunction and even some cancers and increased neurodegenerative disorders. The development of obesity depends in part on the balance between food intake and caloric utilization; but also on the balance between white adipose tissue, which is the primary site of energy storage, and brown adipose tissue, which is specialized for energy expenditure. In addition, white fat in different depots is heterogeneous with respect to its metabolic functions and impact on overall glucose and lipid homeostasis. Accumulation of excess visceral fat (central or "apple-shaped" obesity) is associated with insulin resistance and increased risk of type 2 diabetes and metabolic syndrome, whereas accumulation of subcutaneous adipose (peripheral or "pear-shaped" obesity) does not have such a risk. Both obesity and body fat distribution are heritable traits, however little is known about the developmental origins of adipose tissue; the control of brown versus white preadipocyte commitment; the control of relative amounts and functional differences between white fat cells in different depots; the heterogeneity and intrinsic differences of white fat cells in different depots; and the exact pathways and intermediates between the embryonic stem cell, the mesodermal/mesenchymal stem cell, the preadipocyte (or preadipocytes) and the mature fat cell.
Recently, we and others have begun to gain some insights into early adipose development and patterning and the role of adipose tissue in different depots in disease risk. By comparing gene expression patterns of adipocytes and stromovascular fraction containing preadipocytes from different depots, we identified a potentially important role for fundamental developmental and patterning genes in this process, including a number of Hox genes, Shox2, T-box-15 (Tbx15), Engrailed-1, Glypican-4, and others. Indeed, each fat depot has its own developmental signature, suggesting heterogeneity in the lineage origins of fat in these depots. For three of these genes (Tbx15, Glypican-4, and HoxA5), the level of expression in humans is highly correlated with the level of obesity, as measure by BMI, and the fat distribution as measured by waist-hip ratio (WHR), suggesting that developmental and patterning genes may play important roles in determination of adipose mass and distribution. Overexpression and knockdown of these developmental genes in preadipocytes or Mesenchymal stem cells results in alterations of differentiation and important functions like the balance between lipogenesis and lipolysis, suggesting that these developmental and patterning genes play important roles in determining number and distribution of adipocytes in different fat depots, and the differential function of adipocytes in these depots, which leads to their differential association with diabetes and metabolic syndrome.
We have also shown important roles of insulin, IGF-1 and BMP signaling in control of adipose development, and in differences between white and brown preadipocyte differentiation. In the case of insulin signaling in adipose development, two insulin receptors substrates (IRS-1 and IRS-3) play particularly important roles. Interestingly, knockout of insulin signaling in adipose tissue, which results in selective insulin resistance in fat, has a beneficial phenotype characterized by leanness; resistance to development of obesity, whole body insulin resistance and glucose intolerance

The differences in physiology between central and peripheral obesity appear to be more likely due to differences in the intrinsic properties of adipose tissue in these depots than to their specific locations, and suggests that, contrary to the view that visceral fat is bad, subcutaneous fat may actually have beneficial properties. Thus, transplantation of visceral fat into a normal mouse into either subcutaneous or Intraabdominal depots has little effect on metabolism, while transplantation of subcutaneous fat into either Intraabdominal or subcutaneous locations actually improves metabolism and reduces tendency to gain weight with age. In addition, the latter improves whole body insulin sensitivity, as well as insulin sensitivity on individual tissues including liver and endogenous adipose tissue.
Taken together these data indicate that the adipose organ is more complex than previously believed. There is determined by a complex and divergent pathway of adipocyte determination and differentiation which eventually culminates in not only white and brown adipose tissue, but white adipocytes of different characteristics. These white adipocytes are also differentially distributed in different depots of the body, where they produce different effects on metabolism, normal physiology and disease risk. Dissecting the developmental origins of fat will provide new insights into the pathophysiology of disease and new, unique opportunities for therapy of obesity and its many related medical complications.

Disclosures: C.R. Kahn, *Plexxikon 1, 5; Sirtris Pharmaceuticals 1, 5; Dicerna Pharmaceuticals 1, 5; Eli Lilly 2.*

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Insulin Receptor Signaling in Osteoblasts Regulates Bone and Body Composition

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Insulin and IGF-1 are evolutionarily conserved hormonal signaling pathways with structurally similar ligands and receptors. Despite activating similar signaling pathways, insulin has evolved to serve primarily metabolic functions, whereas IGF-1 primarily influences growth and lifespan. However, in addition to these familiar roles, IGF-1 and insulin also serve overlapping functions. To define insulin actions in bone, we have compared the phenotype of mice which lack either the IGF-1 receptor (ΔIGF-1R) or the insulin receptor (ΔIR) specifically in osteoblasts. Micro-CT analyses revealed decreased trabecular bone volume in the distal femurs of ΔIGF-1R and ΔIR mice at 6 weeks of age. However, histomorphometric analysis of these bones showed striking differences between ΔIGF-1R and ΔIR mice. Whereas ΔIR mice had significantly reduced osteoblast numbers, ΔIGF-1R mice had normal or increased osteoblasts and demonstrated a mineralization defect not observed in ΔIR mice. Primary osteoblasts lacking IGF-1R demonstrated reduced mineralization which could be "rescued" by the addition of insulin. By contrast, osteoblasts lacking IR failed to differentiate and were not rescued by addition of IGF-1. Affymetrix genechip microarray analysis of mRNA from ΔIGF-1R osteoblasts treated with insulin identified osteocalcin (OC) as an insulin regulated target gene. Moreover, OC concentration in ΔIR conditioned media was decreased significantly compared to controls. Interestingly, ΔIR mice, but not ΔIGF-1R mice, developed a phenotype compatible with "metabolic syndrome"; including increased peripheral fat (qMR and fat pad mass), glucose intolerance and insulin insensitivity (GTT and ITT, respectively). These changes were accompanied by decreased serum insulin and adiponectin. Most importantly, circulating undercarboxylated osteocalcin, a recently identified secretagogue for insulin, was decreased in serum from ΔIR mice. Our findings show that insulin receptor signaling regulates postnatal bone acquisition through mechanisms distinct from IGF-1. Moreover, insulin action in osteoblasts also influences fat accumulation, likely by regulating secretion and bioavailability of osteocalcin.

Disclosures: T.L. Clemens*, None*

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FoxO1 Contributes Through its Osteoblastic Expression to the Endocrine Function of Bone

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Molecular pathways regulating development and metabolic homeostasis share many features in common. Among others, a group of molecules that well exemplify this point is the family of ubiquitous transcription factors known as FOXOs. FOXO1, one of three FOXO proteins in vertebrates, is a well known target of insulin signaling and has been extensively studied for its role to regulate glucose homeostasis and insulin resistance through its expression in the pancreas and liver. Based on these observations and given the recent realization that osteoblasts are endocrine cells favoring glucose homeostasis, we examined the potential metabolic functions that foxo1 may exert through its osteoblastic expression in addition to a more traditional role it oculd play in regulating bone mass. Here we shown that deletion of *Foxo1* specifically from osteoblasts ($Foxo1_{ob}$-/- mice) decreases osteoblast numbers, bone formation rate and bone volume without affecting osteoclast numbers. The decrease in osteoblast numbers appeared to be accounted by decreased osteoblast proliferation as evidenced by reduced expression of *cyclins D1* and *D2* and enhanced expression of the cell cycle inhibitor *p27Kip1* in bone. Important, administration of bromodeoxyuridine to newborn mice indicated a decrease in the number of dividing osteoblasts in the *Foxo1*-deficient animals. By contrast, osteoblast differentiation as assessed by analyzing the expression of *Runx2*, *osterix* and *bone sialoprotein* and the ability of calvaria cells from $Foxo1_{ob}$-/- mice to differentiate when cultured in osteogenic medium, was not affected by this mutation. In addition, $Foxo1_{ob}$-/- mice were hypoglycemic and displayed improved glucose tolerance. These effects appeared to stem from an increase in β-cell proliferation, insulin secretion and insulin sensitivity. Additional molecular as well as metabolic studies show that FOXO1, through its expression in osteoblasts, inhibits β-cell proliferation, insulin secretion and insulin sensitivity. These studies indicate that FOXO1 in osteoblasts controls glucose homeostasis and further support the role of the skeleton as a regulator of energy metabolism.

Disclosures: S. Kousteni, *None*

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Regulation of Beta-Cell Differentiation

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Purpose: Differentiation of beta-cells from pan-endocrine precursors is governed by a complex set of signaling events and intrinsic transcriptional regulators. Among the latter are the Forkhead Box, or FoxA transcription factors.
Methods and Results: Using genetic means, we have shown that FoxA1 and FoxA2 are critical players in development, differentiation, and function of the endocrine pancreas. When FoxA2 is ablated in

mature beta-cells using Cre-loxP technology, insulin secretion becomes deregulated due to defects in gene expression of the glucose sensing machinery, but also the vesicle trafficking apparatus. As a consequence, FoxA2 deficient mice develop hyperinsulinemic hypoglycemia.

Conclusions*:* In summary, we have shown that a single transcriptional regulator controls beta-cell fate and function at multiple stages of ontogeny.

Disclosures: K.H. Kaestner, *None*

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ASBMR YOUNG INVESTIGATOR AWARD

Serum Osteocalcin Concentrations, Variants in the Osteocalcin Gene, and their Relationship to Diabetes, Adiponectin Concentration and Visceral Adipose Tissue Volume in Adult Men and Women: The Framingham Study

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Undercarboxylated osteocalcin (ucOC) in animal studies appears to control blood glucose, stimulate adiponectin expression in adipocytes,and increase insulin sensitivity. To examine whether serum OC concentrations are associated and genetically correlated with risk of diabetes, visceral adipose tissue volume (VAT), plasma fasting glucose, insulin, and adiponectin concentrations in adult human, we performed analyses on 1960 men and women from the Framingham Study. Mean (SD) age and BMI were 60.8 (9.1) years old and 28.1(5.1), respectively. Serum OC, included total OC, ucOC and ucOC%, were measured by a radioimmunoassay. We measured VAT by computed tomography. Seven tag SNPs in the OC gene (*BGLAP)* were genotyped. VAT, insulin and adiponectin were log-transformed. A linear regression with adjustment for age, sex and BMI was used. Generalized estimating equations were performed to correct for within-family correlation. We used multivariate variance-components analysis to estimate pairwise genetic correlations (ρG). Total OC was significantly associated with lower VAT, BMI and waist circumference with or without stratification by gender (p=0.03 to <0.0001).Significantly negative associations were found for insulin and glucose level with total OC, ucOC and ucOC% (p=0.05 to <0.0001). Total OCand ucOC were also associated with higher plasma adiponectin concentration (p=0.003 and 0.007, respectively). We then stratified OCconcentrations into tertiles and estimated the OR of prevalent diabetes by logistic regression with adjustment for age, sex and BMI. Compared to subjects in the highest quartile of OC, individuals with lower serum OC had an increased risk of prevalent diabetes. AdjustedORs (95%CI) were 2.2 (1.5-3.2), 1.8 (1.2-2.5) and 1.5 (1.1-2.2) for total OC, ucOC and ucOC%, respectively. Negative genetic correlations were found for total OC and ucOC with BMI, waist circumference, VAT, insulin and glucose (ρG=-0.33 to -0.61). No significant genetic correlations were found for ucOC% with these metabolic risk factors. There was no significant genetic correlation between adiponectin and OC concentrations. Two SNPs (rs1800247 located in the promoter and rs759330 in the 3' flanking region of *BGLAP* gene with LD r2= 0.07) were strongly associated with total OC, ucOC and ucOC%. Subjects with the rs1800247 CC genotype had lower ucOC (p=0.003) and ahigher risk of diabetes (OR=1.3; p=0.04) compared to subjects with the TT genotype. However, after adjusting for OC concentrations, SNPswere not significantly associated with prevalent diabetes. In conclusion, consistent with animal studies, we observed total OC and ucOC level were negatively associated with indices of central obesity and risk of diabetes. In addition to the variants in *BGLAP* gene, negativegenetic correlations provide evidence for shared genetic covariance that regulate in both bone and energy metabolic systems.

Disclosures: Y. Hsu, *None*

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Research Perspective on Clinical Implications of Thiazolidinediones Effects on Bone and Bone Fat Metabolism

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Clinical evidence shows that type 2 diabetes is associated with increased fracture risk and that the anti-diabetic therapy with TZDs further increases this risk. Age and estrogen status are confounding factors for TZD-induced bone loss and increased fracture risk. Rosiglitazone and pioglitazone, two TZDs in clinical use since 1999, increase insulin sensitivity and decrease blood glucose levels *via* activation of peroxisome proliferator-activated receptor (PPARγ). The PPARγ transcription factor is a DNA-binding nuclear receptor, which regulates glucose metabolism, energy expenditure, fat development and bone mass. In animal models, TZDs induce bone loss by affecting the bone remodeling process. TZDs either suppress new bone formation and/or increase bone resorption, depending on age and estrogen status. In older animals bone loss is more extensive and positively correlates with increased expression of PPARγ in bone. In younger animals it results from decreased bone formation whereas in older animals is due to increased bone resorption. Estrogen deficiency has an additive effect on TZD-induced bone loss. Observed bone loss is associated with changes in the structure and function of bone marrow, which includes decreased number of osteoblasts (OB), increased number of adipocytes (AD) and osteoclasts (OC), and increased support for osteoclastogenesis. In summary, animal studies shows that aging and estrogen deficiency augment TZD-induced bone loss and determine its mechanism.

In bone, PPARγ controls lineage commitment of marrow mesenchymal stem cells (MSC) toward OB and AD, and OC recruitment from the pool of hematopoietic stem cells. PPARγ acts as a positive regulator of OC and AD differentiation and as a negative regulator of OB differentiation. The pro-AD and anti-OB activities of PPARγ can be separated by using ligands of different chemical structures indicating a different mechanism governing these activities. A gene expression profile in MSC following rosiglitazone treatment indicates negative effect on the activity of signaling pathways controlling bone homeostasis, among them Wnt, TGF-β/BMP and IGF-1. This effect is strikingly similar to changes observed during aging and leads to speculation that TZDs accelerate the aging of bone.

While affecting bone remodeling, TZDs increase fat content in bone. Bone fat function is largely unknown. Marrow AD express similar set, as extramedullar AD, of genes involved in glucose and lipids metabolism, and produce adipokines such as leptin, adiponectin and resistin, which are hallmarks of fat endocrine activity. Upon TZDs treatment marrow AD acquire a favorable profile in respect to production of insulin sensitizing adipokines and metabolic function. Thus, marrow fat may contribute to the regulation of energy metabolism in peripheral tissues and should be considered as a target for anti-diabetic therapies.

Disclosures: B. Lecka-Czernik, *None*

POSTERS

Novel Endocrine Regulators of Bone Remodeling

M1

Mechanisms of the Skeletal Role in Vascular Calcification Stimulated by Chronic Kidney Disease (CKD)

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The serum phosphorus is a cardiovascular risk factor in CKD. Epidemiologic studies had suggested this, and in vivo studies have demonstrated that the serum phosphorus directly stimulates vascular calcification (VC) and that hyperphosphatemia was indeed a cardiovascular risk factor in CKD through stimulation of VC. Studies of VC have also demonstrated that renal osteodystrophy contributes to hyperphosphatemia in CKD through excess bone resorption, and correction of renal osteodystrophy represents a therapy for VC. Our model of the adynamic bone disorder/osteoporosis in CKD is associated with a leptin mediated loss of obseity and vascular calcification. In multiple interventions in this model, stimulation of bone formation accompanied reduction of VC. We have observed this with BMP-7 (Davies et al, 2005), with sevelamer carbonate (Mathew, S et al, 2007), with calcitriol and paricalcitol {Mathew et al, 2008} and doxercalciferol (Mathew et al, ASN 2008 abstr). This lead us to develop a hypothesis that relates to human observational studies showing an inverse relationship between VC and bone formation or bone mineral density in CKD (London, GM et al, 2004), osteoporosis (Schulz, E et al, 2004) and diabetes (Carr, J et al 2007). The hypothesis is that heterotopic mineralization inhibits orthotopic bone formation. This hypothesis was tested in the studies reported here. In our hyperphosphatemic animal model of CKD stimulated VC and renal osteodystrophy/osteoporosis, we utilized a non-absorbable phosphorus binder that has no known systemic effects besides inhibition of phosphate absorption, reduction of the serum phosphorus and secondarily, PTH levels. CKD induced VC in part through induction of osteoblastic gene transcription in the aorta by stimulating osterix expression. The osterix transcriptome includes dickkoff-1 (Dkk-1), and by CHIP analysis we demonstrated increased osterix binding to the Dkk-1 promoter leading to increased expression and serum levels of Dkk-1 in the CKD animals. Dkk-1 is a Wnt inhibitor, and Wnt stimulated signal transduction controls adult human and mouse bone mass. Thus, when we lowered the serum Pi, osterix expression was inhibited and Dkk-1 was decreased. Treatment with the non-absorbable Pi binder, LaCO3 3% added to the diet, resulted in significant stimulation of bone formation and the adjusted apposition rate, a marker of bone forming unit number. As a result, the osteoporosis of our animal model was corrected through an increase in bone volume and bone mineral density and improved trabecular architecture shown by microCT. We conclude that the induction VC and stimulation of aortic osterix expression by CKD leads to increased Wnt inhibition capacity in the circulation contributing to the inhibition of bone formation in CKD in addition to the leptin mediated pathway of cachexia.

***Disclosures**: **K. A. Hruska**, Shire, Genzyme 5, 2; Stryker 5; Fresenius 2*

M3

Novel FGF-2-Derived peptides for treatment of Osteoarthritis

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Local therapy has improved healing in preclinical trials of articular conditions. Growth factors have shown promising results for improving cartilage repair. Proof of concept has been developed using recombinant growth factors. However the clinical applications of recombinant growth factors have been limited by their short biologic half life, immunogenicity, large dose needed for therapeutic application and high cost of recombinant growth factors. To overcome previously mentioned short comings, we designed novel synthetic peptides derived from Human FGF-2 amino acid sequence for treatment of osteoarthritis. Novel peptide was modified to increase stability, half-life and decrease immunogenicity. We evaluated the effect of FGF-2-Derived peptide and scrambled control peptide upon the regenerative activities of chondrocytes in three dimensional cultures in vitro. FGF-2-Derived peptide significantly enhanced the survival of both normal and osteoarthertic chondrocytes in a dose-dependant manner compared to control. These data provide further motivation to the use therapeutic peptide approach for the treatment of osteoarthritis.

***Disclosures**: **A. Selim**, None*

M5

Sodium/myo-inositol Cotransporter 1 and Myo-inositol Are Essential for Osteoblastogenesis and Bone Formation

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INTRODUCTION. Myo-inositol (MI), a crucial constituent and essential nutrient for living cells, plays an important role in cell signaling and osmoregulation. MI is present at high concentration in the central nervous system (CNS) and is implicated in a number of neurological diseases. Sodium/myo-inositol cotransporter 1 (SMIT1) is the major cotransporter of MI. SMIT1-/- mice had CNS and peripheral nerves mal-development and they died soon after birth due to central apnea-caused hypoventilation (Berry et al., J Biol Chem 2003. 278:18297-302; Chau et al, FASEB J. 2005;19:1887-9). SMIT1 -/- embryos also had abnormal bone phenotypes with short and curved limbs and reduced vertebral height.
OBJECTIVES. To define the role of SMIT1 and MI in bone formation using in vitro cell models and SMIT1-/-mice and to determinate the reversibility of abnormal bone phenotype in SMIT1-/- mice with MI supplementation.
RESULTS. SMIT1 is expressed in multipotential progenitor C3H10T1/2 cells and preosteoblastic MC3E3-T1 cells, and its expression was enhanced during BMP2-induced osteoblastogenesis. SMIT1-/- adult mice had low bone mineral density (BMD), reduced cortical thickness, sparse and thin trabeculae as shown by microCT scanning. Biglycan staining was decreased in bone trabeculae, indicating defective mineralization. SMIT1-/- mesenchymal stem cells (MSCs) had reduced proliferation, decreased ALP activity and impaired bone nodules formation. SMIT1-/- osteoblasts (OBs) had reduced proliferation, maturation and mineralization abilities. Compared to SMIT1+/+, Runx2 and NFATc1 expression was low in SMIT1-/- MSCs, and osteocalcin was down-regulated in SMIT1-/- OBs. The impaired abilities of SMIT1-/- MSCs and OBs to form

bone could be partly reversed by MI supplementation in the culture medium. This was further validated by partial reversal of abnormal bone phenotype in SMIT1 -/- embryos with prenatal maternal MI supplementation.
CONCLUSION. SMIT-1 and MI are essential for osteoblastogenesis, bone formation and BMD determination.

***Disclosures**: **Z. Dai**, None*

Central Control of Bone Remodeling

M7

The Neuromodulator Oxytocin inhibits bone formation through the activation of the sympathetic tone

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The neuropeptide Oxytocin (OT) is an hypothalamic hormone secreted in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. Oxytocin is involved in the regulation of food intake and energy homeostasis. It has been recently reported that oxytocin neurons in the PVN are a component of a leptin-sensitive signalling circuit between the hypothalamus and caudal brain stem. Indeed oxytocinergic neurons of the PVN are hypothesized to amplify and retransmit leptin signalling from the hypothalamus to the nucleus of the solitary tract. Knock out mice deficient in oxytocin (OTKO) display significantly increased body weight and 40% increase in fat pad weight compared with the wild type (WT) littermates. This phenotype is evident at both 4 and 6 months of age. Oxytocin depletion in our murin model also results in a metabolic phenotype characterized by higher fasting glucose levels, insulin resistance and glucose intolerance. Paradoxically the obese OTKO mice don't show any changes in food consumption comparing with WT littermates albeit a highly significant increase in plasma leptin levels. Leptin concentration above a certain level invokes a state of resistance to the hormone that is accompanied by obesity, hypogonadism and paradoxically high bone mass. The paradox of leptin resistance has been the first evidence linking energy and skeletal homeostasis through the activation of the sympathetic nervous system. On this regard has been reported that adrenalin and dopamine activate the oxytocinergic neurons located in different areas of the brain including PVN. To investigate if hyperleptinaemia following OT deficiency had an impact on bone formation we measured the epinephrine levels and performed an evaluation of trabecular bone of both vertebrae and femours in OTKO mice. Interestingly, we found that OT depletion in our murin model leads to a decrease of the sympathetic tone that results three times lower in OTKO comparing with WT. Consequently OTKO mice have a denser appendicular skeleton with increased mineralization of the lumbar vertebrae as well as of the femurs probably due to a "de novo" bone formation by osteoblasts, which are a direct target of the sympathetic nervous system. Based on these results we suggest that the neuromodulator OT can be a new candidate in the central regulation of bone metabolism and a mediator of the antiproliferative action of sympathetic tone on bone formation.

***Disclosures**: **C. Camerino**, None*

M9

Interaction between Neuropeptide Y and Androgens in Control of Bone and Adipose Homeostasis

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Bone and fat mass are regulated by endocrine and neural pathways that originate from the hypothalamus. This spatial relationship in their production suggests the potential for a mechanistic relationship in their action. In keeping with this hypothesis, we have recently shown an interaction between androgens and neuropeptide Y (NPY) in control of osteoblast and adipocyte activity. NPY, a critical component of efferent hypothalamic signaling, acts through central Y2 and peripheral Y1 receptors to regulate bone and fat mass. In bone, the elevated osteoblast activity in NPY Y1 receptor deficient mice (Y1KO) was completely attenuated by orchidectomy (ORX), however, this attenuation was not evident post-ORX in wild type or Y2KO mice. In adipose tissue of Y1KO, ORX induced a marked increase in white adipose tissue mass which was not evident in wild type or Y2KO mice. This differential response between Y1KO and Y2KO mice suggests a receptor-specific interaction between androgens and NPY.
This interaction was further investigated using ORX in NPYKO mice. Mice underwent ORX or sham-operation at 8 weeks of age; skeletal and adipose responses were examined at 16 weeks of age. White adipose tissue (WAT) was decreased post-ORX in wild type (0.98 ±0.08 vs 0.58 ±0.19, $p<0.05$) but not in NPYKO (0.73g ±0.05 vs 0.72 ±0.08), this is in contrast to Y1KO which gained WAT post ORX (0.81 ±0.02 vs 1.28 ±0.14, $p<0.01$). Cancellous bone volume (BV/TV) was reduced by ORX in wild type (7.7 % ±0.9 vs 3.5 ±0.2, $p<0.0001$) and NPYKO (13.4 ±1.8 vs 6.5 ±0.5, $p=0.001$) however, despite this in ORX groups BV/TV remained greater in NPYKO mice than wild type ($p<0000.1$), in contrast to effects in Y1KO mice. The NPY-mediated anabolic phenotype involves greater mineral apposition rate (MAR, um/d). MAR in wild type mice was not affected by ORX (1.1±0.05 vs. 1.2±0.07, ns) but was increased in NPYKO (1.7 ±0.06 vs 2.0 ±0.12, $p=0.02$). This is in contrast to ORX in Y1KO which induced a normalization of BV/TV (wt: 3.9 ±0.8 vs Y1KO 4.8 ±0.8, ns) and MAR (wt: 1.26 ±0.6 vs Y1KO 1.34 ±0.1, ns).
These studies characterise specific interactions between NPY and androgen in fat and bone homeostasis. The similarity between NPYKO and Y2KO responses indicates a central, Y2 dominance in the control of bone and fat post-ORX. However, the unique post-ORX responses involving Y1 receptors indicate an added level of regulatory complexity between androgens and the NPY system which may be of clinical utility.

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

M11 Withdrawn

M13 Withdrawn

M15

Bone phenotype of POMC knockout mice

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The pro-opiomelanocortin (POMC) gene encodes numerous peptide hormones secreted primarily from the pituitary, including alpha-, beta- and gamma-melanocyte stimulating hormones (MSH), adrenocorticotropin (ACTH), beta-lipotropin, and beta-endorphin. Roles for these hormones have been demonstrated in pigmentation, body weight and metabolism regulation, steroid hormone production, and pain modulation. In the hypothalamus, alpha-MSH is secreted in response to elevated leptin levels.
In bone, alpha-MSH has been shown to increase both osteoblast proliferation and osteoclastogenesis in vitro while systemic administration of alpha-MSH reduces bone volume in vivo. Some of the melanocortin receptors as well as the ACTH receptor are expressed in several types of bone cells, but there are few recent studies of the direct effects of ACTH on bone cells, and its activities in vivo are often confounded by the numerous steroid hormones it stimulates. Beta-endorphin is one of several endogenous opioids and this family has generally been shown to be anabolic to bone.
POMC knockout mice (POMC-/-) lack all of the above described peptide hormones, show increased linear growth and develop morbid obesity and pituitary tumors with age. Here we examined this strain for changes in their bone characteristics before the onset of obesity using micro-CT. We found that cortical thickness of tibia was significantly increased in POMC null mice (0.1785mm±0.009SEM vs. 0.1344±0.003, p=0.0139) versus controls. The trabecular region of tibia in POMC null mice trended toward increased trabecular thickness (0.046mm±0.002 vs. 0.044mm±0.0007 controls, p=0.35), with reduced trabecular separation (0.204mm±0.006 vs. 0.230mm±0.03, p=0.45), and showed a trend toward increased bone surface (12.1mm2±2.1 vs. 10.83mm2±0.37, p=0.5). We found average femur length (13.9mm±0.15 vs. 13.4mm±0.23, p=0.16) was increased only slightly in POMC null animals at this stage in development.
Ablation of POMC signaling resulted in changes in bone morphology consistent with some but not all of the constituent POMC hormones. Changes in POMC signaling could impact bone formation during mammalian development and warrants further investigation for possible links to central control of bone metabolism.

***Disclosures**: **J. L. Costa**, None*

Bone, Fat and Brain in the Clinical World

M17

A Novel Locus on Chromosome 11p15 Exerts Pleiotropic and Antagonizing Effects on Bone Mineral Density and Body Weight

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Introduction: Bone mineral density (BMD) is a highly heritable complex trait used to diagnose osteoporosis and assess fracture risk. Increased body weight is associated with increased BMD.
Aim: We performed meta-analysis of five genome-wide association studies on femoral neck (FN) and lumbar spine (LS) BMD in 19,195 Caucasian subjects of European origin and examined the relations with body weight.
Methods: Participants were drawn from the Rotterdam (n=4987;NL; Illumina550), ERF (n=1228;NL; Illumina317), Twins UK (n=2734 women;UK;Illumina317), deCODE (n=6743;IS; Illumina317), Framingham (n=3595;US;Affymetrix500) studies. We analyzed 2,543,686 SNPs imputed with reference to the HapMap CEU panel.
Results: One of the 20 loci identified as associated with BMD at genome-wide significant (GWS, $p<5x10-8$) level includes chromosome 11p15. The top SNP in the region was associated with femoral neck BMD at a GWS level only when adjusting for body weight, (the A allele increased BMD by +0.09 SD, P= 6.4x10-10). In an analysis not adjusting the BMD for weight the effect was 23% lower (+0.07 SD, P=3.1x10-7). The A allele of this SNP was associated with a decrease of 0.6 kg in body weight per copy of the allele in the Rotterdam Study. The closest gene in the region is a transcription factor, part of the conserved high mobility group (HMG) DNA binding domain. The gene is expressed in a wide variety of tissues, most abundantly in skeletal muscle and is a good candidate for BMD determination. No correlation between the SNP and gene expression was observed in human osteoblast lineages. In lymphoblasts, a SNP in high LD (r2=0.95) with the top SNP was associated with expression levels of mRNA levels of NUCB2 (p=9.0x10-4). NUCB2 codes for nesfatin-1, a highly conserved satiety molecule that is associated with melanocortin signaling in the appetite control hypothalamic nuclei in rats.
Conclusion: The A allele of a SNP on 11p15, identified through association with FN-BMD in a meta-analysis of five GWA studies, was associated with an increase in BMD and a decrease in body weight. This region may influence BMD through direct effect on lean mass, while simultaneously regulating food intake by influencing satiety. The compound analysis of expression and genome-wide association allowed us to identify a potential regulatory mechanisms explaining the observed paradoxical inverse relation of this SNP with BMD and body weight.

***Disclosures**: **F. Rivadeneira**, None*

M19

Bone Marrow Fat Quantification using 3T MR Spectroscopy

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Previous studies suggest an inverse association between bone mineral density (BMD) and marrow fat. There is an increasing need to reliably quantify marrow fat, and magnetic resonance spectroscopy (MRS) is a promising option. Previous studies have not evaluated the reproducibility of the MRS measurement of marrow fat. The aim of this study was to evaluate efficacy and precision of MRS at 3T to evaluate vertebral marrow fat.
Ten women (mean age = 59.8 [57 – 67]; mean BMI = 24.1 [18.4 – 33.2]) with low BMD at the spine and/or hip and 5 healthy women (mean age = 37.8 [21 – 55]; mean BMI = 23.1, [21.5 – 24.7]) were studied. The patients' mean BMD T-scores measured with dual-energy X-ray absorptiometry (DXA) at spine and total hip were -1.9 ± 0.7 and -1.4 ± 0.6, respectively. Six subjects (5 controls and 1 patient) were scanned twice to evaluate reproducibility.
MR data were acquired using a GE 3T scanner with a four-channel spine coil. Single voxel MRS were acquired in L1-L4 using a Point RESolved Spectroscopy (PRESS) sequence (TR/TE = 2s/37ms, voxel size = 15×15×20 mm3). The spectral data were reconstructed,

corrected, then fitted with Voigt models for quantifying three peaks using in-house developed software: water (4.65 ppm), unsaturated lipids (2.06 ppm), and saturated lipids (1.3 ppm). Two parameters were calculated:

Fat content (FC) = (Isaturated lipids + Iunsaturated lipids)/(Isaturated lipids + Isaturated lipids+ Iwater) × 100%

Unsaturation Level (UL) = Iunsaturated lipids/(Isaturated lipids + Isaturated lipids) × 100%

The coefficients of variation (CV) of FC and UL were 1.5% and 9.1%, respectively, showing excellent in vivo reproducibility. There was an increasing trend of both FC and UL from L1 to L4 (Figures 1&2) that was significant in both FC and UL between L2 and L3 ($P < 0.05$). The average FC was significantly elevated in patients compared to controls (65.9% ± 7.4% vs. 58.9% ± 7.0%, $P < 0.05$), Figure 3. However, no difference was found in UL between patients and controls in this cohort (16.0% ± 3.0% vs. 15.2% ± 2.7%, $P > 0.05$).

We demonstrated that MRS at 3T is a highly precise method to non-invasively quantify marrow fat contents. Fat contents appear to increase from superior to inferior lumbar vertebrae. Fat content was increased in older patients with low BMD compared with younger healthy controls. We are currently studying a larger cohort to test the relationship between marrow fat and other bone parameters in older women.

Figure 1. Marrow fat content by vertebral level

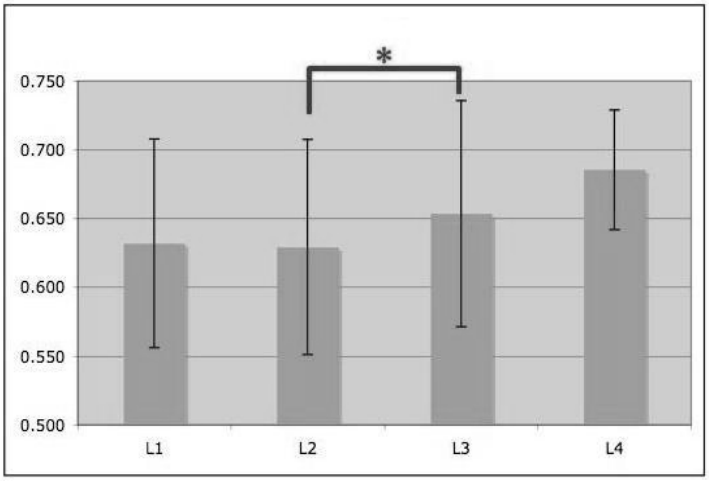


Figure 2. Unsaturation level (L1-L4)

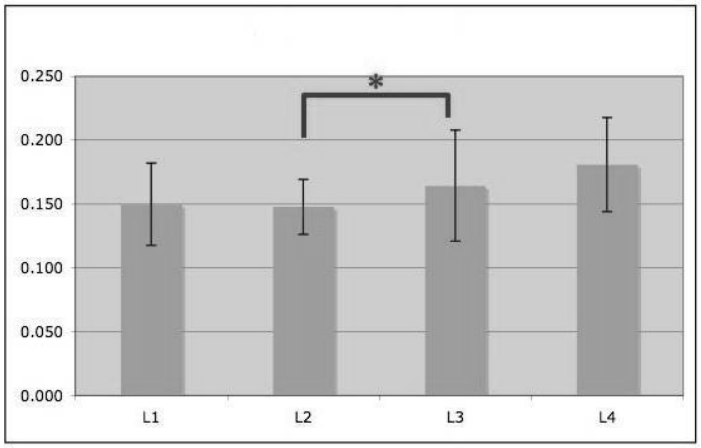
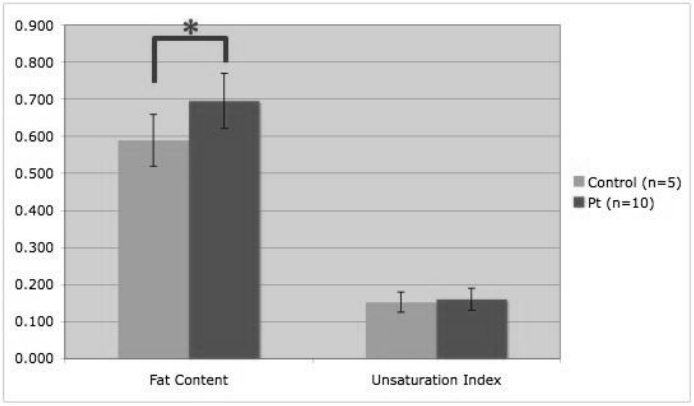


Figure 3. Older patients vs. younger controls



Disclosures: ***A. Schwartz****, None*

M21

Racial differences in effects of glucocorticoids on bone and fat in cardiac transplant recipients

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The risk of osteoporosis and fractures is lower in African Americans (AA) than in Caucasian Americans (CA). It is not clear whether the relative protection from osteoporosis is extended to AA treated with pharmacologic doses of GC. We investigated possible racial differences in bone-fat effects of GC by examining 16 (4 female) AA and 18 (4 female) CA cardiac transplant recipients treated with GC. All subjects had BMD measured at the lumbar spine (LS), femoral neck (FN), total hip (TH) and calcaneus, VFA for diagnosing vertebral fractures, and body composition by DXA (Prodigy, GE Medical Systems). 2 AA (12%) and 3 CA (16%) had vertebral fractures (p=0.8). AA were expected to have higher BMD T-scores based on race as well as tendency towards younger age (55 vs. 60 years, p=0.1), higher weight (101 vs. 92 kg, p=0.3), shorter length of time following transplant (3.5 vs. 4.4 years, p=0.6), and lower prednisone exposure expressed as cumulative grams of prednisone (11 vs. 14 grams, p=0.5), cumulative exposure from over 5 mg/day (8 vs. 10 gm, p=0.06) or current prednisone dose (4.1 vs. 5.6 mg/day, p=0.6). In spite of these factors, AA subjects in our study had similar T-scores at LS (-0.4 vs. -0.3, p=0.8), and tendency towards lower Z-scores at LS (-1.2 vs. -0.1, p=0.056), TH (-1.6 vs. -0.9, p=0.1), FN (-1.3 vs. -1.0, p=0.6) and calcaneus (-0.9 vs. -0.3, p=0.2). When controlling for weight, the Z-scores in AA were lower by 1.4 (p=0.02) at the LS, by 1.0 (p=0.02) at the TH, by 0.5 (p=0.3) at the FN and by 0.7 (p=0.08) at the calcaneus. These differences persisted when controlling for weight gain since transplant, which was similar in AA and CA (9.0 vs. 10.3%, p=0.9), and were even more pronounced when Z-scores were related to self-reported young adult rather than current weight. Similarly, LS Z-scores of AA were lower by 1.3 (p=0.025) when controlling for cumulative prednisone exposure, by 1.1 (p=0.08) when controlling for age, and by 1.3 (p=0.04) when controlling for percent body fat derived from DXA body composition measurement. These findings suggest relatively greater bone loss relative to increase in body fat in AA compared to CA cardiac transplant recipient on chronic GC therapy.

Disclosures*: **T. Vokes**, None*

M23

Trunk Fat Mass and Limb Fat Mass are Both Positively Associated with BMD and Negatively Associated with Bone Turnover

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Previous studies have shown that trunk fat mass is a stronger predictor of cardiovascular and diabetes risk than total fat mass or BMI. Trunk fat mass has also been found to be positively associated with BMD. These results suggest that abdominal fat is more metabolically active than limb fat. We hypothesised that the association of BMD with fat mass is mediated through decreased bone turnover and that bone turnover would correlate more strongly with trunk fat mass than limb fat mass.
The subjects were 304 postmenopausal women, with no disease or medication known to affect bone metabolism, participating in an observational, population-based, cohort study of bone density and fracture. We measured lumbar spine BMD by DXA and regional fat mass with whole body DXA, serum bone turnover markers osteocalcin (OC), procollagen type I C-terminal peptide (PICP), C-telopeptide of type I collagen (CTX) and urine N-telopeptide of type I collagen / creatinine (NTX/Cr).
Data which were not normally distributed were log transformed before analysis. We used regression ("enter" method) of regional fat mass with height squared to correct for body size. Results are shown in the table. All four markers were negatively correlated with trunk fat mass. CTX and OC were negatively correlated with limb fat mass. Lumbar spine and total hip BMD were positively correlated with trunk fat mass and limb fat mass. We calculated 95% confidence intervals of the coefficients for regional fat mass and there were no significant differences in the coefficients for trunk fat and limb fat.
We conclude that bone turnover correlates negatively with trunk and limb fat mass and the association between fat mass and BMD is likely to be mediated through decreased bone turnover. There do not seem to be significant differences between trunk fat and limb fat in their association with bone turnover and bone density.

	n	Trunk fat mass	Limb fat mass
logNTX/Cr	227	0.204**	0.124
logCTX	258	0.197**	0.204**
logOC	295	0.215**	0.184**
logPICP	291	0.177*	0.100
Lumbar spine BMD (g/cm^2)	277	0.339***	0.317***
Total hip BMD (g/cm^2)	303	0.550***	0.510***
R for regression model of marker or BMD on height^2 and regional fat mass * p <0.05, ** p<0.01 ***p<0.001			

***Disclosures**: **J. Walsh**, None*

M25

Fatty Acid Ratios in Osteoarthritic and Osteoporotic Bone

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The purpose of this study was to investigate the ratio of fatty acids in both osteoarthritic and osteoporotic bone.
Recent publications reporting the relationship between an increase in consumption of omega 3 and omega 6 fatty acids (FA's) and the severity of osteoarthritis, have shown a negative effect with omega 6 FA's and a positive effect with omega 3 FA's. Analysis of the fatty acid composition of lipids extracted from osteoarthritic and osteoporotic bone has shown an increase in omega 6 FA's in osteoarthritic bone over that of osteoporotic bone (Plumb and Aspden, 2004).This relationship between omega 6 FA's and OA might be expected as omega 6 FA's are known precursors to the pro-inflammatory eicosaniods.
The adipocyte plays a critical role in energy metabolism, and is derived from the mesenchymal lineage. Excessive fat is known to promote inflammation and arthritic changes in cartilage and synovial membrane. The differential expression of adipogenic genes has been quantified from the trabecular bone of primary hip OA patients. Similarly raised levels of fatty acids have been observed in the cartilage of OA patients. Osteoblasts and adipocytes share a common precursor cell in the bone marrow, and a decrease in bone formation in patients with osteoporosis is the result of enhanced adipogenesis versus osteoblastogenesis from the mesenchymal cells (Justesen et al., 2001). However, a decrease in adipocytes in OA bone marrow compared to OP bone would not be expected if total FA content had increased with that of omega 6 FA's. This study reports the ratio of fatty acids from bone samples collected from patients undergoing total hip replacement for OA, and from hemiarthroplasty for osteoporotic fracture of the femoral neck. Data accumulated from this study will contribute significantly to the understanding of the role of polyunsaturated fatty acids in osteoarthritic patients.
References: Justesen J. Stenderup K. Ebbesen E.N. Mosekilde L. Steiniche T. Kassem M. 2001. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2(3):165-171; Plumb, M.S and Aspden, R.M. 2004. High levels of fat and n-6 fatty acids in cancellous bone in osteoarthritis. Lipids Health Disease 3(12):20-24

***Disclosures**: **J. Humphries**, None*

M27

Follow-up of three sites bone mineral density and bone metabolic markers in osteoporosis patients treated with risedoronate

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Introduction: It is well known that the effectiveness by risedronate administration is a difference among a somatic part. However it is not reported that three sites of bone mineral density (BMD) was measured with the same person at the same time. The purpose of this study is that we clarified association of three sites (lumber spine, proximal femur and radius) of BMD and biochemical markers for bone metabolism by risedronate administration.
Subjects and Methods: 27 Postmenopausal women (age 54-85 yrs. Average 72.5 yrs.) who fulfilled the diagnostic criteria of Japan osteoporosis society were candidated. Three sites of BMD were measured simultaneously using DXA (QDR-4000, Hologic) and serum bone alkaline phosphatase (SBAP) and urinary crosslinked N-teropeptides of thype I collagen (uNTx) were measured before and one year after risedronate administration. Subjects were divided into five groups by increase and decrease every site of BMD. We examined the relation between these proups and markers of bone turnover.
Results: Group A (n=13): all sites of BMD were increased. Group B (n=6): lumber spine and proximal femur were increased. Group C (n=6): limber spine and radius were increased. Group D (n=1): only proximal femur was increased. Group E(n=1): only radius was increased. Risedronate effected on all subjects. Group A had much higher rate of suppressing markers of bone turnover than other

groups. Especially SBAP was significantly reduced on group A against others.
Discussion: Most of the subjects were increased lumber BMD under treatment by oral risedronate, lumber BMD is the appropriate for determination of drug effectiveness. When it is difficult to measure accurately the lumber spine BMD (in case of osteophytes, spondylosis, scoliosis, aorta calcification etc.), you should refer to the other part of BMD and changes of markers of bone turnover.

***Disclosures**: **S. Shimamoto**, None*

M29

The Relationship between Calcium Intake and Calcium Retention in Adolescents is Dependent on BMI

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Incidence of distal forearm fractures in adolescents has doubled over the past 30 years while obesity has almost tripled. In adults, increased weight is associated with increased bone mineral density and decreased fracture risk; however, the opposite seems to be true for adolescents. During puberty, peak height velocity precedes peak bone mineral velocity by about 1 year. This lag in bone consolidation puts adolescents at increased risk for fractures, which may be exacerbated by increased obesity. Furthermore, most adolescents do not meet the recommended intake for calcium, which may contribute to decreased calcium retention and increased fracture risk. We hypothesized that calcium retention would be higher with increasing body-mass-index (BMI) as calcium intake increases.
Pooled data from a series of calcium metabolic balance studies in adolescents conducted between 1990 and 2007 were analyzed to determine the relationship between calcium intake, calcium retention, and BMI in adolescents. Total subjects included were 296 (219 female) adolescents age 10-15y. BMI ranged from 12.6-42.7 kg/m2. Subjects were fed controlled diets with calcium intakes ranging from 600-2400 mg/d. Twenty-four hour fecal and urine samples were collected. Calcium retention was determined by calcium intake minus fecal and urinary calcium losses. Regression and model building techniques were used to determine the relationship between calcium intake, calcium retention, and BMI using Statistical Analysis Software (SAS Institute, Inc., Cary, NC).
Results show that the relationship between calcium intake and calcium retention depends on BMI. As BMI increases, calcium retention is higher with increasing calcium intakes. This implies that increasing calcium intake may mitigate the detrimental impact of overweight on fracture risk in adolescents by increasing bone mass. However, a randomized, controlled trial is needed to test this hypothesis.

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

M31

Regional Fat and Longitudinal Bone Loss in Spinal Cord Injury

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Purpose: Spinal cord injury (SCI) results in increased risk of osteoporosis and low-impact fractures, particularly at the knee. However, during the chronic phase of SCI, the natural history and predictors of longitudinal change in BMD remain incompletely characterized. We conducted a preliminary investigation to assess longitudinal relationships between body fat and BMD at the knee in chronic SCI.
Methods: A convenience sample of 14 persons [age=54.9 (±11) years and injury duration= 21 (±13) years; mean (±sd)] with chronic motor complete (n=10) or incomplete SCI (ASIA D) were recruited from persons participating in a longitudinal health study. A standardized protocol was used to determine BMD at the distal femur and proximal tibia and regional fat analyses a mean of 13.8 months apart (range 12 to 15 months) using a GE Lunar Prodigy or a GE iDXA densitometer (DXA). At the initial scan, %- arm, leg, android (visceral fat), gynoid (hip and thigh fat), and total body fat were calculated. Predictors of annual change in BMD were assessed using linear regression methods.
Results: There was a mean (sd) decrease BMD at the distal femur of [0.051(0.046) mg/cm2/yr or -6.9 (5.2) %/yr, p=0.001] and at the proximal tibia of [0.031(0.055) mg/cm2/yr or -4.8 (7.7) %/yr, p=0.054]. In separate regression models, body mass index (BMI) (p=0.04), %-android fat (p=0.04), and %-total trunk fat (p=0.05) were associated with longitudinal decline in BMD at the distal femur and at the proximal tibia (p=0.06, 0.04, and 0.06, respectively), whereas %-arm, leg, or gynoid fat were not (p=0.20-0.60). The results were similar adjusting for age, duration of injury, and SCI level and severity. Additional predictors of BMD loss were BMD level at the initial exam (p=0.006 at the distal femur and p=0.07 at the proximal tibia). Controlling for initial BMD, the effects of greater BMI on BMD loss was similar at the distal femur (p=0.07), but were diminished for %-android fat (p=0.16), and %-total trunk fat (p=0.20).
Conclusions: Our findings suggest that bone loss occurs at a more rapid rate in chronic SCI than in the general population (~1/%/year after age 30). Factors associated with greater longitudinal decline in BMD included greater visceral fat and greater initial BMD.

***Disclosures**: **L. R. Morse**, None*

Osteoblast and Adipocyte Differentiation

M33

Nocturnin, a peripheral clock gene, regulates bone mass, marrow adiposity, and IGF-I in mice.

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Rosiglitazone (ROZ) treatment of mesenchymal cells (MSCs) suppresses osteoblastogenic genes and increases adipogenic genes. We performed microarray analyses to identify genes whose expression was altered in response to ROZ in MSCs. One such transcript was nocturnin (Noc), a gene originally cloned from Xenopus retinae. Noc is a peripheral clock gene encoding a deadenylase expressed in bone, fat, liver and stem cells. Hepatic Noc mRNA exhibits a circadian rhythm, being highest early at night. RT-PCR from MSCs showed increased Noc expression during adipogenesis but reduced expression during osteoblastogenesis. Thus, we hypothesized that Noc was involved in cell specification and was an important determinant of bone mass. To test this, we analyzed the phenotypes of Noc knockout mice (Noc -/-). Noc -/- mice showed increased trabecular bone volume and increased cortical thickness by microCT, but reduced number of marrow adipocytes. In vitro, calvarial osteoblasts from Noc -/- mice showed enhanced osteoblastogenesis and impaired adipogenesis. Over-expression of Noc in MC3T3-E1 cells suppressed mineralization, while over-expression of Noc in 3T3-L1 cells enhanced adipogenesis. These data indicate that Noc regulates MSC cell fate by favoring adipogenesis

and suppressing osteoblastogenesis. Interestingly, DXA analysis showed that with age Noc-/- mice had progressively enhanced % body fat. At 16 weeks, ovarian fat volume was increased in Noc-/- mice with fewer but larger adipocytes, suggesting that cell hypertrophy rather than number was responsible for increased body adiposity. Next, we focused on downstream targets of Noc. We previously reported that ROZ suppressed IGF-1 expression in MSCs although the mechanism was not known. Calvarial osteoblasts from Noc-/- mice showed increased IGF-1 expression, whereas MC3T3-E1 cells overexpressing Noc (MC3T3-Noc) showed reduced Igf1 mRNA after treatment with actinomycin D. Thus, we hypothesized that Igf1mRNA was a target of Noc de-adenylation. To test this, we isolated the 3'UTR of the Igf1 gene and inserted it into a Luciferase (LUC) vector. When the 6.4 kb full-length 3'UTR was inserted, MC3T3-Noc showed reduced LUC activity, while Noc did not have any effect on the 170 bp short-form 3'UTR. Thus, Noc recognizes a 6.4 kb full-length 3'UTR and degrades IGF-1 mRNA. In summary, Noc has an important role in cell specification of MSCs, and may be regulating bone mass by modulating IGF-1 expression within the skeletal environment.

***Disclosures**: **M. Kawai**, None*

M35

The Role of Cdk6 in Osteogenesis

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Purpose: Previous studies support an important role for the Retinoblastoma protein (pRb) in osteoblast proliferation and differentiation and in bone tumorigenesis. It is also well known that Cdk6 is a direct negative regulator of pRb. Given the above information we decided to explore a potential role of cdk6 in osteogenesis and tumorigenesis of bone.
Methods: In my study I took advantage of cdk6 knock-out and K43M knock-in mutant mouse strains. The K43M mutation renders cdk6 enzymatically dead. Osteoblasts were isolated from calvaria of day 3 post-partum animals, expanded, and used for all in vitro studies. Differentiation assays were done by treating confluent cells with differentiation media containing beta-glycerol phosphate and Ascorbic Acid for 28 days, followed by staining with Alizarin red to detect mineral deposition. Real Time RT–PCR was done by harvesting cells at different points throughout differentiation, followed by conventional protocol. Western blot, immunoprecipitation, kinase assays as well as staining with X-gal for senescence-associated-beta-Gal expression were carried out according to standard protocols.
Results: cdk6-/- primary osteoblasts fail to differentiate in vitro.
cdk6 K43M mutant osteoblasts retain differentiation capacity similar to that from cdk6+/+ mice.
cells isolated from calvaria of cdk6 K43M mutant mice also give rise to adipocytes under osteoblast-specific differentiation conditions.
cdk6-/- cells have a strong proliferative disadvantage in subconfluent and confluent conditions as compared to cdk6+/+ and K43M cultures.
senescence levels in cdk6-/- cells are similar to those in cdk6+/+ cells.
expression of Runx2, a master regulator of osteogenesis, is strongly decreased in cdk6-/-cells as compared to cdk6+/+.
Conclusions: Based on the above results we can conclude that fully functional cdk6 is required for proper osteoblast differentiation in vitro. Its enzymatic activity is dispensable for the initiation and progression of differentiation, but seems to be required for the lineage specification. Cdk6 is crucial for osteoblast proliferation in near confluent or confluent conditions and its absence yields very contact sensitive cells. These results suggest that inactivation of cdk6 may impair de novo bone formation in adult animals, and deregulated cdk6 activity may participate in improper expansion of osteoblasts in certain bone diseases, including cancer.

***Disclosures**: **M. Enos**, None*

M37

Microarray Analysis of Different Cell Populations Derived from Adipogenic Bone Marrow Stromal (BMSC) Cultures

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As a tool to study fat cell differentiation in stromal cell cultures we have used transgenic mice that express GFP under the control of the FABP4 (aP2) promoter. Cells of both the adipose and macrophage/monocyte lineages selectively express this marker in vivo and in vitro. In studies designed to examine gene expression in adipogenic bone marrow stromal cultures we have employed fluorescence activated cell sorting (FACS) to isolate four different populations of cells, based on the presence or absence of GFP versus a fluorescently labeled macrophage/monocyte marker (CD11b-APC) . BMSC cultures from aP2-GFP(topaz) mice were expanded in άMEM+FCS for 10-11 days and then given an adipogenic treatment (ADP; 0.5 □M rosiglitazone, 1 □M insulin). Four days later the cells were harvested, stained with CD11b-APC antibody and separated on a BD FACS Vantage cytometer. The majority of the cells were in the double-negative and the CD11b single-positive (GFP-/CD11b+) populations, with the aP2-GFP single-positive (GFP+/CD11b-) and double-positive (GFP+/CD11b+) populations each accounting for 3-4% of the total cells. Total RNA from each population was extracted for microarray hybridization on the Illumina platform. Intensity values for transcripts from two bioreplicates were normalized and analyzed in Limma and the differentially expressed genes were statistically selected for >1.5-fold changes up or down between any of the 4 different cell populations.
Pattern-based clustering results showed enriched expression of osteoblast-associated genes (e.g. PTH receptor, bone sialoprotein and osteocalcin) in the -/- population. Adipocyte markers such as FABP4, PPAR□, and adiponectin were selectively enriched in both GFP+ populations, while macrophage markers like Emr1 (F4/80) and Csf1r (c-fms) predominated in the CD11b+ groups. These finding are consistent with the cell assignment of each quadrant of the FAC profile. The similarity of the adipogenic signature in the macrophage and adipocyte population was striking. Among genes that distinguished these two cell types are elevated levels of caveolin (Cav1) and transferrin (Trf) in the adipocyte group.
These resutls indicate that it is possible to FACS adipogenic BMSC cultures using an adipocyte-directed GFP transgene plus an established macrophage cell surface marker. This strategy yields sufficient numbers of purified adipose lineage-committed cells for microarray gene expression analyses to be performed.

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

M39

Regulation of Lipid Metabolism-related Genes by Constitutively Active Galpha12 in Osteoblasts

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Heterotrimeric G proteins mediate signaling from cell surface G protein-coupled receptors to downstream effector molecules. The Gs and Gq/11 subfamilies have been associated with differentiation and signaling responses in osteoblastic cells, whereas the role of the G12/13 class in bone has not been well defined. Our previous work

indicated that parathyroid hormone activated phospholipase D through G12/13 and subsequent phosphatidic acid phosphatase action, which was dependent on RhoA and contributed to stimulation of IL-6 promoter activity. In order to further study the function of G12-RhoA-PLD pathway in osteoblasts, we transfected osteoblastic cells UNR106 with constitutively active G12Q226L (CA-G12). The stable transfected cells showed higher alkaline phosphatase activity, increased mineralization and up-regulation of bone matrix proteins (osteopontin and decorin). We then studied the gene expression profiles using real time PCR arrays focus on signaling transduction pathways, which contains 84 genes representing 18 different signal transduction pathways. Fourteen genes were found to be significantly modified in CA-G12-transfected cells compared with empty vector-transfected cells. Most of the genes were involved in mitogenic pathway (c-fos, Junb, Gadd45a, Early growth response 1, Cyclin-dependent kinase inhibitor 1A and Patched 1) and survival pathway (Bcl2, Cxcl1, Telomerase reverse transcriptase and Fas ligand). Most interestingly, several other genes were involved in lipid metabolism pathway, including PPAR gamma, Hepatic nuclear factor 3 beta, C/EBP beta and Hexokinase 2. Using conventional RT-PCR, we confirmed the significant up-regulation of lipid metabolism-related genes in CA-G12 transfected cells. The results suggested that besides the effects on osteoblast differentiation and mineralization, the G12-mediated signaling pathway may also contribute to regulation of lipid metabolism in osteoblasts.

***Disclosures**: **J. Wang**, None*

M41

Regulation of the osteoblast-specific transcription factor Osterix by NO66, a Jumonji family histone demethylase

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Osterix (Osx) is an osteoblast-specific transcription factor required for osteoblast differentiation and bone formation. Osx null mice develop a normal cartilage skeleton but fail to form bone and to express osteoblast-specific marker genes. To better understand the control of transcriptional activation by Osx, we identified Osx-interacting proteins using proteomics approaches. Here we report that a Jumonji C (JmjC)-domain containing protein, called NO66, directly interacts with Osx and inhibits Osx-mediated promoter activation. Knockdown of NO66 in preosteoblast cells triggers accelerated osteoblast differentiation and mineralization as well as a marked stimulation in expression of Osx target genes. We show that NO66 exhibits a JmjC-dependent histone demethylase activity for both H3K4me and H3K36me in vitro and in vivo and that this activity is needed for the regulation of osteoblast-specific promoters. During BMP-2-induced differentiation of preosteoblasts, there is a correlation between the decrease in NO66 occupancy and the increase in Osx occupancy at Osx-target promoters. Our results suggest that the interactions between NO66 and Osx serve to regulate Osx target genes in osteoblasts by modulating histone methylation states.

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

M43

The differentiation of osteoblasts and adipocytes after treatments with parathyroidectomy and cinacalcet hydrochloride in patients with renal hyperparathyroidism

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Purpose: The osteoblast surface increased at 1 week after parathyroidectomy (PTX) for renal hyperparathyroidism, but decreased again at 4 weeks after the surgery (AJKD 2003, NDT 2007). Therefore, the changes of adipocyte volume and number after treatments with cinacalcet hydrochloride (HCl) as well as PTX were investigated. **Methods**: Adipocyte volume (fat volume per unit marrow volume-Fa.V/Ma.V) and adipocyte number (number of fat cells per unit marrow volume-N.Fa/Ma.V), as also osteoblast surface (Ob.S/BS) in cancellous bone were measured before and after PTX in 23 patients with renal hyperparathyroidism. Iliac bone specimens were taken and serum intact PTH (iPTH) levels were measured before and at 1 week after PTX in Group A (n=12) and before and at 4 weeks after PTX in Group B (n=11). In addition, Fa.V/Ma.V, N.Fa/Ma.V and Ob.S/BS were measured before and after 52 weeks of treatment with cinacalcet HCl in patients with renal hyperparathyroidism (Group C, n=4). **Results**: Serum iPTH decreased from 1068.0 (Mean) to 13.5 pg/ml in Group A and from 1235.7 to 14.3 pg/ml in Group B. Ob.S/BS increased from 17.3 to 36.1 % ($p<0.01$); in addition, Fa.V/Ma.V also increased from 14.2 to 25.3 % ($p<0.01$) and N.Fa/Ma.V increased from 14.5 to 18.2 N/mm2 ($p<0.05$) at 1 week after PTX in Group A. Although Ob.S/BS decreased from 20.5 to 11.5 % ($p<0.05$), Fa.V/Ma.V increased from 15.5 to 28.8 % ($p<0.01$) and N.Fa/Ma.V also increased from 14.1 to 19.2 N/mm2 ($p<0.05$) at 4 weeks after PTX in Group B. In Group C, serum iPTH decreased from 1110, 880, 330 and 980 pg/ml to 233, 80, 88 and 116 pg/ml in the 4 patients after the treatment. Fa.V/Ma.V values before/after the treatment were 33.7/34.6 %, 74.7/51.1 %, 75.6/57.9 % and 33.5/34.6 % in these four patients. N.Fa/Ma.V values before/after the treatment were 175.0/177.1 N/mm2, 207.8/143.1 N/mm2, 326.5/283.9 N/mm2 and 174.5/85.6 N/mm2, respectively. Ob.S/BS decreased in all 4 patients after the treatment. **Conclusions**: Although it was reported that marrow stem cells can differentiate into either osteoblasts or adipocytes, it seemed that marrow stem cells simultaneously differentiated into both osteoblasts and adipocytes immediately after PTX (Group A). Long-term hypoparathyroidism may not stimulate the differentiation of osteoblasts; in turn, the adipocytes might suppress the differentiation of osteoblasts (Group B) (Bone 2000). But cinacalcet HCl may have a potential role in reducing the differentiation of adipocytes.

***Disclosures**: **A. Yajima**, None*

M45

Lipoapoptosis of Osteoblasts in vitro could be prevented through the Inhibition of Fatty Acid Synthase

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Purpose: Lipoapoptosis occurs after nonadipose tissues are exposed to an excess of long-chain fatty acids (FA). In the case of aging bone, fat infiltration has been associated with lipoapoptosis of osteoblasts.

However, the mechanisms of osteoblasts lipoapoptosis and its potential prevention have not been elucidated. The purpose of this study was to determine whether osteoblast lipoapoptosis is associated with increased levels of FA, and whether lipoapoptosis in bone could be prevented trough the inhibition of FA synthase.
Methods: We have induced lipoapoptosis in osteoblasts using two different methods: first, we used a two-chamber system to co-culture normal human osteoblasts (NHOst) with differentiating adipocytes; second, we exposed osteoblasts to FA (stearate and palmitate). Both methods were tested in the absence or presence of an inhibitor of FA synthase (cerulenin, 2.5-10 nM). Cell survival, TUNEL assay and caspase 3/7 activity were measured to quantify apoptosis in NHOst.
Results: Direct treatment of NHOst with cerulenin in the absence of adipocytes or FA did not show any effect on osteoblast proliferation and survival. In contrast, NHOst survival was affected by the presence of adipocytes and FA as determined by MTS-Formazan and TUNEL assays as well as higher activation of caspases 3/7. These toxic effects were significantly inhibited (~75%) by addition of cerulenin ($p< 0.001$).
Conclusions: We conclude that lipoapoptosis in bone is associated with the paracrine release of FA within the marrow milieu. Our data indicate that lipoapoptosis of osteoblasts, which may contribute to the age-related changes in bone mass, can be prevented by the inhibition of FA synthase.

***Disclosures**: **P. G. Duque**, None*

M47

A long-term diet enriched in omega-3 fatty acids improves cortical bone structure and mechanical properties in mice

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Purpose: Western diet is characterized by low intake of omega-3 fatty acids, i.e eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A low ratio of omega-3/omega-6 increases risk of chronic disorders, including cardiovascular diseases (CVD) and osteoporosis. In turn, fish oil has been suggested to prevent CVD, bone loss and else. We investigated whether a long-term diet enriched in omega-3s, would improve bone structure and strength in aging mice.
Methods: Thirty female mice received a diet enriched in DHA or EPA (2mg/g of diet) or an isocaloric control diet supplemented with bovine fat (18% of fat), from 3 to 17 months of age. Changes in body composition, bone mass and structure, and biochemical markers of bone turnover were analyzed longitudinally in vivo, whereas biomechanical properties of the femur were analysed by 3-point bending at sacrifice.
Results: DHA and EPA increased fat gain with ageing compared to controls (+76% and +66.5% respectively, vs +42%, $p>0.05$); leptin levels were also higher in EPA and DHA (7.3±0.7ng/ml and 8.5±0.5ng/ml, respectively, vs 4.5±0.9ng/ml in controls, $p<0.01$). EPA, but not DHA, blunted the age-related decline of osteocalcin levels (-70% vs -83% in controls, $p<0.05$), without significant effects on TRAcP5b. However, BMD changes were not affected by either EPA or DHA, nor was the long-term loss of trabecular bone volume in femur (BV/TV -90%, -91%, and -94% in EPA, DHA and controls respectively) or caudal vertebrae. In contrast, cortical volume and thickness increased significantly more with EPA compared to controls (BV: +48.8% vs +35.2%; CtTh: +25.7% vs +18.1%, respectively, $p<0.05$), whereas DHA had no significant effects on these parameters. Accordingly, EPA significantly increased femur bone strength (ultimate stress, 4.3±0.2N-mm vs 5.2±0.4 N-mm; toughness 2515±188N/mm² vs 2075±100N/mm² in controls, all $p<0.05$), and stiffness was not significantly different, whereas DHA also increased ultimate stress (2596±257 N/mm², $p<0.05$).
Conclusions: These data suggest that long-term intake of omega-3s, particularly EPA, may improve structural and mechanical properties of cortical bone in the femur, without affecting trabecular bone loss. These positive effects on cortical bone could be partially explained by an increased of leptin levels and/or bone formation.

***Disclosures**: **N. Bonnet**, None*

M49

The Secreted Protein Ccdc80 Modulates Adipocyte and Osteoblast Differentiation

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Adipocytes and osteoblasts are derived from common mesenchymal progenitor cells and can reciprocally regulate their function via the action of secreted proteins. In this study, we specifically searched for novel genes encoding secreted protein whose expression was modulated during metabolic paradigms (e.g. obesity, fasting), and examined their role during adipocyte and osteoblast differentiation. Using a combination of transcriptional profiling and bioinformatic analysis, we identified coiled-coil domain containing 80 (Ccdc80) as a secreted protein highly enriched in white but not brown adipose tissue. In 3T3-L1 adipocytes, Ccdc80 is expressed and secreted in a biphasic manner during differentiation. Silencing of Ccdc80 by RNAi in adipocytes prevents the induction of C/EBPά and PPARγ, and inhibits lipid accumulation by a mechanism involving, at least in part, blunted repression of T-cell factor-mediated transcriptional activity. The defective adipogenesis in Ccdc80 shRNA-expressing cells was partially prevented by the addition of Ccdc80-containing conditioned medium. Expression of Ccdc80 was also detected in human mesenchymal stem cells derived from bone marrow and C2C12 pluripotent cells. The commitment of C2C12 cells to the osteoblastic but not myogenic lineage increased Ccdc80 expression (2-fold vs non-induced cells). To determine the function of Ccdc80 in osteoblastogenesis, we stably expressed shRNA or transiently transfected siRNA against Ccdc80 in C2C12 cells. Knockdown of Ccdc80 in C2C12 cells resulted in increased alkaline phosphatase activity and expression of the osteoblast-specific transcription factor osterix. The expression of Runx2, another key regulator of osteoblast differentiation, was unaffected by Ccdc80 silencing in C2C12 cells. Taken together, our data suggest that Ccdc80 is a bidirectional modulator of adipocyte and osteoblast differentiation. On the one hand, Ccdc80 is required for the induction of C/EBPά and PPARγ, and lipid accumulation during adipogenesis while, on the other hand, represses osteoblast differentiation by blunting osterix induction downstream of Runx2. Thus, Ccdc80 might be an attractive target for the treatment of bone and metabolic disorders, such as osteoporosis and obesity.

***Disclosures**: **F. Tremblay**, Wyeth 3*

M51

Effects of Strontium on Osteogenic Differentiation in Adipose Tissue Mesenchymal Stem Cells

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Strontium ($Sr2+$) is an alkaline earth trace metal cation that has a high affinity for hydroxyapatite. Strontium ranelate, a drug that has been approved for the treatment of osteoporosis, is composed of two atoms of stable Sr that are combined with ranelic acid. The latter is a carrier, while $Sr2+$ is the active cation with respect to the drug's skeletal effects. The aim of the present study was to evaluate the effect of

Sr2+on osteogenic differentiation of adipose tissue mesenchymal stem cells (AMSCs). Previous work in this laboratory has demonstrated that AMSCs have the same ability to produce bone matrix as bone marrow derived stem cells, while being a better source of stem cells according to their abundance and accessibility. In this study in vitro tests were used to evaluate the ability of Sr2+ to promote the osteogenic differentiation on primary cultures of AMSCs and to evaluate their response in terms of cell proliferation and differentiation.
Cell growth and viability were assessed by [3H]-thymidine incorporation assay and by manually cell counting with a Bürker hemocytometer chamber in the presence of Sr2+ from 1 to 150 µg/ml. Expression of osteoblastic markers (ALP, COLIA1, OCN, OPN, and RUNX2) was examined in all cell lines treated with different concentrations (1-10-100 µg/ml) of Sr2+using quantitative RT-PCR, after 15 and 30 days from osteogenic induction. The data were normalized for GADPH housekeeping genes.
No statistically significant difference was observed in cell proliferation for all primary cell lines cultured in presence of the different concentrations of Sr2+. Regarding gene expression analysis, Sr2+ treatment strongly increased mRNA levels of OCN and RUNX2 in dose dependent manner after 15 and 30 days from osteogenic induction. No effect of Sr2+was observed for the other studied osteoblast differentiation genes.
In conclusion our preliminary results confirm that the Sr2+, at appropriate concentration, can promote osteoblast differentiation of AMSCs throughout the induction of a program of specific marker expression. These findings open the possibility of the use of the Strontium ranelate for the in vivo treatment of cell transplantation in bone regenerations programs.

***Disclosures**: **I. Tognarini**, None*

M53 Withdrawn

M55

Differential Gene Expression in Mechanically Loaded Long Bone Corticies of C57BL/6J and DBA/2J Adult Female Mice

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Age-related osteoporosis affects millions of lives. Identifying genes associated with bone health may increase our ability to prevent and treat bone diseases. Mechanical loading has been shown to impact expression of bone related genes and positively affect bone quality. The aim of this study was to determine the effects of mechanical loading on gene expression in the femoral corticies of adult, female inbred mice. The study examined two strains of mice, C57BL/6J (B6) and DBA/2J (D2). Six mice from each strain were exposed to mechanical loading via tower climbing at 180 days of age for 5 weeks. The mice were motivated to climb by placing their water bottles at the top of the towers. Six control mice of each strain were similarly housed in cages without towers. The left femur from each mouse was removed and cleaned, the marrow was flushed and total RNA within the diaphysis was extracted and labeled. Gene expression was examined using Affymetrix mouse microarrays (chips). Three chips containing similar samples from different mice were used as biological replicates for each strain and treatment (tower vs control) group for a total of 12 chips. The microarray data was analyzed using R and LIMMA. RMA was used for normalization and differential expression was deemed significant at $p \leq 0.05$ after adjustment for multiple comparisons. Bone related genes were differentially expressed across the two strains, including Bmp2, Mepe and Gpnmb, which all had a 2 fold change in expression (Table 1). Interestingly, we found evidence that pleiotrophin (Ptn) expression was increased 2 fold in tower climbers relative to the controls. This change was significant in unadjusted ($p = 0.03$) but not adjusted ($p = 0.29$) data. Ptn has been shown to be a player in regulating bone's response to mechanical loading1 by increasing osteoblast recruitment2. Ptn is also reported to be a negative regulator of adipognesis3, thereby supporting the notion of an inverse relationship between osteogenesis and adipogenesis. In a study of tibia in 10 week old B6 mice, there was a 4 fold increase in Ptn expression due to mechanical loading via 4 pt bending1. The results generated here provide tentative support that Ptn is upregulated in response to more mild exercise-induced mechanical loading in adult mice. Confirmatory real-time PCR studies are ongoing.

1. Xing, et al. J Cell Biochem. 2005; 96:1049-60.
2. Imai, et al. J Cell Biol. 1998; 143: 1113-28.
3. Gu, et al. FEBS Lett. 2007; 581: 382-8.

Table 1 Gene List. Genes differentially expressed relative to strain (B6 vs D2).

Symbol	Name	Function	Results
Bmp2	Bone morphogenetic protein 2	Osteoblast differentiation	D2 > B6
Mepe	Matrix extracellular phosphoglycoprotein	Osteoblast & Osteocyte differentiation	D2 > B6
Gpnmb	Glycoprotein (transmembrane) nmb	Osteoblast differentiation	B6 > D2

***Disclosures**: **M. H. Preston**, None*

M57

Mechanically Biasing the Bone and Fat Phenotype: Long Term (6 month) Exposure to Low Magnitude Mechanical Signals Suppresses Adiposity and Promotes Osteogenesis

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Bone and fat cells are developmentally linked through their precursor, the Mesenchymal Stem Cell. Low magnitude mechanical signals (LMMS) have been shown to be anabolic to bone while suppressing adipose tissue formation, effects achieved at least in part by biasing the bone marrow stromal cell (BMSC) populations towards osteogenesis. Here, the long term impact of LMMS is studied. It is hypothesized that the influence of LMMS will persist over extended periods of time, increasing bone quantity, decreasing adiposity, and enhancing osteogenesis within the BMSC populations. To test this hypothesis, 7w male C57BL/6J mice were exposed to LMMS (90Hz, 0.2g, n=8) or sham loading (n=8) for 24w (15 min/d 5d/w). Over the six month experimental period there was no significant difference in food consumption between groups, nor any significant differences in body weight between control and LMMS animals. At 24w, in vivo µCT (76µm res.) of the abdomen revealed LMMS mice had 35% ($p<0.05$) less total abdominal, 40% ($p<0.05$) less visceral and 25% ($p=0.05$) less subcutaneous fat than controls. At sacrifice, LMMS livers and epididymal fat pads were 9% and 35% lighter than controls ($p<0.05$). Flow cytometry on BMSCs stained with SCA1 and CD90.2 antibodies, showed that LMMS increased the percentage of stem cells (small SCA1+ cells), and osteoprogenitors (larger complex cells SCA1+/CD90.2+) by 24% and 38% ($p<0.05$). µCT (12µm res.) of the proximal tibial metaphysis showed an 11% ($p<0.05$) increase in the cortical bone area of LMMS mice with body mass as a covariate. In this experiment the mechanical suppression of adipogenesis persisted through 24w. Given the similarities in weight and food consumption, and the very small mechanical signal, it appears that suppression of the adipose phenotype is exerted through mechanical influences on the precursor cell population rather than metabolic changes in the resident cell population. The LMMS driven shift in BMSC allocations towards bone formation precludes the commitment of these cells to the adipose lineage, while the lower liver weight in

LMMS animals implies downstream benefits for other physiologic systems. The potential of persistent, systemic benefits from a non-invasive intervention which developmentally suppresses adiposity may hold implications in the treatment of obesity, osteoporosis, and diabetes, and inherently implies that their etiology is linked.

***Disclosures**: **B. Adler,** None*

M59

Cyclic AMP Signaling in Mesenchymal Stem Cells Has Reciprocal Effects on Their Ability to Differentiate into Mature Osteoblasts vs. Mature Adipocytes

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Limited information is available on the role of the cAMP signaling in regulating the differentiation of mesenchymal stem cells (MSCs) into mature osteoblasts and adipocytes. To investigate this, we treated mouse bone marrow stromal cells with the cyclic AMP elevating agent forskolin (fsk), and determined the effect on osteoblast and adipocyte differentiation. In one set of studies, MSCs were induced to differentiate by the addition of ascorbate and beta-glycerophosphate, and cells were then treated with fsk (0.1 mM) for 5 days. Fsk treatment produced a marked reduction in the number of mineralized colonies (control: 56±7.5 vs. fsk: 6.7±1.7) measured by Von Kossa (VK) staining at day 21 in culture. Continuous exposure of cells to hPTH(1-34) (100 nM) after the addition of ascorbate and beta-glycerophosphate completely blocked the appearance of mineralized colonies at day 21. In a second series of studies, we examined the effect of cAMP signaling prior to the induction of differentiation. MSCs were exposed to fsk for 5 days prior to the initiation of differentiation. Fsk was then removed, and osteogenesis or adipogenesis (addition of rosiglitazone, dexamethasone, insulin and IBMX) was induced. Treatment of MSCs with fsk followed by induction of osteogenesis resulted in a dose-dependent increase in the number of VK-positive mineralized colonies at day 21. At a maximal dose (0.1 mM), fsk increased VK-positive colony number by 12-fold over control. Fsk produced no change in the number of alkaline phosphatase-positive colonies, indicating that the effect of fsk treatment was manifest at a relatively late stage of osteoblast differentiation. Treatment of MSCs with fsk followed by induction of adipogenesis resulted in a dose-dependent decrease in adipogenesis measured as the total area of Oil Red O staining. At a maximal dose (0.1 mM), fsk reduced subsequent adipogenesis by over 80%. In summary, once osteogenesis is initiated, cAMP signaling markedly inhibits progression to the late stages of osteoblast differentiation, and this effect can be replicated by continuous exposure to PTH. Strikingly, activation of cAMP signaling in MSCs conditions the cells to more fully differentiate into mature osteoblasts and to less efficiently differentiate into mature adipocytes. It will be of great interest to determine the mechanism underlying this cAMP-dependent conditioning, and to identify the G protein coupled receptors in MSCs that may initiate this response in vivo.

***Disclosures**: **R. Kao**, None*

M61

Mechanical Signals Increase the Pool of Osteoprogenitor Cells During Disuse, Suppress Bone Marrow Adiposity and Accelerate Bone Recovery During Reambulation

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Loss of weight-bearing will bias bone marrow stromal cells towards adipogenesis, ultimately compromising the regenerative capacity of the stem cell pool. Consequences of disuse on the marrow environment are sustained even upon returning to function, thus impeding the rapid and full recovery of bone mass and architecture. Here, it was evaluated whether low-magnitude high-frequency vibrations slow the deterioration of the stem cell population caused by disuse and whether this intervention could help restore the loss in bone mass following reambulation. Male C57BL/6J (n=24) mice were subjected to hindlimb unloading for 3w, followed by 3w of reambulation (normal cage activity). 50% of the disuse mice were subject to daily vibrations (90Hz@0.2g, 15min/d). Following 3w of disuse, vibrated mice had a 91% ($p<0.05$) greater osteogenic bone marrow stromal cell population, but similar trabecular morphology, marrow adiposity and body mass compared to mice that were subjected to disuse without the mechanical intervention. After disuse plus 3w of reambulation, trabecular bone of vibrated mice had a 30% greater bone volume fraction, 98% greater marrow osteoprogenitor population, 83% greater osteoblast surfaces, 59% greater bone formation rates, and a 235% greater ratio of bone lining osteoblasts to marrow adipocytes (all $p<0.05$). Interestingly, body mass of mice not subject to the mechanical intervention during disuse showed a rapid increase in weight upon reambulation, significantly differing from vibrated mice at the end of first week of reambulation, a difference sustained through the 3w recovery period (5-6%, $p<0.05$). These data indicate that the mechanical intervention helped maintain the osteogenic potential of bone marrow cells during disuse, even though there was no apparent response at the tissue level. Continued exposure to the mechanical signal upon reambulation biased the differentiation of bone marrow cells to osteoblasts, as evidenced by the drive towards restoration of bone mass and morphology evident in the vibrated group. By contrast, disuse mice deprived of the mechanical signal, rapidly added fat mass on recovery, indicating a population of stem cells irreversibly committed to an adipogenic lineage. The rapid recovery of bone mass during reambulation as potentiated by low-magnitude vibrations indicated an enhanced regenerative potential and emphasized the critical importance of the mechanical environment on defining the bone/fat phenotype.

***Disclosures**: **E. Ozcivici**, None*

M63

Assessment of Bone Marrow Adiposity in Young Outbred mice

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The physiological function of bone marrow adiposity is unclear. The goal of the present study was to develop complementary approaches to measure marrow fat in different skeletal sites (tibia and femur) and in different regions of each site (proximal and distal) in outbred mice. For the assessment of marrow fat by microcomputed tomography (microCT), femurs and tibiae of 4-week-old CD-1 mice were fixed in formalin, decalcified in EDTA, soaked in 2% osmium tetroxide/5% potassium dichromate and scanned to visualize osmium staining. For the assessment of gene expression in marrow, the epiphyses of the tibiae and femurs were removed and the bones were placed distal end down in a microcentrifuge tube containing an internal sleeve. Bones were centrifuged briefly to remove marrow, which was then extracted for RNA. To evaluate gene expression in marrow derived from the proximal and distal regions, tibiae and femurs were cut into two equal segments at the mid-diaphysis; marrow was harvested from each segment and extracted for RNA. The mRNA expression of adipocyte associated genes was assessed by real-time PCR. For histological assessment, bones were fixed in

formalin and decalcified for 3 weeks. Paraffin-embedded sections were stained with hematoxylin/eosin to visualize adipocyte lacunae. Qualitatively, osmium staining appeared greater in tibiae compared to femurs, and staining was higher in the distal end of the tibia compared to the proximal end. An endocortical arrangement of the osmium staining was seen. Likewise, histological sections showed more abundant adipocyte lacunae in the distal end of the tibia. The ratio of gene expression in tibiae compared to femurs (n=3) was 1.7±0.3 for adiponectin (p=0.05), 1.2±0.2 for fatty acid binding protein 2 (Fabp2) (NS) and 0.9±0.1 for CCAAT/enhancer binding protein alpha (Cebpa) (NS). A preliminary experiment (n=1) showed that expression of adiponectin mRNA was higher in the distal end of the tibia. Taken together, the alternative methods used to assess marrow fat indicate that the distal region of the tibia has greater fat content than the proximal region. Adiponectin expression appears to be a better discriminator of marrow fat compared to Fabp2 and Cebpa.

***Disclosures**: **K. Elias**, None*

M65

Bone Marrow Adipogensis is Affected by Insulin-like Growth Factor-1 Complexes

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Bone marrow fat, mainly adipocytes, accumulates during aging, displaces mineralized tissue and reduces the mechanical integrity of bone, thus increasing fracture risk. Adipocytes provide stroma for maintenance of mesencymal stem cells (MSC) and reside at sites of bone turnover (i.e., endosteal surfaces). There exists an inverse relation between adipocyte and osteoblast genesis with MSC differentiating into either. The production of these cells includes a role for the growth hormone (GH) / insulin-like growth factor-1 (IGF-1) axis. GH is lipolytic, and IGF-1 increases pre-adipocyte replication and differentiation. IGF-1 bioactivity is affected by binding proteins (BP), including an acid-labile subunit (ALS), that form IGF complexes. The purpose of this study was to investigate relationships between adiposity and IGF-1 bioavailability.

We used knockout (KO) mouse models of IGF-1 deficiency: liver-specific IGF-1 deficient (LID), IGFBP-3 (BP3KO) and ALS (ALSKO). All show decreased bone formation [1,2]. We evaluated the differentiation potential of marrow-derived MSCs in primary culture. Cells were FACS analyzed and sorted. Osteoblasts were co-cultured with control non-adherent cells to test ability to support osteoclastogenesis. Studies were approved by IACUC. All mice were 8-16 weeks old and backcrossed >5 generations to C57BL6/J background.

Osteoblastogenesis (alkaline-phosphatase activity) in cultures from ALSKO and BP3KO was significantly greater than in control. ALSKO adipogenesis (adipocyte number) was also significantly greater (Fig. 1). Expression of C/EBPα was at least 1.5 fold greater in marrow from all 3 KOs compared to control. IGF-1 or ALS addition to cultures stimulated or decreased adipogenesis, respectively. ALSKO osteoblasts did not support osteoclastogenesis (Fig. 2).

Ablation of IGF-1 complexes appears to increase adipogenic-potential and to depend on ALS. ALSKO exhibit reduced osteoblast number, surface, and mineral apposition rate compared with LID and control [2]. The differences in osteoblast and adipocyte activity in ALSKO, absent differences in pre-osteoblast IGF-I secretion, lead us to the conclusion that regulation of IGF-1 bioactivity plays a role in determining MSC fate. In vivo examination of these gene deletions will further our understanding of mechanisms behind correlations of low serum levels of IGF-1, increased adiposity and fracture risk.

[1]Yakar et al., 1999, PNAS, 96:7324–9 [2]Yakar et al., 2006, J Endocrin, 189:289–99

Fig. 1. Adipocyte number in MSC cultures.

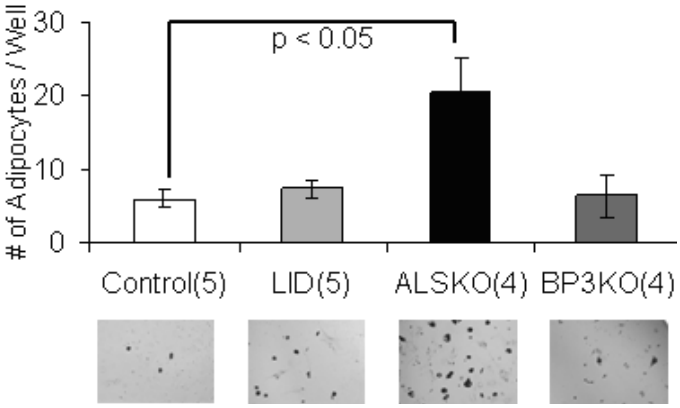
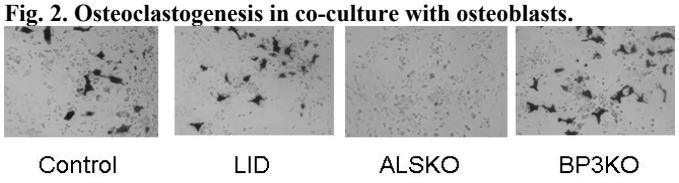


Fig. 2. Osteoclastogenesis in co-culture with osteoblasts.



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

M67

Adipocyte Regulation of Early Heterotopic Ossification

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We have previously reported the rapid appearance and expansion of brown adipocytes in a model of heterotopic ossification (HO). This model relies on induction of HO by delivery of adenovirus transduced cells expressing BMP2 to the quadriceps muscle of a mouse. Microarray and immunohistochemical analysis of this model, as compared to a control that received similar cells transduced with an empty cassette adenovirus, shows the immediate elevation, within 48 hours, of key genes involved in energy metabolism, oxygen regulation, and adipogenesis. We postulated that these cells function to regulate localized oxygen tension through their characteristic robust aerobic respiration, which plays a functional role in patterning of the newly forming bone and cartilage. The study presented here extends this study by attempting to characterize the origin of the brown adipocytes, the regulation that may surround their differentiation and function, and the crosstalk that these cells have with other inflammatory molecules, including production of lymphatics. The brown adipocytes are defined by positive expression of uncoupling protein 1 (UCP1) which is thought to be restricted to this cell type. We further demonstrate the co-expression of two other markers, preadipocyte factor-1 (Pref-1), and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) in these cells, confirming the adipocyte phenotype. To determine the origin of the cells we have employed a tracking mouse which expresses beta-galactosidase in cells which express or have expressed fatty acid

binding protein-4 (FABP4) at one point of their lifespan. Interestingly, we observe the expression of this marker on the sheath of adjacent peripheral nerves. These nerve associated cells, which also express Pref-1 and UCP1, appear to be migrating from the nerve sheath towards the site of new bone formation. Interestingly, at a slightly later time frame we observe the expression of osterix within these migrating cells. From this preliminary data, we hypothesize that the nerve may house tentative progenitors essential for production of HO. Finally, we are investigating the role of inflammatory mediators in this process. Determination of the functional role of adipose, inflammation, and innervation in bone formation, may provide novel targets for the design of drugs that can inhibit HO, as well as targets for molecular imaging agents that can detect HO prior to the deposition of matrix.

***Disclosures**: **M. E. Salisbury**, None*

M69

Role of Conjugated Linoleic Acid in Osteoblast, Adipocyte and Bone Marrow Stem Cell Proliferation

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Recent research suggests a connection between osteoporosis and obesity as low bone mass may be caused by increased proliferation and maturation of adipocytes within the bone marrow. Many nutritional factors are being studied on a cellular level for their possible role in the prevention of osteoporosis and obesity. One of them is conjugated linoleic acid (CLA), a natural component of milk fat, with cis-9, trans-11 (9,11-CLA) and trans-10, cis-12 (10,12-CLA) as the dominant isomers. The objective of this research is to investigate if physiologically relevant levels (20 μM, equivalent to three servings of dairy) of individual isomers or isomer blends (as found in milk) of CLA benefit bone and reduce adipogenesis by using three cell lines: osteoblasts (MC3T3-E1); adipocytes (MC3T3-L1); and stromal stem cells (ST2). Fresh media containing a final concentration of 20 μM of purified individual or CLA isomer blends (Nu Chek Prep Inc., Elysian, MN) were added and cells were grown for 6 days. All experiments were repeated 4 times. A resazurin salt (Sigma-Aldrich, St. Louis, MO) assay was used to determine cell numbers. ANOVA was performed using Graphpad Prism with variation attributed to concentration of treatment compounds and $P < 0.05$ was considered significant. In osteoblastic cells, proliferation was reduced by all combinations of CLA except for the 80% 9,11-CLA/10% 10,12-CLA blend. Similar results were seen for adipocyte proliferation, except the 90% 9,11-CLA/5% 10,12-CLA isomer blend had no effect. In stromal cells, proliferation was significantly reduced only by the 90% 9,11-CLA/5% 10,12-CLA blend. In addition, there was a tendency for proliferation to decrease as the ratio of 9,11-CLA to 10,12-CLA increased indicating the isomers may influence opposing proliferation pathways. Overall, the results show that CLA may negatively regulate the proliferation of all three cell lines at low concentrations (20 μM as in dairy products) therefore, it may be the key isomer in inhibiting adipogenesis. As the studies showing beneficial effects of CLA on bone typically involved higher concentrations, this suggests that CLA may be dose dependent and the Western diet may not have enough of these natural 'bioactive' fatty acids to promote osteoblastogenesis.

***Disclosures**: **O. J. Kelly**, None*

M71

Recapitulating Osteogenesis with Electrospun Fibrinogen and Adipose Stem Cells

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Intro: Addressing the strong need for generating new bone in the clinic and for research, we explore using adipose stem cells (ASC) coupled with electrospun fibrinogen (Fg) nanofibers for synthesizing bone de novo. As ASC hold remarkable osteoblastogenic potential, and that fibrinogen is a primordial wound healing matrix naturally deposited in broken and diseased bone, overwhelming potential exists for creating new bone for research models and for therapeutic grafts in this highly biomimetic approach.
Methods: Electrospun discs of Fg, polydioxanone (PDO) and Fg:PDO blends were seeded with early passage ASCs, BJ fibroblasts, or MG63 osteosarcoma cells and grown for 21 days in osteogenic or regular growth media. For rapid matrix cellularization, ASCs were electrosprayed into a concurrently electrospinning Fg:PDO. Punches of the cellularized spun Fg:PDO where placed in 48 well plates or in NASA designed bioreactors with regular growth or osteogenic media for 21 days. Constructs where harvested weekly and assayed for bone-like histological and morphological features with alkaline phosphatase (AP), Alizarin Red S (ARS), for Osteocalcin expression and also imaged under scanning electron microscopy (SEM).
Results: The appearance of regular, porous, bone-like structures were seen in osteogenic induced ASC seeded scaffolds beyond 14 days with SEM, yet only observed on scaffolds containing Fg. ARS staining indicated pronounced mineralization throughout bone-induced ASC scaffolds with the red staining intensity increasing with Fg content. Unseeded (acellular) and BJ seeded scaffolds negatively stained for ARS and AP, yet all scaffolds showed excellent cellularity via H&E and DAPI. Osteogenic induced ASCs on Fg scaffolds also stained for AP and Osteocalcin. Further, at 21 days of culture, Fg scaffolds with ASCs in osteogenic media became hard and brittle phenotypically. Trichrome stains indicated robust new collagen synthesis and matrix remodeling on all Fg scaffolds, with increased staining seen with time. Remarkably, simultaneous electrospraying ASC within spinning matrix rapidly produced (in 20 minutes) the cellularity observed with over 2 months of culture with conventional seeding of cells atop the scaffold.
Conclusion: Electrospun fibrinogen is an excellent material for ASC growth, proliferation and osteogenic differentiation, providing an outstanding system for furthering basic bone model-based research and for regenerative medicine advances.

***Disclosures**: **M. Francis**, None*

Bone as an Endocrine Organ

M73

The transcription factor ATF4 regulates glucose metabolism through its expression in osteoblasts

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The demonstration that the osteoblast is an endocrine cell type regulating energy metabolism implies that it also expressed cell-specific regulatory genes such as transcription factors involved in this process. ATF4 is a transcription factor that accumulates

predominantly in osteoblasts in vivo where it virtually regulates all functions linked to the maintenance of bone mass such as collagen production, bone formation and osteoclast differentiation. Remarkably, Atf4-/- mice have also very small fat pads, an observation that prompted us to analyze whether ATF4 affects energy metabolism. Here we show that ATF4 normally inhibits insulin secretion and decreases insulin sensitivity in liver, fat and muscle, as a results insulin secretion and sensitivity is increased in Atf4-/- mice. Several lines of evidence indicate that this function of ATF4 occurs through its osteoblastic expression. First mice overexpressing Atf4 in osteoblasts only (a1(I)Collagen-Atf4 mice) display a decrease in insulin secretion and are insulin insensitive as assessed by metabolic studies and molecular markers. Second, this a1(I)Collagen-Atf4 transgene could correct the energy metabolism phenotype of Atf4-/- mice. Third and more definitely mice lacking Atf4 only in osteoblasts present with the same energy metabolism abnormalities as mice lacking Atf4 in all cells. At the molecular level, ATF4 favors expression of Esp, a gene decreasing the bioactivity of osteocalcin, an osteoblast-specific hormone whose roles are to increase insulin secretion and sensitivity. Taken together, these results identify ATF4

as a global transcriptional regulator of all the known functions of the osteoblast and add further credence to the notion that osteoblasts are endocrine cells.

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

M75

Local Regulation of Osteoblast Activity via NPY Signaling

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Similarities exist between osteocytes and neuronal cells; both share a dendritic morphology and the ability to form a network of processes, which is a vital component of neuronal regulatory function. In addition, osteocytes actively participate in the release of regulatory proteins that can modulate osteoblast lineage activity. To elucidate differential gene expression between osteoblasts and osteocytes we completed a comprehensive analysis of their gene profiles. Selective identification of these two populations was achieved by utilization of visual markers of bone lineage cells. We have developed dual GFP reporter mice in which osteocytes are expressing GFP (topaz) directed by a DMP-1 promoter, while osteoblasts are identified by expression of CFP (cyan) driven by 2.3kb of the Col1a1 promoter. Histological analysis of 5-day-old neonatal calvaria confirmed the expression pattern of DMP1-GFP in osteocytes and Col2.3GFP in osteoblasts and osteocytes. Distinct populations of cells were isolated by fluorescent activated cell sorting, and gene expression was analyzed using an Illumina WG-6v1 BeadChip. Results from our micro-array analysis demonstrated elevated expression levels genes encoding proteins that function as part of synaptic signaling and the neurotransmitter-gated ion channels. Significantly higher levels of cholinergic receptors Chrna1, Chrnb1, Chrng and Chrna4 were observed in DMP1topaz+ cells compared to the osteoblast population. In addition we observed an increase in expression of neuropeptide Y (NPY), a neurotransmitter with hormonal functions in fat metabolism and in central regulation of bone mass. Microarray and real-time PCR analysis of RNA demonstrated a 4 fold and 8 fold respectively higher NPY mRNA in the preosteocyte/osteocyte fraction (DMP1topaz+ cells) compared to the osteoblast (Col2.3 Cyan+/DMP1topaz-) cells. NPY immunostaining confirmed the expression of NPY in osteocytes as well as in the mature osteoblasts. More importantly, we observed that the expression of NPY was reduced by 30-40% in models of mechanically loaded bones and in primary calvarial osteoblast cultures. Screening of NPY receptors Y1 and Y2 revealed the presence of Y1 mRNA in long bone and cultured calvarial osteoblasts, while the Y2 mRNA was only detected in the brain. Furthermore, mouse calvarial osteoblasts treated with 1 and 10 nM NPY resulted in the phosphorylation of extracellular regulated kinase 1/2 (ERK1/2). NPY treated cells demonstrated a reduction in the levels of intra-cellular cAMP and osteoblast differentiation markers (osteocalcin, BSP, and DMP1) were also decreased. These results indicate a potential for the existence of a novel regulation of the osteoblast linage cells by local NPY signaling, and therefore support the notion that the osteocytes can function as a neuronal/endocrine entity within bone tissue.

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

M77

FOXO1 Regulates Glucose Homeostasis through its Expression in Osteoblasts

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The recent realization that osteoblasts are endocrine cells favoring glucose homeostasis raised the question of how this function is regulated at the transcriptional level. Because FOXO1 has been implicated in glucose homeostasis and it is expressed in osteoblasts, we were interested in examining whether it is involved in the endocrine function of these cells. Here we show through cell – specific gene deletion and molecular as well as metabolic studies that FOXO1, through its expression in osteoblasts, inhibits beta – cell proliferation, insulin secretion and insulin sensitivity. Indeed, mice with osteoblast-specific inactivation of Foxo1 (Foxo1ob-/- mice) were hypoglycemic and displayed improved glucose tolerance. These effects appeared to stem from increases in β-cell proliferation, insulin secretion and insulin sensitivity. These studies indicate that FOXO1 expressed in osteoblasts controls glucose homeostasis and further support the role of the skeleton as a regulator of energy metabolism.

***Disclosures**: **M. T. Rached**, None*

Other

M79

Treatment with a Form of a Soluble Activin Receptor Type IIB Prevents Androgen Deprivation-Induced Changes in Body Composition

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Androgen deprivation therapy (ADT) is used to stop or slow growth of testosterone sensitive prostate cancer cells, but is associated with undesired side effects, including increased adiposity and decreased lean tissue. ADT also induces significant bone loss accompanied by increased fragility fracture risk. Previous work describes the activin receptor type IIB (ActRIIB) as an important signaling receptor for ligands modulating growth of muscle, fat and bone. Treatment with a non-signaling, decoy ActRIIB effectively inhibits the action of these ligands and results in increased bone and lean mass and decreased adiposity. To determine whether inhibiting ActRIIB signaling attenuates ADT-induced effects on muscle, fat and bone, we treated sham-operated (SHAM) and orchiectomized (ORX) mice with RAP-031, a fusion protein comprised of a form of the extracellular domain of ActRIIB linked to a murine Fc. Both groups received either

vehicle (VEH) or 10mg/kg RAP-031 (RAP) for 10 weeks. NMR and microCT scans were used to assess body composition and bone microarchitecture, respectively. In SHAM mice, RAP-031 significantly increased lean tissue and bone mass while decreasing adipose mass compared to VEH. As a result of androgen deprivation, ORX mice lose lean tissue and bone mass as well as gain fat mass. Compared to VEH-SHAM, VEH-ORX mice had 18.0% less lean tissue and 41.6% more fat at study completion. In contrast, RAP-ORX mice had comparable lean tissue and fat mass to the VEH-SHAM group and significantly increased lean tissue mass and decreased fat mass compared to VEH-ORX groups (Lean: VEH-ORX: 15.59±0.26g, RAP-ORX: 19.78±0.26g, $p<0.05$; Fat: VEH-ORX: 7.12±0.53g, RAP-ORX: 4.57±0.28g, $p<0.05$). These data illustrate that RAP-031 treatment attenuates ORX-induced effects on lean and adipose tissues. ORX also resulted in significant bone loss. The VEH-ORX group had reduced trabecular bone volume fraction (-65.3%, $p<0.001$), trabecular number (-46.3%, $p<0.001$) and trabecular thickness (-17.4%, $p<0.03$) compared to VEH-SHAM controls. In contrast, RAP-ORX trabecular bone volume fraction (+300%, $p<0.001$), trabecular number (+120.4%, $p<0.001$) and trabecular thickness (+29.6%, $p<0.001$) were significantly increased compared to VEH-ORX mice and were not significantly different from VEH-SHAM controls. These data support the hypothesis that treatment with a form of soluble ActRIIB can offset many of the negative side effects associated with ADT.

***Disclosures**: **J. Lachey**, Acceleron Pharma 3*

M81

Thigh muscle adiposity and age-related white matter lesions by brain MRI in elderly subjects: the Age Gene/Environment Susceptibility Study-Reykjavik

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Purpose: The purpose of this study was to investigate a potential linkage between Whilte Matter Lesions (WML) and alterations in adipose infiltration of the thigh muscle. WML are thought to result from small vessel ischemic damage and may potentially disrupt neural circuitry responsible for motor control and regulation of other properties of musculoskeletal tissues. **Methods**: 4187 participants (aged 66-95 years, 2446 women, 1741 men) had brain MRI (1.5T system, including T1, PD/T2 and FLAIR sequences) to detect WML. Subcortical (sWML) and periventricular (pWML) lesion loads were assessed semi-quantitatively from MR images. CT images of the mid-thigh were analyzed for the mean Hounsfield unit (HU, an adiposity measure) of the muscle after removal of fat deposits, as well as the intermuscular fat area (IntFat) within the muscle bundle. The cohort was stratified into quartiles of sWML and pWML, and multi- regression (SASv9.2) was used to examine trends across sex-specific quartiles of WML and to compare the HU and IntFat values in the highest WML quartile (HI) to the other three (REF). Adjustments were: age, height, self-reported knee or hip OA, self-reported congestive heart failure, self-reported diabetes or impaired fasting blood glucose, self-reported pulmonary disease, average systolic blood pressure, stroke history, history of transient ischemic events, BMI, coronary calcium, and self-reported level of current moderate/vigorous physical activity. To test if relations between WML and muscle fat were mediated by muscle strength, data were also adjusted for leg strength. **Results**: Higher WML quartile tended to correspond to lower HU (more intramuscular fat) and to higher intFat, but trends were significant only for men ($p<0.05$). Men and women in the HI quartile of pWML and sWML had lower HU but only men had higher IntFat (Table). Muscle strength adjustment attenuated the relation between WML load and HU but not IntFat. **Conclusions**: In our analyses, subjects with higher WML load had fattier thigh muscle even after adjustment for age, BMI, multiple health indices, and physical activity. Broadening this analysis to include other indices of brain pathology and other musculoskeletal tissue compartments may further illuminate the correspondence between changes in brain structure and alterations in musculoskeletal tissue adiposity.

*: $p<0.05$ **: $p<0.01$		Men		Women	
		HU	IntFat (cm^2)	HU	IntFat (cm^2)
pWML	HI	41.6*	18.3**	38.8*	18.7
	REF	42.2	17.3	39.2	18.5
sWML	HI	41.9	17.3*	38.8 *(p=0.08)*	18.7
	REF	42.2	18.2	39.2	18.5

***Disclosures**: **T. F. Lang**, None*

M83

Endurance Training Minimizes Alterations in IGF-I and Skeletal Mass, but not Fat Mass or Leptin, during Prolonged Energy Restriction

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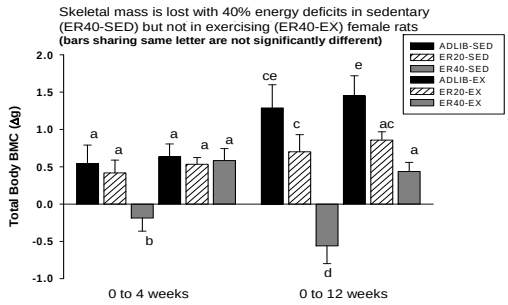
Dietary energy deficits leading to weight loss can induce loss of bone in both humans and rodents; reductions in both circulating estradiol and leptin have been implicated as mechanisms for the reduced bone mass. We sought to test whether endurance exercise protects against decrements in bone mass in female rats subjected to a chronic energy deficit (-40%) and hypothesized that bone mass changes would be linked more tightly to endocrine regulators of energy metabolism than to estradiol. Forty-eight Sprague-Dawley virgin female rats (5-mo-old) were acclimated to AIN-93M purified diet for 8 wks and then block assigned by body weight and proximal tibial volumetric bone mineral density (PT vBMD) to experimental groups. For the next 12 wks, control rats (ADLIB-EX & ADLIB-SED) were fed AIN-93M ad lib. Energy restriction in exercised rats (ER40-EX) and sedentary rats (ER40-SED) was achieved with AIN93M diets modified to provide 30% and 40% less energy, respectively, with 100% of all other nutrients provided. Treadmill running for 4 d/wk, 90-100 min/d (~60% VO2 max) in EX rats increased weekly energy expenditure by 10%. On day 0 and after 12 wks, peripheral quantitative tomography (pQCT) scans assessed PT cancellous vBMD; dual-energy x-ray absorptiometry (DEXA) measured changes in body composition (fat, lean mass) and total body bone mineral content (TB-BMC). Serum leptin and IGF-I were quantified by ELISA from leg vein blood samples at weeks 0 and 12. After 12 weeks, body mass, fat mass and TB-BMC declined by 15%, 90%, and 7%, respectively, in ER40-SED animals. By contrast, ER40-EX rats maintained body mass, with increases in lean mass balancing the 45% decline in fat mass; mean TB-BMC increased by 3%. Although all groups exhibited aging-related loss of PT cancellous vBMD, ER40-SED rats lost significantly more over 12 weeks. Increases in serum leptin in the ADLIB rats were abolished in both ER40 groups; serum IGF-I declined over 12 wks only in ER40-SED animals (by 27%). There were no effects of exercise or energy restriction on changes in serum estradiol or on relative uterine weights at 12 weeks. Serum leptin and IGF-I values at 12 weeks, but not estradiol values, were strong predictors of TB-BMC. These data suggest that endurance training fundamentally alters the IGF-I response to chronic energy restriction,

maintains total body mass and thereby protects against bone decrements usually observed with chronic negative energy balance.

Bloomfield et al_Total Body BMC Changes in Energy Restricted Adult Female Rats



***Disclosures**: **S. A. Bloomfield**, None*

M85

Imaging of Heterotopic Ossification in Advance of Bone Formation.

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Heterotopic ossification (HO) is significant problem in traumatic shock, spinal cord injury, and hip replacement. We have used PET and optical imaging using an MMP9 substrate to visualize heterotopic ossification before the onset of mineralization of osteoid. We have also investigated MMP9 and the related molecule, monocyte chemoattractant protein 1 (CCL2) during BMP2-induced heterotopic ossification by immunohistochemistry and microarray analysis. We find that the peak of MMP9 detected by imaging techniques is on the fourth day after injection of BMP2-producing cells into the mouse quadriceps. However, by microarray analysis and immunohistochemistry we find MMP9 staining as early as the second day after injection of BMP2-producing cells. However, since only active MMP9 is measured during imaging, it is conceivable that inactive forms are synthesized at earlier times. This data is consistent with the known role of MMP9 in vascular invasion since this begins around days 3-4. Detection of HO by X-ray or microCT analysis is not observed until day 6 or later. Our analysis of BMP2-mediated heterotopic ossification indicates that at least some of the stem cells for this process appear to be derived from the sheath of local peripheral nerves. Interestingly, MMP9 is observed between days 2 and 4 in close association with local peripheral nerves. The role of MMP9 in demyelination in multiple sclerosis is well known. Based on the close association of MMP9 with peripheral nerves and the similarity of the microarray profile observed in BMP2-mediated heterotopic ossification with that of multiple sclerosis leads us to speculate that MMP9 may be involved in demyelination of local peripheral nerves enabling release of stem cells albeit at a level undetectable by imaging. Currently, HO is untreatable. However, it is conceivable that early detection may enable novel treatments to be devised. We report here a first step in that direction.

***Disclosures**: **E. Sevick-Muraca**, None*

M87

Genetics of Trabecular Bone Morphology, Obesity, and Leptin

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Studies on mutant mice show that obesity and leptin, a hormone produced by adipose tissue, affect trabecular bone morphology through the central nervous system. We map quantitative trait loci (QTLs) in a population of Recombinant Inbred Lines (RI) formed from the LG/J by SM/J intercross. Here we examine dietary, genetic, and random environmental relationships between trabecular bone morphology, obesity, and leptin levels in 512 LGXSM RI mice. We estimated heritabilities, genetic correlations, and QTLs for bone trabecular morphology. MicroCT scans of the third lumbar, fourth caudal vertebra, and the proximal tibia were used to measure total medullary area (TA), bone volume per total volume (Bv/Tv), connectivity density (CD), structure model index (SMI), trabecular number (TbN), thickness (TbTh), and separation (TbSp) in 8 males and 8 females reared on low or high fat diets from 16 RI strains.
Diet effects were stronger in females than in males, and animals on a high fat diet had higher Bv/Tv, more CD, and more closely-spaced, thicker, and plate-like trabeculae, all indicative of increased trabecular bone mass. Heritabilities were moderate, ranging from 20% to 70%. Genetic correlations among trabecular features followed the same pattern within and between each bone; TA was independent while Bv/Tv, CD, TbN and TbTh were all positively correlated with one other and negatively correlated with SMI and TbSp, which were positively correlated with each other. These correlations indicate that the major axis of genetic variation is in trabecular bone volume, contrasting strains having many, closely-spaced, thick, interconnected, plate-like trabeculae with strains having relatively few, sparsely distributed, thin, unconnected, rod-like trabeculae. Significant negative genetic correlations were found between leptin and lumbar (rG = -0.80) and caudal TA (rG = -0.52) with a trend in the same direction for tibial TA (rG = -0.37). Total fat depot weight was also negatively correlated with lumbar TA (rG = -0.43). Random environmental correlations between leptin levels and TA were not different from zero. Thus, genes, but not random environmental effects, that result in higher leptin levels and obesity yield smaller medullary cavities, especially in the vertebrae. QTL mapping found 45 QTLs for trabecular traits on all chromosomes, 36 of which correspond to locations affecting obesity and leptin levels previously mapped in these strains.

***Disclosures**: **E. A. Carson**, None*

M89

Powerful Bivariate Genome-wide Association Analyses Identified the BIG2 Gene Influencing both Obesity and Osteoporosis Phenotypes

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Genome-wide association (GWA) has been successfully used to identify genetic factors related to common and complex diseases, such as obesity and osteoporosis. Current GWA studies are normally implemented in a univariate framework and analyze different phenotypes in isolation. This univariate approach ignores the potential correlation between important disease traits. Hence it is difficult to detect pleiotropic genes, which may exist for obesity and

osteoporosis, two common diseases that are closely correlated genetically. To identify such pleiotropic genes and the key mechanistic links between the two diseases, we conducted the first bivariate GWA study of obesity and osteoporosis. Our study group included 1,000 unrelated homogeneous Caucasians. Using the sample, we searched for genes underlying co-variation of the obesity phenotype, body mass index (BMI)-a measure of body fat, with the osteoporosis risk phenotype, hip bone mineral density (BMD). We identified eight SNPs of the brefeldin A-inhibited guanine nucleotide exchange protein 2 (BIG2) gene, which were bivariately associated with both BMI and hip BMD. These SNPs, clustering in a tight haplotype block, achieved bivariate association p values at the level of 10-7. In particular, one of the SNPs, rs2295580, located in exon 27, achieved a genome-wide significant bivariate p value of 1.38 × 10-7 in sliding window haplotype analyses. Another SNP, rs730544, which is in nearly complete linkage disequilibrium (LD) (r2= 0.98) with this exonic SNP, also achieved a genome-wide significant bivariate p value of 2.60 × 10-7. Interestingly, BIG2 controls TNF (tumor necrosis factor) bioactivity through regulating the extracellular release of TNFR1 (TNF receptor). Given the importance of TNF for the pathogenesis of both obesity and osteoporosis, our findings suggest a novel mechanism functioning through the interaction between BIG2 and the TNF pathway for co-regulation of these two important diseases.

***Disclosures**: **L. Zhao**, None*

M91

Human articular chondrocytes express 15-lipoxygenase-1 and -2.Potential role in osteoarthritis

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Objective: To evaluate the expression of 15-lipoxygenase (LOX)-1 and -2 in articular chondrocytes and to investigate the effects of their metabolites, 13-HODE and 15-HETE, on IL-1-induced MMP-1 and -13 expression.
Methods: The expression of 15-LOX-1 and -2 was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting in chondrocytes, and by immunohistochemistry in cartilage. Chondrocytes were stimulated with IL-1 in the absence or presence of 13-HODE and 15-HETE and the level of MMP-1 and -13 protein and mRNA expression were evaluated by immunoassay and real-time RT-PCR, respectively. The role of PPARg was evaluated using transient transfection experiments and the PPARg antagonist GW9662.
Results: Articular chondrocytes express 15-LOX-1 and -2 at the mRNA and protein levels. 13-HODE and 15-HETE dose-dependently decreased IL-1-induced MMP-1 and -13 protein and mRNA expression. This effect does not require de novo protein synthesis. 13-HODE and 15-HETE activated endogenous PPARg and GW9662 prevented their suppressive effect on MMP-1 and -13 production, suggesting the involvement of PPARg in these effects.
Conclusion: This study is the first to demonstrate the expression of 15-LOX-1 and -2 in articular chondrocytes. Their respective metabolites, 13-HODE and 15-HETE, suppressed IL-1-induced MMP-1 and -13 expression in a PPARg dependent pathway. These data suggest that 15-LOXs may have chondroprotective properties by reducing MMP-1 and -13 expression.

***Disclosures**: **N. Chabane,** None*

M93

Quantatative Osteocyte Cell Process Stimulation: A Novel Technique Using a Stokesian Fluid Stimulus

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The fluid flow within the lacunar-canalicular system is widely accepted to elicit necessary forces to initiate mechanotransduction complexes in osteocytes that communicate this excitation to adjacent or nearby cells via metabolic agents, solute and ionic molecules, and second messengers. The osteocyte is implicated as the mechanosensing cell in bone, where its cell process is 600 times stiffer than the cell body. Long dentritic processes that protrude from the cell body are equipped with mechanotransductive mechanisms that convert biophysical forces into biochemical and electrical responses, and are the basis of many physiological functions for bone maintenance. We have developed a technique that implements a Stokesian Fluid Stimulus (SFS) probe to better characterize signal transduction mechanisms in the cell process by which they can communicate changes in their dynamic microenvironment throughout their lacunar-canalicular network in vivo. Osteocyte-like MLO-Y4 cells are cultured at low density to prevent forming an interconnected network for the simplicity of single cell experiments. A patch clamp circuit with a holding potential of -60 mV is applied to a single cell, and the conductance of the isolated MLO-Y4 cell is observed and recorded for the duration of the experiment. The SFS microelectrode is filled with external solution and attached to a picospritzer set at 25 psi with a pulse duration of 100 ms. The placement positions of the microelectrodes relative to the MLO-Y4 cell are shown in Figure 1. An SFS is applied to discrete locations along the cell process and cell body to elicit pN level forces that are physiologically relevant according to theoretical models. The force profile of the SFS is calculated by relating the shape of the extruded bolus to the micropipette tip Reynolds number, as shown in Figure 2. The SFS can create forces gentle enough to mimic forces the osteocytes are believed to experience in-vivo, and these forces are quantifiable in the pN range. Efflux or influx of ions through channels can be observed using the SFS applied to specific locations along the cell process. The new SFS technique elicits responses, shown in Figure 3, that can be correlated to pN level forces at discrete locations on the cell process, and can be used to explore channel activity, integrin complexes, and purinergic receptor activation associated with a mechanotransduction event in the osteocyte.

Figure 1: Microelectrode Positions During a Patch Clamp Experiment

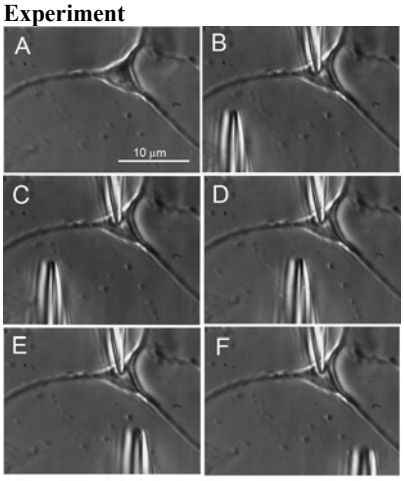

Figure 1: Microelectrode Positions During a Patch Clamp Experiment

Figure 2: Calibration of SFS Force Profile

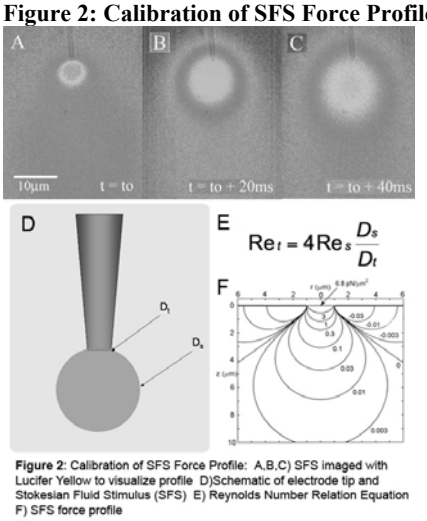

Figure 2: Calibration of SFS Force Profile: A,B,C) SFS imaged with Lucifer Yellow to visualize profile D)Schematic of electrode tip and Stokesian Fluid Stimulus (SFS) E) Reynolds Number Relation Equation F) SFS force profile

Figure 3: SFS Recordings of an MLO-Y4 Cell

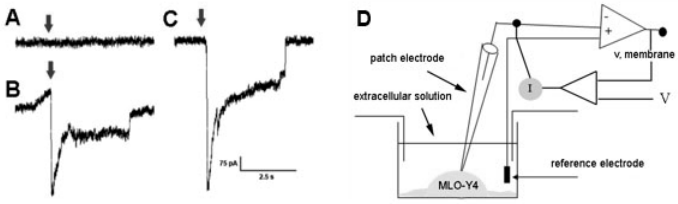

Figure 3: SFS Recordings of an isolated MLO-Y4 of the A) cell body B) one process, and C) another process of the same cell D) Patch clamp circuit schematic

***Disclosures**: **D. Wu,** None*

Novel Endocrine Regulators of Bone Remodeling

T2

Adiponectin Affects Bone Cells In Vitro and In Vivo

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Fat mass impacts on both bone turnover and bone density, and is a critical risk factor for osteoporotic fractures. Adipocyte-derived hormones may contribute to this relationship, and adiponectin is the principal circulating adipokine. However, its effects on bone remain unclear. We have, therefore, investigated the direct effects of adiponectin on bone cells in vitro, and determined the bone phenotype of adiponectin-deficient mice. Adiponectin was dose-dependently mitogenic to primary osteoblasts (60% increase at 10 µg/mL), and markedly inhibited osteoclastogenesis (by 26% and 54% at 1 and 5 µg/mL, respectively). It had no effect on bone resorption in isolated mature osteoclast assays.
In adiponectin-knockout (AdKO) male C57BL/6J mice, trabecular bone volume and trabecular number (assessed by micro-computed tomography) were increased at 14 weeks of age, by 30% (p=0.02) and 38% (p=0.0009), respectively. Similar, non-significant trends were observed at 8 and 22 weeks of age. Biomechanical testing showed lower bone fragility and reduced cortical hardness at 14 weeks.We conclude that adiponectin acts directly on bone cells, but that these actions do not explain the bone phenotype of the knockout animals. Thus, it must also have indirect effects on bone, possibly through modulating growth factor action, or insulin sensitivity. Since adiponectin does influence bone mass in vivo, it is likely to be a contributor to the fat-bone relationship.

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

T4

Leptin may be Important in the Unique Skeletal Metabolism of Ursine Hibernation

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Ursine hibernation uniquely combines prolonged skeletal unloading, anuria, pregnancy, lactation, protein recycling, and lipolysis. Hibernating bears are mostly in non-REM sleep, a state of sympathetic nervous system (SNS) suppression, with periods of REM sleep and wakefulness. Biochemical and radiographic evidence indicates that in hibernating bears, bone turnover is balanced, bone resorption is relatively less than expected for an unloaded skeleton, and unloading- induced bone loss does not occur.1,2
Leptin has anabolic and catabolic skeletal effects, mediated through the hypothalamus by SNS stimulation and CART production. The SNS may mediate bone acquisition in mechanical loading and bone loss in mechanical unloading. This study investigated the skeletal role of leptin during ursine hibernation.
Serum from hibernating (winter 2006) and active (spring 2005, 2006 and 2007), free-ranging female black bears was analyzed using assays validated for black bear, including leptin by RIA, CTX and PTH by ELISA, and BSALP by wheat germ lectin precipitation and colorimetric assay.
Leptin (mean[SD] ng/ml) was greater ($p < 0.001$) in hibernating (3.7[1.1], n =35) than active (2.1[1.3], n = 21) bears. Leptin did not differ ($p = 0.5$) between hibernating lactating (n = 14) and non-lactating (n = 21) bears. In non-lactating, hibernating bears, leptin positively correlated with age-adjusted BSALP ($r2 = 0.24$, $p = 0.02$). In lactating, hibernating bears, leptin positively correlated with CTX ($r2 = 0.30$, $p = 0.04$). In hibernating bears, PTH (mean[SD] pg/ml) was less ($p = 0.04$) in lactating (14[10], n = 15) than non-lactating (23[16], n = 27) bears. In lactating bears, PTH negatively correlated with leptin ($r2 = 0.28$, $p = 0.05$, n = 14).
In hibernating bears, leptin may enable prolonged fasting with preferential adipose metabolism, and support reproduction. The balance of leptin's skeletal effects was tipped toward catabolism in lactating bears, where it may facilitate milk production, and toward anabolism in non-lactating bears, where it may help to prevent unloading-induced bone loss. Leptin's catabolic effect may be counteracted by SNS suppression associated with non-REM sleep. Lactating bears may experience less non-REM sleep, and SNS stimulation by suckling. Leptin-induced hypothalamic production of CART may inhibit bone resorption in hibernating bears.
Refs: 1. Seger et al., JMNI 2008;8(4):360. 2. Seger, U of Maine 2008.

***Disclosures**: **R. L. Seger**, None*

Central Control of Bone Remodeling

T6

Brain-derived serotonin favors bone mass accrual

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The recent demonstration that gut-derived serotonin is a potent inhibitor of bone formation is an incentive to determine whether brain-derived serotonin also affects bone remodeling. To address this question we generated Tph2-deficient mice that are unable to synthesize serotonin in the brain. These mutant mice were born at the expected Mendelian ratio and had a normal life span. Unexpectedly, histological and histomorphometric analysis performed in 6 and 12 week-old mice showed that the absence of serotonin in the brain resulted in a low bone mass phenotype due in part to a decrease in bone formation. This observation indicating that serotonin exerts opposite function on bone formation depending on its site of synthesis prompted us to ask which of the two sources of serotonin exerts a dominant influence on bone remodeling. To that end we generated mice lacking both Tph1 and Tph2 thus unable to synthesize serotonin anywhere in the body. Tph1-/-; Tph2-/- mice were also born at the expected Mendelian ratio and had a normal life expectancy. Histological and histomorphometric analysis showed the existence of a low bone mass phenotype due to a decrease in bone formation in these double mutant mice. Hence these results indicate that the influence of brain-derived serotonin on bone remodeling is dominant over the one of gut-derived serotonin even though it accounts for only 5% of the total pool of serotonin in the body. These results are an incentive to better understand the mechanism of action whereby brain-derived serotonin regulates bone remodeling.

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

T8

Perturbed Osteoclastogenesis in the CB2 Receptor Null Mouse Model

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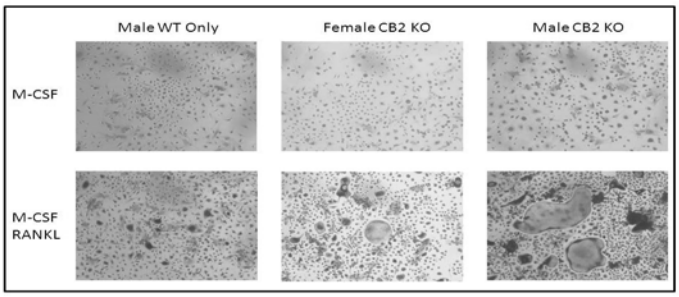
Recent work has shown that neuroendocrine pathways and neurotransmitters have a key role in the regulation of bone remodeling. We investigated the role of the endocannabinoid system in the differentiation of bone marrow progenitors obtained from mice with targeted inactivation of either cannabinoid receptor 1 (CB1KO) or cannabinoid receptor 2 (CB2KO). Osteoclasts (OC) are derived from the monocyte-macrophage lineage and osteoblasts (OB) are derived from the stromal-mesenchymal lineage of cells within the bone marrow. Osteoclastogenesis occurs in response to signals from circulating hormones, and local regulators like receptor activator of NF-□B ligand (RANKL) and macrophage colony stimulating factor (M-CSF). Bone marrow specimens were obtained from the femurs of ~1 year old wild-type (WT) or CB1 or CB2 knock-out mice on the C57BL/6 background. Bone marrow cells were flushed from the shaft of femurs in 0.5 ml □-MEM (containing 10% FBS and 1% p/s antibiotic). After 24h in culture, the non-adherent monocyte fraction (medium) was separated from the initially adherent marrow stromal cells (MSC). The MSC fraction was tested for OB differentiation using Alizarin Red staining following treatment with osteogenic medium and adipoblast differentiation using Oil-Red O staining following treatment with NH AdipoDiff Medium (Miltenyi). Bone marrow monocytes were cultured in the presence of M-CSF (50 ng/ml) for 3 days to form adherent bone marrow macrophages (BMMs). To generate OCs, BMMs (4x104 cells) were seeded in 24-well plates in the presence of M-CSF (30 ng/ml) or M-CSF plus RANKL (100 ng/ml) for 7 days. OCs were assessed by positive TRAP staining. We found that there was equal potential for osteoblastogenesis using MSC of CB1KO and CB2KO mice, compared to WT mice. However, adipogenesis occurred more rapidly and in greater numbers for CB2KO mice. No apparent differences were observed between CB1KO and WT mice. In contrast, osteoclastogenesis of BMM from CB2KO mice was more rapid, beginning at day 4 versus day 6 for WT and CB1KO BMM, and although we saw no increase in the overall number of converting progenitors, the OC that formed from CB2KO BMM were perturbed and oversized compared to WT. These results indicate that CB1 participation in bone remodeling is via an indirect mechanism. By distinction, the participation of the CB2 receptor is directly associated with normal development of OC and may also predispose stromal cell differentiation within the OB lineage.

Figure 1



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

T10

The Impact of Ovariectomy on Genes Encoding the Core Circadian Regulatory Proteins in Murine Bone Tissue

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The core circadian regulatory proteins (CCRP) play a critical role in synchronizing metabolic activities throughout the body. The genes encoding the CCRP have been reported to display an oscillatory expression profile in murine calvarial bone under physiological conditions in vivo. Furthermore, up to 20% of metabolic transcriptome in such peripheral tissues oscillates in a similar manner. In vitro studies using bone marrow mesenchymal stromal/stem cells have demonstrated that the CCRP is present and regulated within osteoblast progenitors. The current study tested the hypothesis that osteoporosis is associated with either an attenuation and/or temporal

phase shift of the CCRP oscillatory expression in skeletal tissues. An ovariectomized murine model was used to approximate the pathological conditions associated with post-menopausal osteoporosis. Measurements of uterine weight, physical activity, and bone histomorphometry provided confirmatory evidence that ovarian hormone deficiency resulted in bone loss and attenuated diurnal activity levels. Two weeks after surgery, the sham operated and ovarietomized female C57BL/6 mice were euthanized at 4 hour intervals. The serum levels of corticosterone and osteoprotegerin, which have each been reported to display a circadian oscillation in humans, were reduced and attenuated in the ovariectomized relative to the sham operated cohort. The oscillatory expression of CCRP mRNAs was attenuated in the femoral and vertebral samples from the ovariectomized mice relative to controls; however, the acrophase of individual genes differed between the two skeletal tissues. The presence of marrow within the vertebral bodies, as opposed to the marrow-flushed femurs, may account for this difference. A subset of osteoblast biomarker mRNAs displayed oscillatory expression in the femur that was attenuated with ovarian hormone deficiency; however, their circadian profile was less pronounced in the vertebral specimens. A subset of osteoclast biomarker mRNAs displayed a phase shift in the ovariectomized femur relative to controls. The study demonstrates that ovarian hormone deficiency modulates the expression profile of the CCRP and its potential metabolic downstream targets within a 2 week period in female mice. Further studies will be necessary to define the relative contribution of centrally- and peripherally-mediated circadian mechanisms to osteoporosis.

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

T12

Reduction in bone resorption after GLP-2 injection is neurologically mediated independently of PTH

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Injection of GLP-2 in healthy individuals results in an acute and significant reduction in bone resorption as measured by the biochemical marker, serum CTX (s-CTX). This reduction coincides with a similar acute reduction in plasma PTH. PTH is rapidly reduced to 70% relative to baseline and remains low (80% relative to base line) for several hours after injection of GLP-2. It has therefore been speculated that the positive effect of GLP-2 on s-CTX is a result of the reduced levels of endogenous PTH, an indirect regulator of osteoclast activity.
In order to study the mechanism of action for GLP-2 in reduction of bone resorption 1.6mg GLP-2 was administered to spinal cord injury (SCI) patients and both sCTX and PTH measured by ELISA. This study shows that the GLP-2 induced reduction of s-CTX is missing in patients with a complete injury (American Spinal Injury Association (ASIA) Impairment Scale (AIS) A, n = 4), which also involve impaired intestinal function. However, in patients suffering from an incomplete injury with some sensory, motility and intestinal function (AIS C + D, n = 8), the reduction in s-CTX level is maintained. Notably, in both groups of SCI patients, the PTH level was reduced (~75% relative to baseline) to the same extend as seen in healthy individuals, regardless of the severity of the SCI. This indicates that a reduction in serum PTH level, seen in response to supra-physiological concentrations of GLP-2, is not sufficient to reduce sCTX. Further, it lends support to the notion that the reduction in sCTX seen in response to exogenous GLP-2 is dependent on CNS as the reduction in sCTX is lost in patients with severe SCI.
Other possible regulatory mechanisms, including a potential transmission of a neuronal signal from the intestine to the brain, are discussed.

***Disclosures**: **C. Bornaes**, None*

T14

Both Caloric Restriction and High Fat Diet Negatively Influence Acquisition of Bone Mass During Growth

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Leptin signaling via the hypothalamus is deleterious to trabecular bone, while the reported anabolic effects of leptin in cortical bone remain controversial. Yet, if both mechanisms are present, then changes in leptin levels should have different effects on each compartment. To explore this, we determined the effect of diet-induced changes in leptin on the skeleton in young mice.
Methods: At 3 wks of age we weaned male C57Bl/6J mice (N=8-12/grp) onto normal (N, 10% cal/fat), 30% caloric restriction (CR, 10% cal/fat), or high fat diet (HF, 45% cal/fat). Outcomes at 6 and 12 wks of age included body mass, femur length, serum leptin and IGF-1, whole body bone mineral density (WBBMD) via PIXImus, cortical and trabecular bone architecture at midshaft and distal femur via μCT, and bone formation and cellularity via histomorphometry (at 6 wks of age).
Results: Serum leptin was 52% and 88% lower in CR and 58% and 60% higher in HF vs. N at 6 and 12 wks of age ($p<0.05$ for all). Serum IGF-1 was 14% lower in CR and 26% higher in HF vs. N ($p<0.01$ for both) at 6 wks of age. CR mice were smaller, with lower WBBMD, trabecular and mass-adjusted cortical bone properties. Bone formation rate and osteoblast number (Ob.N) were decreased, while eroded surface and osteoclast number were increased ($p<0.01$ for all) in CR vs. N. In comparison, HF mice were heavier, but had lower WBBMD and mass-adjusted cortical bone properties ($p<0.05$), with no trabecular bone differences vs. N. Bone formation rate was not significantly changed, despite decreased Ob.N vs. N.
Conclusion: Bone acquisition during growth is impaired in CR mice, even relative to their smaller size, indicating that low leptin and/or IGF-1 levels are deleterious to both cortical and trabecular bone. Cortical acquisition is impaired in HF mice, with reduced WBBMD and cortical bone properties despite high IGF-1, leptin, and body mass. Expected negative effects of high leptin on trabecular bone were not seen in HF, perhaps due to greater mechanical loading with high body mass. Overall, these data show that altered leptin levels during growth affect cortical and trabecular bone mass and architecture, in some cases independently of body mass. However, high serum leptin was not associated with trabecular bone loss, nor anabolic effects on cortical bone, while low leptin appeared deleterious to both cortical and trabecular compartments. These data reflect the complexity of leptin action during skeletal acquisition.

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

Bone, Fat and Brain in the Clinical World

T16

Impact of Clinical and Genetic Factors on Bone Mass in Rett Syndrome

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Rett syndrome (RTT) is an X-linked neurological disorder caused by different mutations in the Methyl-CpG-Binding Protein (MeCP2) gene on chromosome Xq28. RTT patients experience the early onset of neurologic signs with cognitive defects, seizures and a movement disorder of the trunk and extremities with stereotypic hand wringing motions. Osteopenia is reported in RTT. However, a relation to a specific MeCP2 mutation has not been demonstrated. This is a cross-sectional study of lumbar spine bone mineral content (BMC), bone mineral density (BMD) and RTT-associated clinical parameters to MeCP2 mutations in RTT children and adults.
Methods: This study includes 54 girls ages 1.9-17, a male age 6, and 5 RTT females, ages 20-33 years. DXA BMC results were compared to age-related reference curves for females (Kalkwarf, 2007). BMD standards were supplied by Hologic. Blood cells, lymphoblast or fibroblast cell lines were analyzed for MeCP2 mutations (Hoffbuhr, 2001).
Results: BMC measurements were < 10 th %-ile in 68 % of young females in whom adequate scans were achieved and in the male. BMD values were < 2 SD in 72 % of young females. L1-L4 BMD Z-scores for adult females were between -4.4 and -2.3, total hip BMD Z-scores were -2.3 to -4.6. 11% in this group had a history of fracture. Mutational analysis showed the following, (n=patients): R106W, (3); R133C, (5); R168X, (2); T158M, (13); R255X, (1); K266X,(8); R270X,(3); R294X, (5); R306C, (10); R206 H (3) and R306P, (1). For BMC associated with these mutations, the lowest L1-L4 BMC occurred in the 13 patients carrying the T158M mutation (p=0.003). BMC was not correlated with large or small deletions. Several factors may contribute to low bone mass in RTT. 54 % used anticonvulsants. The average body mass index was 15.9 ± 3.3 suggesting poor nutritional state. Ht. and wt. Z-scores were not related to BMC. There was no relationship between BMC and scoliosis, the patient's ability to ambulate or the RTT severity score.
Conclusion: Here, two-thirds of young RTT females and 5 RTT adult females have BMC and BMD values significantly low for chronologic age with increased fracture rate. Low L1-L4 BMC was correlated with the T158M MeCP2 mutation. It is not known whether MeCP2, which binds methylated promoters to form a multiprotein repressor complex, directly or indirectly, affects osteoblast/osteoclast function in RTT patients which has potential therapeutic significance.

***Disclosures**: **J. Shapiro**, None*

T18

Calcium Deficiency and Total Vitamin D-Deficiency Alter Calcium and Fat Absorption and Bone Tissue, but not Body Weight

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Vitamin D and/or calcium have been thought as involved only with bone health, but are now known to have broader metabolic functions. High vitamin D and calcium intakes are reported to facilitate weight and fat loss by subjects on hypocaloric diets. Also, levels of PTH are said to be positively and of 25(OH)vitamin D [25(OH)D] to be negatively related to body weight, but it is unclear whether these findings are consequences or causes of overweight. Using a model of experimentally induced osteomalacia, we now report how calcium and vitamin D deficiency affected plasma 25(OH)D levels, calcium retention, bone mineral, fat absorption and body weight. Twenty adult female minipigs were switched from a standard regimen with 9 g calcium and 2000 IU vitamin D3 per kg to one with 6 g calcium and 6500 IU vitamin D3 (control diet) or one with 2 g calcium per kg and no vitamin D (deficient diet). Plasma 25(OH)D levels on the control diet rose for 10 months from a base value of 74.8 ± 10.8 (SEM) nmol/L to 255.4 ± 40.2 nmol/L. Thereafter the plasma values plateaued for 3 months and then decreased to 206.1 ± 10.8 nmol/L at the end of the study (15 months). Plasma 25(OH)D levels (nmol/L) in animals on the deficient diet dropped significantly from a base value of 60.2 ± 11.4 to 20.5 ± 8.2 after 5 months and to 15.3 ± 3.4 after 10 months. Plasma levels of 1,25(OH)2-vitamin D in the minipigs on the deficient diet were persistently higher than in the controls. After 15 months the animals on the deficient diet were in negative calcium balance (-3.65 ± 2.23 g/7d), having lost significantly more bone mineral density (-51.2 ± 14.7 mg/cm3) than the controls (-2.3 ± 11.8 mg/cm3) whose calcium balance was positive (1.32 ± 1.26 g/7d; $p<0.08$). The bone moisture content of the animals on the deficient diet also was higher. These animals had lost an insignificant amount of weight, even though they absorbed slightly but significantly ($p<0.009$) more fat (379.8 ± 1.4 g/7d) than controls (370.9 ± 2.8 g/7d), whose body weight remained constant.
Our findings indicate that a diet deficient in calcium and vitamin D affects energy metabolism via increased fat absorption, but does not lead to a higher body weight. An increase in fat at the expense of lean mass or an accumulation of abdominal fat at the expense of peripheral fat cannot, however, be excluded.

***Disclosures**: **K. Scholz-Ahrens**, None*

T20

Bone Marrow Changes in Adolescent Girls with Anorexia Nervosa

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Early osteoporosis and fracture risk in adolescent girls with anorexia nervosa (AN) may be caused by increased bone marrow fat and premature conversion of red marrow (RM) to yellow marrow (YM). We performed T1 relaxometry and qualitative analysis of knee MRI in 20 adolescents with AN and 20 healthy age- and race-matched controls, age (mean + SD) 15.8+1.5 yr. Subjects underwent right knee MRI in a 1.0 T extremity scanner to obtain coronal T1W images for qualitative analyses. Two blinded radiologists visually assessed RM, designated as low signal intensity (SI), in the distal femoral (DFM) and proximal tibial metaphyses (PTM) according to the following scale: 0=homogenous hyperintensity (HI), no RM; 1=few HI, mild RM; 2=scattered HI, moderate RM; 3=more diffuse HI, extensive RM; 4=all dark, complete RM. Scores were decided by consensus. Visual inspection in controls revealed consistent patterns of lower SI, presumably residual RM, within the medial aspect of the DFM and the central aspect of the PTM. Patients with AN showed higher SI in these four anatomical locations. Consistent patterns of higher SI, presumably YM, were observed in the femoral and tibial epiphyses. Mean T1 was recorded in these four anatomical locations

for all subjects. Visual inspection showed higher T1 SI (less RM) in the knee metaphyses in the patients with AN. The adjusted mean qualitative score was lower in the AN group than controls for the femur (0.6 vs. 1.2, p=0.03) and trended lower for the tibia (0.5 vs. 1.0, p=0.08). A saturation recovery protocol generated images with repetition times from 350 to 3000 msec for quantitative T1 analyses (ONI Med Sys). T1 maps were generated from the saturation recovery images via a two-parameter-fit algorithm. Mean T1, adjusted by factorial analysis of variance for pair matching, intra-subject correlation, and age (-26.3 msec/yr, SE 4.9, p< 0.004) was higher overall in RM than YM by 95.6 msec (SE 9.1, p<0.001). The AN group showed a lower mean T1 than controls by 26.1 msec in RM, but higher by 21.1 msec in YM, a net difference of 47.2 msec (p=0.01). These results show that adolescents with AN exhibit more yellow marrow conversion within the knee metaphyses than healthy controls. This study suggests earlier more widespread hematopoietic to fatty marrow conversion in AN, perhaps due to adipocyte over osteoblast differentiation in the mesenchymal stem cell pool which may result from hormonal abnormalities.

***Disclosures**: **C. Gordon**, None*

T22

Psychiatric Hospitalization during Mid-life Predicts Elevated Risk for Osteoporosis

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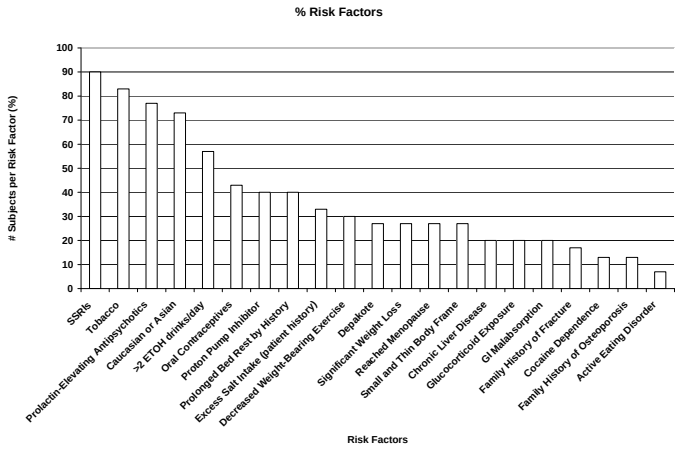
Purpose: This pilot study evaluated global historical risks for osteoporosis in middle-aged psychiatric inpatients with severe mental illness as part of a personalized screening and preventive education intervention pilot protocol.
Methods: Subjects ages 40-60 years were recruited from 3 acute care inpatient units in an academic metropolitan psychiatry department. Seventy percent (30 of 47) completed the protocol. Chart review and structured subject interviews captured demographics, historical, lifestyle, and medication exposure risk.
Results: Thirty largely single, disadvantaged ethnically diverse women and men completed the study of 47 referred (70%). Seventy percent had a major mood disorder (51% depressive disorders; 32% major depression and 15% bipolar disorder) or primary psychotic disorder (19%) as their primary psychiatric diagnosis. Seventy-two percent had a comorbid substance disorder. Subjects had a mean of 7.8 osteoporosis risk factors (range 4-13). Twenty-seven percent had a history of fracture. Only 2 had been diagnosed with osteoporosis and given any prevention recommendations. The top 5 risks, prevalent in up to 90% of subjects, were selective serotonin reuptake inhibitor (SSRI) use, smoking, prolactin-elevating antipsychotic exposure, gender, and alcoholism (Figure 1).
Conclusions: 1) A history of psychiatric hospitalization during mid-life is a summary predictor for elevated risk for osteoporosis. 2) Middle-aged psychiatric inpatients, including those with severe mental illness, are motivated for, comply with, and benefit from bone health assessment and preventive education. 3) Interventions to reduce fracture risk in those with severe mental disorders are underutilized although not different from recommendations for the general mid-life population. These recommendations begin with screening via appropriate laboratory and bone density testing, followed by clinical prevention, which includes calcium and Vitamin D supplementation, enhanced physical activity, and coordinated primary care follow-up. 4) A comprehensive research agenda is needed to address bone health in those with severe mental disorders, focusing on identifying a) whether the incidence of osteoporosis is shifted toward an earlier age; b) relative contributions of mental illness, its treatment, and lifestyle factors toward altered bone metabolism; and c) tailored osteoporosis screening, prevention, and intervention programs.

Figure 1 - Prevalence of Historical, Medical and Lifestyle Risk Factors for Osteoporosis



***Disclosures**: **B. Gracious**, None*

T24

Low Vitamin D Status Among Floridian Overweight/Obese Postmenopausal Women and its Relationship With Obesity Markers

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Low vitamin D status has been documented in both the young and old, and has been associated not only with bone health but with metabolic derangements including higher insulin levels and fat mass. Serum 25(OH)D is the best measure of vitamin D status although guidelines for acceptance of normal range levels are less clear. Our aim was to examine serum 25 (OH) D levels and their association with dietary intake, insulin, and body fat in healthy, overweight/obese, postmenopausal Caucasian women in Florida. Serum 25(OH)D and insulin were analyzed (by ELISA, Alpco Diagnostics, Salem, NH) in 94 women aged 55.8±4.1 with a body mass index (BMI) of 31.9±4.8 kg/m2 (mean±SD). Body composition was measured by iDXA, including total, android and gynoid fat, while dietary intake was assessed by 3-day food records. Mean serum 25(OH)D and insulin concentrations were 56.2±25.1 nmol//L and 9.3±8.8 microIU/ml, respectively. Hypovitaminosis D, based on the cut-off of <75 nmol/L, was present in 75.3% of our cohort, and negatively associated with body fat and BMI (p<0.05). 25(OH)D and insulin were also significantly inversely related. Vitamin D intake was below the DRIs in 98% of the cohort and was nearly significantly related to 25(OH)D (p=0.07). Body fat (total, android and gynoid fat) was negatively correlated with both serum 25(OH)D and vitamin D intake (p's <0.05). After stratifying the participants according to BMI (<30, group 1; and ≥30, group 2), ANOVA demonstrated a significant difference in dietary calcium (Ca), serum 25 (OH)D and insulin such that Ca intake and 25 (OH)D were higher and insulin was lower in group 1 (all p's <0.05). Additionally, hypovitaminosis D was present in 80.7% of group 2 participants. Our data indicate the strong inverse relationship of serum 25(OH)D and (to the extent) dietary vitamin D intake with insulin, BMI, and body fat, as well as that low vitamin D status might be ubiquitous, even in the sunny state of Florida. Our results are consistent with some other findings suggesting that

vitamin D might have a bigger role in weight/body composition management, from the aspect that it is being sequestered in adipose

tissue and that it can inhibit adipogenesis. However, it is not clear what is a cause or effect. Funded: USDA/CSREES/NRI #2004-05287

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T26 Withdrawn

T28

Higher Body Fat is Associated with Lower Bone Mineral Content at Multiple Skeletal Sites in Women within Body Mass Index Categories

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Purpose: Increasing body weight (BW), comprised of fat (FM) and fat-free soft tissue (FFSTM) masses, is beneficial for maintenance of bone health. FM can protect against bone fractures in women. Yet, emerging evidence demonstrates that excess adiposity is associated with low bone mineral content (BMC) and increased incidence of osteopenia, osteoporosis and non-spine fractures in pre- and postmenopausal women. Adipose tissue secretes proinflammatory adipokines that promote bone resorption when released in high concentrations. BW that is disproportionately comprised of FM may be detrimental to BMC and skeletal integrity. Therefore, the association between FM percentage (FM%) and BMC in women within body mass index (BMI) categories was examined.
Methods: Women (N=198), aged 25-86 years, underwent dual-energy X-ray absorptiometry (DXA) scans for measurement of total body (TB) BMC (g), FM (kg), FFSTM (kg) and FM%. Further, BMC of the lumbar spine (LS, L1-L4), total proximal femur (TPF) and total forearm (FA) were measured. Women were categorized into BMI (kg/m2) groups, including healthy (BMI=18.5-24.9, n=97), overweight (BMI=25-29.9, n=58) and obese (BMI=≥30, n=43), based on BW and height. Differences in participant characteristics by BMI group were analyzed by one-way ANOVA (Table 1). Bivariate relationships between FM; FFSTM; FM% and BMC were analyzed using Pearson's correlation coefficient.
Results: BMI ($p<0.05$), BW ($p<0.05$) and TB FM ($p<0.01$), FFSTM ($p<0.01$) and FM% ($p<0.01$) were incrementally higher across groups of healthy, overweight and obese women. Although BMC was not significantly different among BMI groups, FM% was significantly negatively associated with LS ($p<0.01$) BMC in all 198 women. FFSTM was positively associated with TB, LS, TPF and FA BMC (all $p<0.01$) for the entire sample. Women in the healthy BMI group had a negative association between FM% and TB, LS, TPF and FA BMC (all $p<0.01$). Likewise, women in the overweight BMI group had a negative association between FM% and TB, LS, TPF and FA BMC (all $p<0.01$). For women in the obese BMI group, FM% was inversely related to BMC at all measured sites but not significantly so. Within BMI groups, FFSTM was positively and significantly associated with BMC at all skeletal sites, including the TB.
Conclusion: Excess adiposity, measured as FM%, may be detrimental while FFSTM may be protective for bone in women regardless of age and even within a healthy BMI status.

Table 1. Descriptive Characteristics of Women (N=198) by Body Mass Index (BMI) Classification

Characteristic	Healthy (BMI=18.5-24.9) (n=97) Mean ± SD	Overweight (BMI=25-29.9) (n=58) Mean ± SD	Obese (BMI=≥30) (n=43) Mean ± SD	*p*-value
BMI (kg/m^2)	22.3 ± 1.6	27.2 ± 1.5	36.0 ± 5.1	<0.05
Age (yr)	46.9 ± 12.0	50.7 ± 11.0	50.7 ± 11.0	NS
Height (cm)	164.6 ± 6.5	164.5 ± 5.7	163.2 ± 7.3	NS
Weight (kg)	60.5 ± 6.5	73.5 ± 6.9	95.1 ± 15.4	<0.05
TB FM (kg)	18.0 ± 4.2	27.6 ± 3.9	40.8 ± 9.6	<0.01
TB FFSTM (kg)	43.0 ± 4.7	46.4 ± 7.1	54.6 ± 7.6	<0.01
TB FM%	29.4 ± 5.2	37.1 ± 3.5	42.4 ± 4.9	<0.01
TB BMC (g)	2084 ± 316	2172 ± 307	2202 ± 322	NS
LS BMC(g)	55.8 ± 14.2	58.8 ± 11.0	54.9 ± 13.5	NS
TPF BMC (g)	32.2 ± 3.1	30.8 ± 4.5	32.1 ± 5.6	NS
FA BMC (g)	11.7 ± 1.9	12.1 ± 1.8	11.8 ± 2.0	NS

TB=total body; FM=fat mass; FFSTM=fat-free soft tissue mass; FM%=fat mass percentage; BMC=bone mineral content; LS=lumbar spine; TPF=total proximal femur; FA=total forearm; *p*-values from one-way ANOVA.

***Disclosures**: **K. E. Piehowski**, None*

T30

Pref-1 Predicts Marrow Adiposity and Low Bone Mass

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The regulation of bone cell lineage is important in understanding states of low bone mass. Adipocytes and osteoblasts originate from a common progenitor human mesenchymal stem cell (hMSC), and marrow adipocytes may affect osteoblast differentiation. Low bone mineral density (BMD) and increased marrow fat are characteristic of anorexia nervosa (AN), a state of self-imposed starvation. Pref-1, a member of the EGF-like family of proteins expressed in hMSCs, preadipocytes and bone, is a negative regulator of adipocyte and bone cell differentiation. Osteoblast-specific Pref-1 over-expression in a mouse model results in reduced BMD. We hypothesized that Pref-1 would be elevated in AN and would predict marrow fat and low BMD. We studied 30 females: 20 with AN and 10 controls of comparable age (26.8y + 1.5 vs. 29.2y + 1.7, respectively) and differing BMI by design (17.6 + 0.2 vs. 21.9 + 0.5). We measured Pref-1, leptin, an adipokine markedly decreased in AN, and BMD of the AP lumbar spine, lateral spine (LS), and total hip by DEXA in all subjects. Bone marrow fat content by proton MR spectroscopy was measured in 10 women with AN and 10 controls. Pref-1 was significantly higher (0.47 vs. 0.37 ng/mL, p=0.03) and log leptin lower (p=0.01) in AN as compared to controls. There was a negative correlation between log Pref-1 and BMD of the AP spine (R= -0.54, p=0.003) and LS (R= -0.44, p=0.02) and positive correlation between leptin and BMD of the AP spine (R=0.39, p=0.04) and hip (R=0.42, p=0.03) in all subjects. In AN, log Pref-1 was negatively correlated with BMD of the AP spine (R= -0.57, p=0.008) and LS (R= -0.55, p=0.01) and log leptin was positively correlated with hip BMD (R=0.50, p=0.02), while in controls log leptin was negatively correlated with BMD of the AP spine (R= -0.8, p=0.0095) and hip (R= -0.72, p=0.029). There was a positive correlation between log Pref-1 and marrow fat of the proximal femoral metaphysis (R=0.53, p=0.018) and negative correlation between leptin and L4 marrow fat

(R= -0.45, p=0.046) in the entire group. These data support the hypotheses that Pref-1, a negative regulator of adipocyte and osteoblast differentiation, is 1) elevated in AN and 2) predicts low BMD and increased marrow fat. In contrast, leptin is a positive predictor of BMD in AN and negative predictor of marrow fat. These data suggest that Pref-1 may contribute to the pathogenesis of low bone mass states by regulating the differentiation process of hMSCs into bone and fat.

***Disclosures**: **P. K. Fazeli**, None*

T32

Android fat distribution is inversely associated with bone mineral density

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Background: Limited and conflicting information is available regarding the association between fat distribution and bone mineral density (BMD). We examined this relationship and potential influences of body mass index (BMI), age, gender, site of BMD measurement and type of fat distribution.
Design: In 2631 participants from the Erasmus Rucphen Family (ERF) study, BMD was measured by dual energy x-ray absorptiometry (DXA) at the hip, lumbar spine and total body. Fat distribution was assessed using waist-to-hip ratio (WHR), waist-to-thigh ratio (WTR) and the classical trunk-to-leg fat ratio and newer android-to-gynoid fat ratio by DXA. Analyses were stratified by gender and median age (48.0 years in women and 49.2 in men). Linear regression analysis was performed with BMD as dependent and fat distribution as independent variables with adjustments for age, height, smoking, use of alcohol and additionally for BMI to explore its potential role as confounder.
Results: Without adjustment for BMI, most relationships between fat distribution and BMD were significantly positive, except for the WTR that had significantly negative relations with total body bone mineral content (BMC) in women and with total body BMC, femoral neck BMD and lumbar spine BMD in men. After adjustment for BMI, most significant correlations were negative except for the trunk-to-leg fat ratio with lumbar spine BMD. Inverse associations were stronger in males than in females. No consistent influence of age or menopausal status was found. Differences in the relation between fat distribution and total body BMD vs. BMC decreased after adjustment for BMI.
Conclusions: Android fat distribution as assessed by circumference ratios and the android-to-gynoid fat ratio by DXA is inversely associated with BMD once the effect of BMI is taken into account. This contrasts the current view that android fat deposition is beneficial for bone. These findings may provide better insight into underlying biological mechanisms determining the relation between fat and bone tissue and may be relevant for the enhancement of fracture risk prediction.

***Disclosures**: **M. Zillikens**, None*

Osteoblast and Adipocyte Differentiation

T34

Effects of CB1 and CB2 Receptors on Skeletal Development and Osteoblast Differentiation

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Recent studies have demonstrated endocannabinoids and their G protein-coupled cannabinoid receptors type 1 (CB1) and type 2 (CB2) in the skeleton. Although it has become clear that this system is functional in bone, the precise mechanisms of action are only beginning to emerge. In osteoblasts and their progenitors, there is definitive evidence for expression of CB2 receptors, though the expression of CB1 is debatable. CB2-deficient mice demonstrate an acceleration of age-related bone loss, whereas CB1-deficient mice display a variable skeletal phenotype. In this study, we characterized the skeletal phenotype in CB1/CB2 double receptor knockout (CB1/2 null) mice. In addition, we also utilized specific CB1 and CB2 receptor antagonists to examine their effects on osteoblast differentiation in culture. Micro-CT analysis of femurs from 12 wk-old CB1/2 null mice demonstrated a significant reduction in trabecular bone volume compared to wild-type (WT) mice. Trabecular number and thickness were significantly decreased while trabecular separation was increased in the CB1/2 null mice. There was also a significant decrease in cortical thickness in the CB1/2 null compared to WT mice. To examine the functional significance of CB1 and CB2 receptors on osteoblast differentiation, we established primary osteoblast cultures utilizing newborn wild-type calvaria (Arnott et al., Bone 42:871, 2008). When cultured under osteogenic conditions (i.e. ascorbic acid and b-glycerophosphate), these cells differentiate and produce a mineralized matrix. Measurement of alkaline phosphatase (ALP) activity was assessed to evaluate the effects of specific CB receptor antagonists on osteoblast differentiation. Treatment with the CB1 antagonist (SR141716A or AM281) or the CB2 antagonist (SR144528) caused a significant, dose-dependent inhibition of ALP activity. When cultures were treated with both CB receptor antagonists simultaneously, the inhibition of ALP activity was more pronounced compared to either antagonist alone. Our results suggest that both CB1 and CB2 are functional receptors during osteoblast differentiation in vitro and in the regulation of bone mass in vivo. The observation that combined CB1 and CB2 receptor antagonism/deletion has a more dramatic effect compared to single receptor deletion warrants additional studies to evaluate intracellular signaling subsequent to ligand activation of CB1 and CB2 in osteoblasts.

***Disclosures**: **S. Popoff**, None*

T36

Osteogenesis regulated by adipocytes

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Progressive bone marrow adipogenesis has been seen associated with reduced osteoblastic bone formation and bone loss. But the role of bone marrow adipocytes remains controversial. Recent literature directs considerable attention to the function of bone marrow adipocyte in the process of osteogenesis. Some studies have demonstrated a reciprocal switch between the differentiation of adipocytes and osteoblasts. Further studies found that adipocytes

derived from bone marrow may regulate the proliferation and differentiation of osteoblasts. However, the mechanism by which adipocytes may possibly regulate osteogenesis is not clear. In the present study, we analyzed osteogenesis regulation by adipocytes through two different types of co-cultures: one with intercellular contact and the other without intercellular contact. Under conditions of uniform osteogenesis stimulation and different adipogenesis stimulation (C, no stimulation; L, low stimulation; and N, normal stimulation), the alkaline phosphatase (ALP) positive area gradually decreased in percentage from the Control to the Normal Group in both co-cultures. The ALP positive area reduced further in the intercellular contact co-culture. Yet, the numbers of adipocytes showed the opposite tendency. Reduction in osteogenesis activity was accompanied by a decrease in Runx2 expression, and, increase in Ppar-□ expression. To find out what adipocyte-derived factors trigger this change in osteognenesis, we applied mRNA microarray and proteomics techniques to analyze the mRNA and protein profiles, respectively. The mRNA microarray results revealed that the Notch1 level was significantly decreased, while PIK3 and smad6 levels were significantly increased across Control to Normal groups. The proteomic analysis led to the identification of 12 differential expressed proteins, among which, 7 protein expression levels were gradually increased and 5 protein expression levels were gradually decreased across Control to Normal groups. Among them, the gradually decreasing proteins, S100A6 and AnnexinA2, were reported to promote bone formation and facilitate mineralization respectively. Meanwhile, the gradually increasing proteins, calreticulin, plays an important role in inhibiting the Wnt signal pathway through the osteogenesis process. Our results indicate that adipocytes regulate osteogenesis mainly through an indirect manner by up-regulating the expression of Ppar-γ and down-regulating the expression of Runx2.

Table 1 Identification of proteins differenatially expressed in adipocytes regulated osteogenesis

Sample spot	Accession number	protein name	Change tendency	MW/pI	Biological process
1	P12109	Collagen alpha-1(VI)	↓	108,529/5.26	cell-binding protein
2	P07355	Annexin A2	↓	38,604/7.57	Calcium-regulated membrane-binding protein
3	P06703	Protein S100-A6	↓	10,180/5.32	proliferation and differentiation
4	P09382	Galectin-1	↑	14715.7/ 5.33	Apoptosis and differentiation
5	P04179	Superoxide dismutase	↓	24,722/ 8.35	Anti-oxidation
6	P08758	Annexin A5	↑	35,937/ 4.94	anticoagulant protein
7	P27797	Calreticulin	↑	48,142/ 4.29	calcium binding chaperone
8	P50454	Serpin H1	↓	46,441/ 8.75	Binds specifically to collagen
9	P60174	Triosephosphate isomerase	↑	26,669/ 6.45	Carbohydrate biosynthesis
10	P18669	Phosphoglycerate mutase 1	↑	28,804/ 6.67	Glycolysis
11	P04406	Glyceraldehyde-3-phosphate dehydrogenase	↑	36,053/ 8.57	Glycolysis
12	P30041	Peroxiredoxin-6	↑	25,035/ 6.00	Lipid degradation

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

T38

The regulation of signal transducers and activators of transcription5 (STAT5) by C/EBPα, β and PPARγ during adipogenesis

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STAT1, 5A, and 5B proteins are expressed and regulated by cytokine-mediated phosphorylation and nuclear translocation during adipogenesis of human bone marrow derived stromal cells (BMSCs). We found that STAT5A and STAT5B expression is regulated by C/EBPα, C/EBPβ and PPARγ during adipogenesis of BMSCs and 3T3-L1 preadipocy. Bone marrow was aspirated from iliac crest and BMSCs were cultured in DMEM containing 10% FBS in low density. For adipogenesis, BMSCs were cultured in adipogenic medium for 14 days. For immunoblot, we used GAPDH, C/EBP, PPAR, STATs antibodies. To determine DNA and protein binding, we performed electrophoretic mobility shift assay and chromatin immunoprecipitation assay. To measure transcription activity, we performed luciferase assay. The expression of STAT5A and STAT5B increased time-dependently during adipogenesis. RNA interference with STAT5A completely blocked the differentiation, while inhibition of STAT5B partially blocked the differentiation. To determine whether C/EBPα/β or PPARγ activates the STAT5A and STAT5B promoter through DNA binding, the luciferase assay was performed. The transcriptional activity of STAT5A was induced by PPARγ, and the transcriptional activity of STAT5B was induced by C/EBPα and C/EBPβ. To precisely localize the region which is responsible for the transactivation by C/EBPα, C/EBPβ and PPARγ, several truncated STAT5A and STAT5B promoter-luciferase constructs were made. As a result, the constructs pGLSTAT5B-118/+45 lost responsiveness to C/EBPα or C/EBPβ, and the constructs pGLSTAT5A-101/+57 lost responsiveness to PPARγ. To determine whether C/EBPα/β or PPARγ binds to the STAT5A and STAT5B promoter, cells were differentiated to adipocytes after which ChIP analyses were performed. C/EBPα and C/EBPβ bound to STAT5B promoter at day 1 after which binding is maintained until at least day 3 following the induction of differentiation, and PPARγ bound to STAT5A promoter. In EMSA, C/EBPα and C/EBPβ proteins bound to the STAT5B promoter, as configured by supershift using anti-C/EBPα and C/EBPβ antibodies. We observed that C/EBPα and C/EBPβ directly regulate the transcriptional activity of STAT5B, and PPARγ regulates the transcriptional activity of STAT5A. Inhibition of STAT5A expression completely blocked the differentiation, while inhibition of STAT5B only partially blocked the differentiation. Therefore the expression of STAT5A is a necessity, while the expression of STAT5B is supplementary in adipogenesis.

***Disclosures**: **H. Jung**, None*

T40

Sprouty1 Expression Influences Allocation Of Mesenchymal Stem Cell Populations

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Adipose tissue has a critical role in body homeostasis because of its endocrine, autocrine and paracrine influences on blood pressure, immune function, angiogenesis, and energy balance. Bone and fat arise from the same progenitor, the MSC. MSC differentiation is dependent on microenvironmental cues in the bone marrow, acting in

an autocrine or paracrine fashion. One critical regulator of adipogenesis and osteogenesis is PPAR□. In addition, members of the Sprouty (Spry) family of tyrosine kinase inhibitors affect craniofacial development and chondrogenesis, suggesting a potential impact on osteogenesis. Potential roles of Sprouty in adipogenesis are unknown. To understand its role in MSC differentiation in vivo, mSpry1 was conditionally expressed in mice under control of the aP2 in a Cre-activated transgene. Expression of mSpry1 activated by aP2-Cre markedly decreased the number of fat cells, and resulted in hypertrophy of adipocytes in abdominal fat. Densitometric analysis revealed a corresponding increase in total bone mineral density in the mSpry1 transgenic mice. To test if Spry1 expression influenced the MSC population, bone marrow and ATSCs were differentiated in vitro and evaluated for their adipogenic and osteogenic potential. ORO, ALK and vonKossa staining of 1 and 2 wk cultures reflected enhanced osteogenesis and decreased adipogenesis. qRT-PCR for selected marker genes was performed to determine the changes in gene expression pattern during differentiation comparing mSpry1 transgenics with wild type gender and age matched littermate controls. There were several significant differences in gene expression, but transcript levels for TAZ (transcriptional coactivator with PDZ-binding motif), a transcriptional modulator of MSC differentiation, were most notable, elevated 80-fold vs control. We postulate that mSpry1 acts via inhibition of PPARg2 expression to repress adipocyte differentiation in MSC, pushing them towards the osteogenic lineage. Further, the demonstration of TAZ regulation by Spry1, possibly via inhibition of FGF signaling, provides a potential mechanism for understanding lineage allocation of MSCs between bone and fat. Our findings also support the theory of plasticity in lineage commitment and the reciprocal relationship of bone and fat cells, suggesting that subtle changes in the microenvironment of the bone marrow, such as a low level of Spry1 expression, can lead to significant phenotypic changes in the skeleton.

***Disclosures**: **S. Urs**, None*

T42

Tracking Expression of a Smooth Muscle Alpha-Actin-Red Fluorescent Protein (SMAA-RFP) Reporter in Bone Marrow Stromal Cell Cultures

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In view of mounting evidence that vascular pericytes serve as multipotent progenitor cells for various mesenchymal lineages, we developed a line of transgenic mice that express the RFP mCherry under the control of an SMAA promoter+first intron fragment. These mice were crossed with an adipocyte reporter line (aP2-GFPcyan) and the double positive offspring were used to investigate the expression of these markers during osteogenesis and adipogenesis in vitro.
Bone marrow stromal cells were expanded in □MEM+FCS for 10-11 days and then given either adipogenic (ADP; 0.5 μM rosiglitazone, 1 μM insulin) or osteogenic (OST; 50 ug/ml ascorbic acid, 8 mM βGP) treatments. Scanning fluorescence microscopy revealed the gradual emergence of widespread red fluorescence that resolved into bright, polygonal patterns in the ADP group. aP2-Cyan+ cells appeared to be contained within these polygonal structures. At higher magnification, the cells within this region showed populations of red cells of widely varying intensities along with vacuolated cells that were either cyan+ alone or both cyan+ and cherry+. Switching ADP differentiated cultures to an OST treatment led to a rapid loss of brightness of both transgenes and cultures showed large, diffuse areas of cherry+ expression resembling control OST cultures. Untreated and ADP treated BMSC cultures were also analyzed by flow cytometry following staining with the macrophage marker CD11b-APC. Over 60% of the cells in these cultures were positive for CD11b+, indicating a large macrophage population. Consistent with microscopy, ~50% of the total cells in culture were SMAA/Chry+. The brightest of the cherry+ cells are large by forward scatter and may represent smooth muscle myocytes. In the CD11b- population, >90% of the cells in untreated control cultures are SMAA/Chry+, as compared to ~67% of the cells in ADP treated cultures. aP2-Cyan+ only cells account for ~5% of the CD11b- population after 2-7 days of ADP treatment, while double positive cell numbers were relatively invariant (1-2%) over this time.
These results indicate that while SMAA-Chry transgene expression preceded both adipogenesis and osteogenesis, it also persisted during differentiation in a significant subset of adipocytes and macrophages. Thus, the utility of this transgene as an early mesenchymal lineage marker may lie in the ability to sort SMAA/Chry+ cells from early BMSC cultures and follow markers such as aP2/GFP during subsequent manipulations.

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

T44

A Method for Quantitative Imaging of Adipocyte Distribution in Murine Bones

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Marrow fat content is a phenotypic characteristic in studies of progenitor cell differentiation to the osteoblast versus adipocyte lineage. Our goal was to develop a method for measuring marrow fat volume and distribution in murine bones. Heavy metal staining of lipid using osmium tetroxide (OsO4) has been used for decades in histological assessments. With the advent this decade of benchtop micro-focus X-ray computed tomography (μCT), we investigated whether the spatial resolution afforded by μCT imaging (~10 μm) could be used in conjunction with heavy metal staining of lipid to visualize and quantify adipocyte volume and distribution within the marrow of intact excised mouse bones. Preliminary histological studies revealed that aqueous OsO4 solutions could not penetrate undecalcified cortex. Thus, a relatively simple protocol was developed to first decalcify specimens to allow diffusion of OsO4. Whole intact bones with encapsulated marrow were dissected from 10 week old inbred and outbred mice to survey skeletal sites typically used for documenting bone morphometry (femur, tibiae, vertebrae). The humerus, sternum, and metatarsals were included as candidates for supplementary analysis of marrow fat. Intact bones were fixed in 10% formalin 2-5 days, allowing bone morphometric analysis via μCT while in fixative. Following decalcification in 14% EDTA for ~5 days, specimens were stained in aqueous 1% OsO4 with 2.5% potassium dichromate. Volumetric images were acquired via μCT, providing ultra-high contrast and segmentation of discrete metal-stained adipocytes distributed through the marrow.
Very few adipocytes were observed in the humerus and sternum, whereas high concentrations of fat were seen in metatarsals. Substantial fat volumes were observed in femurs and tibiae, providing measurable differences in adipocyte number, distribution, and volume. Adipocytes were more concentrated toward the metaphyses, with large numbers in the distal tibia. In the diaphysis adipocytes were distributed circumferentially at the endocortical surface. Applying the method to C57Bl/6 versus C3H/He inbred mice, marrow fat content and density in the distal femur demonstrated a positive correlation to trabecular bone mass and bone formation rate within the same compartment.
Overall, the method appears to be robust and forgiving, with potential for altering the aqueous OsO4 cocktail without detriment to adipocyte staining. Attention must be given to identifying and excluding external fat within residual soft tissue, as well as the potential for modest staining of large vessels after longer OsO4 exposures.

Incorporation of an additional treatment step to eliminate external fat would allow this technique to be automated for accurate high-throughput quantitation of whole bone marrow adiposity within murine bones.

***Disclosures**: **D. J. Adams**, None*

T46

Alendronate Reverses Loss of Bone Mineral Density and Strength Associated with Rosiglitazone in Ovariectomized Rats

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Evidence has recently emerged that diabetes treatments targeting peroxisome proliferator-activated receptor-γ (PPARγ), such as rosiglitazone (RSG) and pioglitazone, are associated with an increased risk of fracture. This study was designed to investigate the underlying mechanisms of RSG-associated fracture and if changes in bone parameters associated with RSG could be prevented by treatment with an antiresorptive agent.

Nine-month old Sprague-Dawley rats underwent ovariectomy (OVX) or sham operation. OVX animals were randomized into 8 groups of 12 each receiving vehicle, metformin (PO, 300 mg/kg/day), alendronate (SC, 0.03 mg/kg/twice weekly), 17β-estradiol (SC pellet, 0.01 mg), RSG at two different doses (PO, 3 or 10 mg/kg/day), RSG (10 mg/kg/day) + alendronate, or RSG (10 mg/kg/day) + 17β-estradiol for 12 weeks. Bone mineral density (BMD), bone mineral content (BMC), and bone turnover markers were measured and biomechanical testing was performed.

Over the study period, BMD and BMC (whole body, lumbar spine, and proximal tibia) significantly decreased for RSG-treated animals relative to OVX controls. Percentage change in BMD from baseline in the lumbar spine (LS mean; assessed by DXA) with OVX was –4.65% versus –12.48% and –15.30% in the RSG-3mg and RSG-10mg groups, respectively; both $P < 0.001$ versus OVX). The pQCT analysis indicated the decreases in BMD for RSG-treated animals were mostly trabecular bone loss. Three-point bending and compression tested were assessed. Slight decreases were noted in bone strength parameters at sites rich in trabecular bone (lumbar spine and calcaneus). No effects were noted on bone resorption markers or levels of osteocalcin for RSG-treated animals. Slight decreases in P1NP levels were noted relative to OVX controls.

Combination treatment with alendronate completely prevented bone loss and bone strength reductions associated with RSG treatment whereas 17β-estradiol treatment only partially attenuated the reductions in these parameters. Metformin treatment did not affect BMD or strength parameters compared to OVX controls.

The results indicate that oral administration of RSG exacerbates OVX-induced bone loss at sites rich in trabecular bone. Concomitant treatment with alendronate is effective in preventing RSG-induced bone loss in this model.

***Disclosures**: **L. A. Fitzpatrick**, GlaxoSmithKline 3*

T48

BMD QTL on Mouse Chromosome (Chr) 1 Interacts with Dietary Fat

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Intercrosses of low BMD C57BL/6J (B6) and high BMD C3H/HeJ (C3H) mouse strains, followed by congenic strain analyses, have demonstrated strong regulation of femoral vBMD and distal trabecular structure in female mice (Beamer et al. 200_; Shultz et al. 200_). The B6.C3H-1-12 (1-12) congenic strain carries a 4 Mb C3H region on distal Chr 1 that changes several phenotypes compared with the B6 background. At 16 wks, 1-12 females show increases in: body weight, % fat, femoral vBMD, mid-shaft periosteal circumference, distal femoral trabecular BV/TV and Tb.th, as well as histomorphometric measures (BV/TV, Tb.th, Ms.Bs, BFR). Faber et. al. (2009) reported that high fat (42%) fed B6C3F2 females and males, measured by PIXImus, did not show the strong BMD regulation on distal Chr 1 previously reported. These findings suggest that the distal Chr 1 BMD QTL may represent a gene that interacts with environmental factors, such as dietary fat. To test whether BMD regulation in 1-12 mice could be altered by dietary manipulation, groups (n=11) of 1-12 females by split litter design were placed on their 'normal' (6%) or high fat (42%) diet for 8 weeks, beginning at 8 weeks of age. At 16 weeks, mice were measured for whole body aBMD by PIXImus and for femoral vBMD by pQCT. The data from PIXImus showed that % fat and total tissue mass (body mass) were reduced in the high fat group compared with controls, while aBMD was not different from controls. The data from pQCT showed a significant decrease in femoral vBMD in the high fat group compared with controls, a difference that is not affected by the change in body weight as a covariate. We also observed that femur length was increased as a consequence of high fat diet treatment. The results suggest that the QTL on distal Chr 1 does in fact respond to the dietary manipulation by loss of body fat, body mass, and reduction of femoral vBMD. We conclude that this response to high fat diet is not explained by an aversion of the mice to the diet, since total bone area was not different for either group, and femur length increased in the high fat group, suggesting skeletal growth was not disturbed.

The mechanism(s) supporting the 1-12 responses to dietary manipulation are unknown. However, the 4 Mb region in 1-12 has been partitioned into sublines 1-12-1 and 1-12-2. 12-1 is 0.865 Mb of C3H and carries the vBMD regulation, whereas 12-2 is 3.695 Mb and does not carry vBMD regulation as determined by pQCT. Replicate studies with these sublines should show whether the regulation of vBMD and % fat co-segregate. Such a result would demonstrate that environmental regulation of bone and fat occurs through genes in this Chr 1 region.

***Disclosures**: **W. G. Beamer**, None*

T50

Loss of Inhibitor of Differentiation 3 leads to Decreased Adiposity and Increased Bone Density in Male Mice

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Purpose: Both the adipocyte and osteoblast lineage are known to derive from a common mesenchymal stem cell progenitor. However, the exact pathways that regulate cell determination are not well understood. Previous studies provide evidence for a role for Inhibitor of differentiation 3 (Id3) in both osteoblast and adipocyte

differentiation through upstream regulation by the transcription factor SMAD4. Our goal was to evaluate the effect of loss of Id3 on adipose tissue and bone formation in vivo and to validate an in vivo system that may allow us to identify downstream Id3 targets that could mediate lineage commitment.
Methods: Male Id3 knockout (KO) and C57BL/6J (Bl/6) litter mate control mice were placed on chow or obesity inducing (OI) diets (Id3 KO chow n=9, OI n=10; bl/6 chow n=10, OI n=8) at 4 weeks of age. DXA scans were performed after 4, 8, 12 and 16 weeks of diet. Measurements of body fat percentage and bone mineral density (BMD) were obtained. Expression quantitative trait locus (eQTL) analysis and causality modeling, using adipose microarray profiles from C57BL/6J x C3H/HeJ F2 mice (N=295), were used to identify genes regulating Id3 expression.
Results: After 16 weeks of diet, DXA scan results indicated that on both the chow and OI diet Id3 KO mice had reduced total body fat (TBF) percentages compared to the Bl/6 mice (15.1% and 17.3% (p=0.044) respectively for chow; and 31.8% and 38.9% (p=0.0352) respectively for OI). Total body weight (TBW) was not different on the chow diet, but was significantly lower in the Id3 KO mice compared to the Bl/6 mice on the OI diet (p=0.035). Analysis of femoral areal BMD in these mice at the same time points demonstrated higher BMD in the KO mice compared to controls on both diets (p=0.0482 and p=0.0095 respectively). In addition, based on coincident eQTL and causality modeling we confirmed Smad4 as an upstream regulator of Id3 expression.
Conclusions: These data suggest a preference for the development of bone over adipose tissue in the Id3 KO mouse and provide evidence that eQTL analysis and causality modeling can identify known regulators of the Id3 differentiation pathway. Future directions will include utilizing this system to identify downstream Id3 targets that direct mesenchymal precursor cells to the adipocyte lineage.

***Disclosures**: **A. Cutchins**, None*

T52

Increased Mesenchymal Progenitor Cells in Bone of RB1-/- Mice

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The retinoblastoma pathway is deregulated in 90% of human cancers. Yet, the molecular pathogenesis of tumors that directly target pRb (retinoblastoma and osteosarcoma) are poorly understood. To understand the role of pRb in tumor formation we have focused on a tissue where RB is commonly deleted, bone. Human osteosarcoma cells in general exhibit no signs of terminal differentiation, and are defined by bone formation. We hypothesize that osteosarcoma is caused by molecular/genetic disruptions of the osteogenic differentiation pathway. Homozygous null RB mice die (E11.5-13.5)before any significant skeletal development or mineralization occurs. Therefore, we utilize the cre/lox system to create a bone targeted RB1 knockout animal. RB1-/- mice display defects in bone formation. Isolation and characterization of primary calvarial cells (dorsal skull cells) from these animals show that pRb abates progression of pre-osteoblasts to mature osteoblasts. We asked if diminished differentiation resulted in an increase in mesenchymal progenitor cells (MSC). MSCs are pluripotent cells that can differentiate into a variety of cell types. I have shown in the absence of RB1, calvarial cells can differentiate into multiple lineages including bone and fat, where WT littermates can are restricted to the osteoblast lineage. Colony formation assays, a functional in vitro assay, suggest RB1-/- cells have increased progenitor cell number, and through sphere formation assays have shown an increased self-renewal capacity. There is also a significantly larger population of SCA-1 (a stem cell marker)positive cells in RB1-/- cultures compared to WT littermates. In addition, utilization of microarray technology suggests a unique RB1 dependent gene expression pattern in osteogenesis. This is consistent with our previous work showing the necessity of the pRb/Runx2 interaction to turn on osteocalcin transcription, a late marker in differentiation. We have begun to identify a possible mechanism for pRb regulation early in osteogenesis through the hedgehog signaling pathway that explains the increased pluripotency of mesenchymal cells in the absence of RB1. Therefore, we have identified an increased number of bone progenitor cells that are pluripotent from RB1-/- mice. Our studies suggest a novel function of pRb in tissue differentiation of adult stem cells, which is critical for controlling not only the proliferation of progenitor cells, but that of differentiating bone cells as well.

***Disclosures**: **E. Kong,** None*

T54

An interaction between PPAR gamma and polyunsatured fatty acids influences changes of bone microstructure in aging mice

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A Western diets characterized by low ratios of omega-3/-6 fatty acids (FA) have been associated with age-related chronic disorders. Mechanisms of PPAR γ activation, increased adiposity and decreased osteoblastogenesis. In B6.C3H-6T (6T) congenic mice, which express higher levels of PPAR γ compared to genuine C57BL/6J (B6), bone loss induced by a fat-rich diet is magnified (Ackert-Bicknell et al, JBMR 2008). Purpose: We hypothesized that the level of PPAR γ would specifically influence the effects of omega-3/-6 on bone mass and structure.
Methods: 6T and B6 female mice aged 3 months were given either a fish oil diet (omega-3/-6 ratio: 7.9) or an isocaloric safflower oil diet (omega-3/-6 ratio: 0) for 8 months (n=10). Changes in body composition and bone mass were analysed by Piximus, and microstructure in caudal spine and tibia by in vivo microCT.
Results: After 8 months, body weight and % fat were lower with fish oil compared to safflower in 6T (-24.9% and -35.4%, respectively, $p<0.05$) and B6 (-30.0%, -27.6%, respectively, $p<0.05$). Spine BMD was significantly improved in response to fish oil in B6 (71±3mg/cm2 vs 54±3mg/cm2 with safflower, $p<0.01$), whereas femoral BMD was unaffected. In contrast, in 6T, vertebral BMD did not significantly differ between the two diets whereas femoral BMD was significantly lower in the fish compared to safflower group (76±2mg/cm2 vs 83±1 mg/cm2, $p<0.05$). In caudal spine of B6 mice, fish oil inhibited loss of BV/TV and trabecular number (TbN) (-6.0% and +2.2% versus -32% and -11.4% in the safflower group, respectively, all $p<0.05$), whereas in 6T, fish oil did not have significant effects. In the proximal tibia, the loss of BV/TV and TbN with aging was not prevented by fish oil in B6. However, in 6T, fish oil further increased the loss of BV/TV and TbN (-93.7% and -94.4%, versus -84.8% and -87.3% in safflower group, respectively, all $p<0.05$). At the tibia midshaft, cortical bone volume gain was significantly reduced by fish oil in B6 (-1.7% vs +8.0% in safflower group, $p<0.05$) and 6T (-1.7% and +5.6% in safflower group, $p<0.05$). These differences did not remain significant after adjustment for body weight.
Conclusions: Compared to a diet rich in omega-6s, fish oil prevents spine BMD and trabecular bone loss. These beneficial effects are lost, and eventually reversed, in mice expressing PPAR γ gene at higher levels, suggesting that the effects of FA on bone microstructure may depend on interactions with PPAR γ genotypes. Moreover, the effects of FA on trabecular microarchitecture appear to be site dependent.

***Disclosures**: **N. Bonnet**, None*

T56 Withdrawn

T58

Characterization of the Lipodystrophic Phenotype that Accompanies High Bone Mass in the Ebf1 Deficient Mouse

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Early B cell factor-1 (Ebf1) is a transcription factor known to be important for B cell development, but mice deficient for Ebf1 exhibit markedly increased bone density with increased numbers of osteoblasts, bone formation rate and serum osteocalcin. In addition to the large number of osteoblasts on bone surfaces, the bone marrow of Ebf1-/- mice is striking in its increased marrow adiposity, which is independent of the lack of B cells. Therefore, the purpose of this work was to characterize the lipodystrophic phenotype that accompanies the altered bone morphology of Ebf1-/- mice using a combination of molecular and metabolic techniques. While marrow adiposity was increased in the knockouts (KOs) compared to the wild-type (WT) controls, deposition of white adipose tissue to other regions of the body, both subcutaneously (40-50%) and abdominally (80-85%), was severely reduced at all ages. At the same time, brown adipose tissue was not affected. Transcriptional profiling of white adipose tissue showed a marked decrease in the expression of PPAR□ and C/EBP transcription factors in Ebf1-/- tissue compared to WT. Consistent with the observed decrease in the body-wide white adipose tissue, circulating levels of leptin were decreased in KO animals of all ages compared to their litter-mate controls (decreased 65-95%). Interestingly, however, the levels of a second adipokine, adiponectin, were comparable to controls at all ages after 2 weeks of age. Serum analysis also found the KO animals to be hypoglycemic and hypotriglyceridemic. This was accompanied by normal or reduced circulating insulin levels, while circulating glucagon was significantly increased (increased 1.7-8.5 fold). In conclusion, it appears that the Ebf1 KO animals exhibit lipodystrophy that manifests as global decreases in white adipose tissue with increased marrow adiposity. This was not accompanied by insulin resistance, but instead persistent hypoglycemia that is not resolved by elevated circulating glucagon. The striking early increase in all bone formation parameters and the unexpected concomitant accumulation of adipocytes in the bones of Ebf1-/- mice identifies Ebf1 as a heretofore-unrecognized transcription factor required for the regulation of osteoblast development and adipose tissue allocation.

***Disclosures**: **J. Fretz**, None*

T60

Role for Tartrate Resistant Acid Phosphatase as an Inducer of Osteoblast/Adipocyte Lineage Cell Differentiation and Activity

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Mesenchymal stem cells (MSCs) can give rise to diverse tissues among them bone and fat. Osteoblast and adipocyte lineage cells share or respond oppositely to factors regulating their proliferation/differentiation, suggesting a close ontogenetic relationship between these cell types.
One factor of potential relevance in this regard is tartrate-resistant acid phosphatase (TRAP) alias purple acid phosphatase (PAP) or type 5 acid phosphatase (AcP5). TRAP is predominantly expressed by osteoclasts and macrophages with suggested roles in bone resorption and innate immune responses. Importantly, proteolytic processing of a monomeric biosynthetic precursor generates the enzymatically active protein that has been implicated in regulation of cell adhesion and migration, formation of oxygen radicals and T-helper cell polarization. Recently, we described (Lång et al. PLoS ONE 3: 3; e1713, 2008) that expression of monomeric TRAP induces obesity due to adipocyte hyperplasia through secretion from adipose tissue macrophages, suggesting that the action of TRAP is partly controlled by the proteolytic status of the molecule. As earlier studies suggested that TRAP induce differentiation of osteoblasts in culture, the aim of this study was to further investigate the in vivo role of TRAP in bone remodelling.
This was achieved by the use of a previously described lean TRAP overexpressing mouse model (Angel et al. JBMR 15:103-110, 2000) in which expression of the construct is dependent on the TRAP promoter thus only overexpressing TRAP in cells normally able to express TRAP. Unexpectedly, these mice did not show signs of altered bone resorption i.e. serum CTx, osteoclast number and morphology were normal in the transgenic mice. However, they did exhibit increased bone mineral content (BMC) and density (BMD) along with increased bone formation markers such as serum PINP and mRNA levels of osteocalcin, Cbfa-1/Runx2, collagen I, alkaline phosphatase and bone sialoprotein. The major cell types overexpressing TRAP in this model was not the osteoclast but rather osteoblasts and osteocytes. These results indicate that TRAP overexpression in osteoblastic cells causes an increase in osteoblast differentiation and/or activity. These data together with the recent finding that TRAP induces proliferation and differentiation of adipocyte progenitors suggests that TRAP can act as an inducer of osteoblast/adipocyte lineage cell differentiation and activity in vivo.

***Disclosures**: **P. Lång,** None*

T62

Progressive Lipodystrophy in the Osteosclerotic Mice Over-expressing Fra1

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A link between bone and fat mass has been established the last few years suggesting that common systemic and/or local molecular mechanisms are shared between osteoblast and adipocyte differentiation. We therefore analyzed the role the osteogenic transcription factor Fra1 in fat. In vitro, the adipogenic differentiation of primary osteoblast (POBs) isolated from the calvaria of pups over-expressing Fra1 (fra1-tg mice) was found to be drastically reduced when compared to wild type littermates. This effect was Fra1-dependent since constitutive or inducible over-expression of Fra1 in an adipogenic cell line strongly inhibited adipocyte differentiation. It was not due to decreased cell growth. At the molecular level, the response to insulin as measured by ERK and AKT activation was unaffected, as was the induction of the glucocorticoid-regulated genes Gilz, Per1 and Adrb2. Although, the expression of key regulators of mesenchymal cell fate decision (i.e. Runx2, Sox9, MyoD, C/eblβ and C/ebp□) was unchanged indicative of a normal commitment potential of the cells; we found the adipogenic transcription factor C/ebpα to be down regulated in fra1-tg POBs and in the adipogenic cell line over-expressing Fra1. Consequently, Pparγ was also down-regulated in Fra1 over-expressing cells. The relevance of these observations was established by the finding that fra1 transgenic mice that developed progressive osteosclerosis due to increased osteoblast differentiation were also developing a parallel drastic progressive lipodystrophy

leading to absence of white fat tissue (WAT). The phenotype was found to be more pronounced in the females than in the males. Histologically, when present the white adipose tissue that expressed high level of Fra1 appeared immature and expressed lower level of the adipogenic marker aP2 and Glut4 as well as C/ebpα. In contrary, despite a similar increased in the level of Fra1 expression, the histology of brown adipose tissue (BAT) and the expression of its typical markers Ucp1, Pgc1 and Pgc1β were unchanged. Thus, our data add to the known common systemic control of fat and bone tissue, a new cell autonomous level of control of cell fate decision by which an osteogenic transcription factor such as Fra1 is promoting bone formation and opposing adipocyte differentiation. This latter effect is being caused by C/EBPα down-regulation. In addition, they demonstrate that increased bone mass can also be observed in lean mice.

***Disclosures**: **J. Luther**, None*

T64

Adult Murine Mesenchymal Stem Cells Respond to Mechanical Strain with an Anti-Adipogenic/Pro-Osteogenic Program

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Exercise combats obesity while promoting the formation of bone. Since adipocytes and osteoblasts originate from a common precursor, the mesenchymal stem cell (MSC), effects of exercise on both fat and bone may occur at the stage of mesenchymal lineage selection. We have investigated the usefulness of MSCs cloned from adult murine marrow in the study of mechanical regulation of MSC lineage. We first confirmed that MSCs made from C57BL/6 adult males would undergo controlled differentiation. After 4 days in adipogenic medium, MSCs expressed PPARγ and adiponectin protein and stained positively for oil-red-O. Adipogenesis was accompanied by a reduction in β-catenin levels. MSCs grown in osteogenic medium for 5 days showed positive alkaline phosphatase staining and increased osterix expression, while bone nodules stained by alizarin red were present after 21 days. Hematopoietic stem cells were excluded from the MSC cultures, as assessed by FACs analysis. In adipogenic medium, application of strain (3600 cycles, 2%, daily for 3 d) reduced PPARγ and adiponectin mRNA by 30% and 50%, with reduction confirmed by Western. The decrease in β-catenin accompanying adipogenesis was prevented by the strain regimen. These results are consistent with our prior work showing that strain prevented adipogenesis and involved β-catenin in C3H10T1/2 cells. We next evaluated MSCs made from adult Caveolin-1 null mice and found that adipogenesis was retarded: after 3 days in adipogenic medium, PPARγ and adiponectin mRNA levels in Cav-1 null MSCs were only 5% and 1% compared to concurrently differentiating caveolin-1 replete MSCs. However, adipogenesis had progressed in the Cav-1 null MSCs by 7 days. Importantly, strain effectively inhibited adipogenesis in the Cav-1 transgenic MSCs. We next considered strain effects on MSCs grown in osteogenic medium for 3 days. Application of strain for 6 hours increased COX2 and WISP1 mRNA by 212% and 199% in wild-type MSCs, consistent with strain activation of β-catenin we previously described in pre-osteoblasts. In contrast, strain failed to increase COX2 or WISP1 in Cav-1 null MSCs, and these MSCs did not form bone nodules, suggesting that osteogenic differentiation was perturbed in the absence of caveolin-1. In conclusion, we have shown that C57BL/6 MSCs, which effectively undergo adipogenesis and osteogenesis, respond to mechanical strain in a manner similar to our prior work in murine cell lines. That the caveolin-1 null MSCs respond differently suggests that MSCs from transgenic mice can be expected to provide further insights into the mechanisms by which mechanical strain influences mesenchymal lineage selection.

***Disclosures**: **N. D. Case**, None*

T66

Adipocytes Express a Functional System for Serotonin Synthesis, Re-Uptake and Receptor Activation

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Serotonergic pathways in the central nervous system (CNS) are activated in the regulation of food intake and body weight. In the search for an efficient treatment of obesity, numerous drugs that interact with the serotonergic system have been developed. In this study we hypothesized that adipocytes, like other cells of mesenchymal origin, possess serotonin receptors, and thus could be regulated by peripherally circulating serotonin.
Using RT-PCR analyses, we found that human, mouse and rat adipocytes express serotonin receptors, the serotonin transporter (5-HTT), and the rate-limiting enzyme in serotonin synthesis, tryptophan hydroxylase 1 (Tph1), indicating that adipocytes are able to respond to serotonin and to regulate serotonin availability themselves. In vitro experiments demonstrated that serotonin induces adipocyte proliferation through binding to 5-HT2A/C receptors with subsequent activation of the PKC pathway, and that serotonin regulates synthesis of leptin in mature adipocytes. In vivo experiments showed that hyperserotoninergic Sprague Dawley rats had lower plasma leptin levels than controls, after both short- and long-term serotonin treatment, whereas ghrelin levels were unaffected. Long-term serotonin treated rats had, as expected, a significantly lower body weight than controls.
These findings are of clinical importance as they show that serotonin regulates adipocyte function in a direct manner via the blood circulation and/or paracrine and autocrine mechanisms, and not only indirectly via the CNS as previously assumed.

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

T68

Direct Regulation of Secreted Phosphoprotein-24 Gene Expression by IGF in Bone Progenitor Cells Lines

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Secreted Phospoprotein-24 (Spp24) is a 24kDa non-collagenous bone matrix protein of a currently unresolved function. Data from our lab has shown that adenoviral overexpression of Spp24 in human mesenchymal stem cells (MSC) yields a potent increase in the osteoblast phenotype over Ad-LacZ control when cultured in differentiation media. The purpose of this study was to investigate what molecule(s) are upregulating Spp24 in bone. To elucidate the signaling mechanisms of Spp24, our lab has cloned the 1.7Kb upstream promoter sequence into a luciferase reporter vector and overexpressed it inside two bone progenitor cell models, C2C12 murine myoblasts and HS-5 human stromal cells. We have found that out of a number of known osteogenic molecules (IGF-I, IGF-II, HGF, PTH, VD, BMP, Forskolin), significant direct regulation was seen only by IGF-I and IGF-II. While HGF was unable to stimulate Spp24 activity by itself, it did have a significant increase when treated simultaneously with IGF-II. In order to verify the action of

IGF at the transcriptional level we treated hMSCs with IGF-I. RT-PCR showed an increase in Spp24 mRNA levels in a dose responsive manner, confirming our promoter data. We next sought to determine the mechanism by which IGF-I and IGF-II were increasing Spp24 activity. Since IGF1R shows partial preference for IGF-II, a combination treatment of IGF-I and IGF-II was given to progenitor cells transfected with the Spp24 promoter. No increase in regulation over normal IGF treatment, either additively or synergistically, was observed, suggesting that the two molecules were both acting through the same mechanism. A number of putative binding sites for the AP-1 transcription factor, which has been shown to stimulate osteoblastogenesis, have been found on the Spp24 promoter in silico. It is known that c-fos/Jun, two major components of the AP-1 transcription factor, are commonly upregulated by activation of the IGF1R pathway. Thus, it is our current hypothesis that enhancement of Spp24 is activated by the AP-1 transcription factor through direct protein-DNA promoter interactions, resultant from IGF-I (or IGF-II) action on its receptor, IGF1R. Collectively, these data show that Spp24 is a novel downstream molecule in the GH/IGF axis that is directly regulated by IGF. In light of Spp24's actions, it is likely that this interaction is important to IGF action on bone, especially, the IGF-mediated enhancement of osteoblast formation.

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

T70

Alpha-Linolenic Acid as a Potential Inhibitor of Adipogenesis

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The Western diet has a high n-6 to n-3 PUFA ratio which is believed to be responsible for many inflammatory diseases, including obesity and osteoporosis. Consequently, there is great interest in increasing n-3 PUFA in the diet and in particular alpha-linolenic acid (ALA), high in flax seeds. Additionally, ALA is the primary n-3 PUFA in dairy products existing as a blend of low n-6 (linoleic acid):n-3 (ALA) ratios. The aim of this research is to investigate if ALA increases osteoblastogenesis and reduces adipogenesis by using three cell lines; osteoblasts (MC3T3-E1); adipocytes (MC3T3-L1); and stromal stem cells (ST2). The concentrations of ALA in our experiments were based on the total triglyceride concentration of bovine serum (culture media) as fatty acid-free serum is not readily available. For ST2 and MC3T3-E1 cells, fetal bovine serum contained 54µg lipid/ml complete media. Therefore, to achieve total lipid:ALA ratio of 1:1, the media was supplemented with 54µg/ml of ALA. Similar calculations were used to obtain other lipid:ALA ratios. The molarity of the supplemented ALA ranged from 200 µM (1:1) to 40 µM (5:1). For MC3T3-L1 cells, the new-born calf serum contained 9.8µg lipid/ml media, therefore 9.8µg of ALA was added to obtain a 1:1 ratio, etc. giving a range of 35 µM (1:1) to 7 µM (5:1). Thus, the ratio of total lipids to ALA was similar for all cell lines but the molarity was different. All experiments were repeated 4 times. A resazurin salt (Sigma-Aldrich, St. Louis, MO) assay was used to determine cell numbers. ANOVA was performed using Graphpad Prism with variation attributed to concentration of treatment compounds and $P < 0.05$ was considered significant. ALA at all ratios significantly reduced osteoblast and adipocyte proliferation. For ST2 cells, ALA at a ratio of 1:1 significantly inhibited proliferation but as the ratio of total lipid to ALA increased proliferation returned to control levels. This indicates that ALA may inhibit proliferation when present in relatively high concentrations but the mechanism is unclear. As ALA inhibited pre-adipocyte proliferation at all ratios it may be effective at reducing adipogenesis at more physiologically relevant concentrations (35 µM to 7 µM) while having little impact on osteoblast or stem cell proliferation. These results emphasize that the actual ratios of n-6:n-3 fatty acids are critical and may be independent of individual fatty acid concentrations in regulating cell metabolism.

***Disclosures**: **O. J. Kelly**, None*

T72

WNT Signaling during Adipocytogenesis of Human Bone Marrow Stromal Cells

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From the body of information available about mechanisms of adipocyte differentiation with murine preadipocytes, it is known that 1) Wnt10b inhibits adipogenesis and stimulates osteoblastogenesis, and 2) Wnt4 and Wnt5a stimulate adipocytogenesis. Because little is known about WNTs and human adipocytogenesis, we tested the hypothesis that WNT signaling regulates adipocytogenesis of human bone marrow stromal cells (hMSCs); we assessed canonical and non-canonical WNT and effects of a small molecule stimulator of β-catenin (SB-216763) during adipocytogenesis.
Bone marrow samples were obtained with IRB approval as discarded femoral tissue. Low-density mononuclear cells were isolated by density centrifugation on Ficoll/Histopaque 1077. Adherent hMSCs were expanded 2 passages. Upon near-confluence of hMSCs, medium was changed to α-MEM, 1% FBS-HI with adipocytogenic supplements (1 µM dexamethasone, 0.5 mM 1-methyl-3-isobutylxanthine, and 10 µg/ml insulin). The effect of adipocytogenic medium on the expression (RT-PCR) of adipocyte maker genes and WNT genes was performed at intervals to 10 days. The effects of SB-216763 on expression of adipocytogenic genes during differentiation were tested. Eighteen days after treatment, adipocytes were counted in cells positive for staining with 0.3% Oil Red-O. hMSCs from six subjects were used to assess reproducibility.
Analysis of adipocyte marker genes indicated that the expression level of PPARγ2 and LPL was detectable after 1 day in adipocytogenic medium and increased thereafter. The expression of adipsin and leptin also increased with time. Upon adipocytogenic differentiation of hMSCs (day 1), the expression of canonical WNT genes (2, 10B, 13, and 14) decreased, whereas non-canonical WNT genes (4 and 11), but not WNT5A increased. WNT11 was the only one to change prior to upregulation of adipocyte signature genes. SB-216763 (5 µM), which increased β-catenin levels (Western immunoblotting) inhibited adipogenesis by blocking induction of PPARγ2, LPL, and adipsin. Consistent with the molecular effects, SB-216763 inhibited generation of Oil Red-O adipocytes with duration- and dosage-dependence.
These study indicate that for human adipocytogenesis, non-canonical WNT11 and 4 may be major enhancers and that activation of canonical WNT signaling pathway prevents hMSCs from differentiating into adipocytes. Thus, there may be fundamental species differences in WNT signaling during adipocytogenesis.

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

Bone as an Endocrine Organ

T74

Relationship Between Osteocalcin and Glucose and Energy Metabolism -- Data from the ECKO Trial

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Purpose: Recent data suggests that a vitamin K-dependent bone protein, osteocalcin, may play a role in glucose and energy metabolism. We explored the relationship between osteocalcin and glucose and energy metabolism by analyzing serum samples collected in the ECKO trial.
Methods: The ECKO trial is a 2 to 4-year randomized double-blind placebo-controlled trial of vitamin K (5mg/day) supplementation versus placebo in 440 postmenopausal women with osteopenia. We analyzed stored baseline and 1 year serum samples from consented women without diabetes for total osteocalcin, undercarboxylated osteocalcin, degree of undercarboxylation, fasting glucose, fasting insulin and total adiponectin levels. We calculated HOMA-IR, a measure of insulin resistance. Using Spearman correlations and multivariable regression analyses, we assessed the relationship between osteocalcin and parameters of glucose and energy metabolism.
Results: Three hundred women (mean age = 59.6 years; mean BMI = 26.2; 92% Caucasians) were included in the study. At baseline (before any vitamin K or placebo supplementation), adiponectin levels were higher among Caucasians than other groups (13.0ug/ml vs. 9.3ug/ml). Baseline adiponectin was correlated with BMI ($r = -0.32$, $p<0.0001$) and HOMA-IR ($r = -0.49$, $p<0.0001$). Total osteocalcin was weakly correlated with adiponectin ($r = 0.17$, $p=0.003$) and HOMA-IR ($r = -0.12$, $p=0.04$), but there were no relationships between undercarboxylated osteocalcin (or degree of undercarboxylation) and fasting insulin, HOMA-IR, adiponectin, or BMI. At 1 year, the vitamin K supplemented group (n=139) had significantly lower total osteocalcin (21ng/ml vs. 24ng/ml, $p<0{\cdot}0001$), undercarboxylated osteocalcin (-52·8% vs. -3·5%, $p<0{\cdot}0001$) and degree of undercarboxylation (-21·4% vs. –2·0%, $p<0{\cdot}0001$) when compared to the placebo group (n=161). However, these perturbations in osteocalcin and undercarboxylated osteocalcin did not result in any changes in fasting glucose, fasting insulin, HOMA-IR or adiponectin.
Conclusions: Our data do not support osteocalcin, especially undercarboxylated osteocalcin, as a significant regulator of glucose and energy metabolism in healthy postmenopausal women with osteopenia. Further research is needed to clarify whether osteocalcin and undercarboxylated osteocalcin have endocrine functions in humans.

***Disclosures**: **A. Cheung**, None*

T76

Once a Day Injections of Osteocalcin Improve Glucose Tolerance and Insulin Sensitivity and Prevent Type 2 Diabetes

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We have shown previously that the uncarboxylated form of the osteoblast-specific secreted molecule osteocalcin functions as a hormone favoring glucose handling and increasing energy expenditure. As a result, the absence of osteocalcin leads to glucose intolerance in mice, and genetically modified mice with an increase in uncarboxylated osteocalcin are protected from type 2 diabetes and obesity. To assess the therapeutic relevance of these observations we tested whether osteocalcin injected once daily could affect glucose metabolism in wildtype (WT) mice. We show here that injection of osteocalcin (3 or 30 ng/g/day) once a day significantly improved glucose tolerance, as assessed by glucose tolerance tests (GTT), in WT mice fed a normal chow. Moreover, insulin sensitivity was also improved after 4 weeks of this regimen as measured by insulin tolerance tests (ITT). Blood glucose after feeding was significantly reduced following osteocalcin daily injections. Remarkably, daily injections of osteocalcin increased β-cell proliferation, β-cell mass and insulin secretion. Thus, daily injection of osteocalcin has a beneficial effect on glucose metabolism in mice fed a normal chow.
We next asked whether daily injections of osteocalcin could have these beneficial effects on glucose tolerance and insulin sensitivity in a model of diet-induced type 2 diabetes. To that end WT mice were fed a high fat diet (HFD) for 8 weeks and then injected once a day with osteocalcin (30 ng/g/day) for 8 weeks. Osteocalcin daily injections increased energy expenditure thereby preventing body weight gain. Moreover, fasting blood glucose and serum insulin levels were markedly reduced when compared to placebo-treated mice, and insulin sensitivity, as measured by the Homeostasis Model Assessment (HOMA), was partially restored. As a result, glucose tolerance was significantly improved in animals maintained on HFD and injected once a day with osteocalcin. Finally, the hepatic steatosis induced by HFD was completely absent in mice fed a HFD and receiving osteocalcin daily. Overall these results provide evidence that, at least in mice, osteocalcin injected once a day does improve glucose handling and prevents the development of type 2 diabetes.

***Disclosures**: **M. Ferron**, None*

T78

Gene variation within aromatase regulates estrogen production and thereby bone structure in elderly women

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Variation in the aromatase gene has been shown to be an important determinant of estrogen activity in postmenopausal women. The aromatization of androgenic precursors in adipose tissue is the main source of estrogens after the menopause. Production of estrogen at this local level may control the differentiation of human mesenchymal stem cells (hMSCs) to either osteoblasts or adipocytes, making variation within the aromatase gene a key component in osteoblast generation and pre-adipocyte accumulation. We have previously shown that a missense mutation within exon 5 of the aromatase gene (CYP19A1) increases Vmax an in vitro assay by 400%. This study aimed to study the effect of the gene variant on the bone structure of a population cohort and its effect in a relevant cell type, the pre-adipocyte. Study subjects were members of a population-based cohort comprising of 1,185 Caucasian women aged

between 70 and 85 years. BMD was measured by DXA (Hologic QDR 4500). Serum estradiol was measured by RIA (Orion Diagnostica, Finland). Genotyping of the T201M site used Taqman (Applied Biosystems, Foster City, CA). Statistical analysis was performed using SPSSv15. The frequencies of the genotypes in the population were CC = 0.83, CT = 0.16, TT = 0.1. Using a dominant model the variant (T) was associated with increased estradiol for the heterozygote and rarer homozygote combined (CC: 25.5 ± 14.9 pmol/L vs. CT, TT: 38.3 ± 27.4 pmol/L; P = 4.5 x10-21). In addition, the variant allele was associated with increased BMD at the total hip (CC: 806 ± 126 mg/cm2 vs. CT, TT: 831 ± 129 mg/cm2; P = 0.015), femoral neck (CC: 686 ± 104 mg/cm2 vs. CT, TT: 706 ± 109 mg/cm2; P = 0.021). In vitro aromatase activity assays of cultured primary pre-adipocytes from omental and subcutaneous fat are in progress. Early data is suggestive of large variation in aromatase activity which may be associated with aromatase genotype variation. These data confirm previous findings on the importance of gene variation in the estrogen pathway and identify a specific aromatase variant having a substantial effect on bone structure that also occurs at high frequency in the population.

***Disclosures**: **E. J. Payne**, None*

Other

T80

Genetic and Environmental Effects on Cortical Bone Morphology, Obesity, and Leptin

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Cortical bone properties, such as bone area (B.Ar), moment of inertia (J) and cortical thickness (Ct.Th), are associated with measures of overall body weight and obesity, such that obesity is seen as protective against osteoporosis. We examine the genetic and environmental bases for this relationship in 512 mice from a set of 16 Recombinant Inbred (RI) mouse strains formed from the LG/J by SM/J intercross. Eight animals of each sex and strain were reared on high or low fat diets and fat depots weighed at necropsy at 24 weeks. MicroCT scans were obtained for the radius and femur and B.Ar, J and Ct.Th measured. The effects of diet, genes, and random environment were measured using MANOVA and quantitative trait locus (QTL) mapping.
Dietary effects on both femoral and radial cortical morphology were observed primarily in females and were stronger in the femur than in the radius. Animals reared on a high fat diet had larger bones than those reared on a low fat diet. Heritabilities were moderate (45%-60%) for femoral features and about 25% lower (13%-33%) for the same features in the radius. This may be due to the smaller size of the radius relative to measurement errors. Cortical features were strongly positively genetically correlated ($0.65 < rG < 0.96$). Correlations across the bones were also substantial, indicating that genetic factors affecting various aspects of cortical morphology are held in common. Leptin levels are negatively genetically correlated with femoral B.Ar and J, but positively correlated with Ct.Th (rG = 0.34). Random environmental factors lead to no correlation between leptin levels and femoral morphology. Total fat pad weight (rG = 0.64) and body weight (rG = 0.47) are also positively genetically correlated with femoral Ct.Th. Leptin levels are not significantly genetically correlated with radial cortical properties but total fat pad weight and body weight are positively genetically correlated with B.Ar (rG = 0.51; 0.51) and J (rG = 0.64; 0.52) and IYY (rG = 0.61; 0.48). Random environmental factors do not lead to a relationship between obesity and leptin and cortical bone properties. 42 quantitative trait loci (QTLs) were mapped for cortical features on all chromosomes except 19, and of these, 32 coincided with locations previously found to harbor genes affecting fat pad weights and leptin levels in these strains. Chromosomes 9 and 10 harbored male-specific loci.

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

T82

Low-Level Whole Body Vibrations that Suppress Adiposity and Increase Bone Mass do not Increase Activity Levels and Fuel Utilization in Mice

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Exposure to extremely low-amplitude whole-body vibrations suppresses the increase in adiposity during growth and enhances bone mass and architecture in mice. The underlying putative mechanism has been linked to changes in the mesenchymal stem cell pool in the bone marrow in which the application of high-frequency mechanical signals favors the differentiation of stem cells into osteoprogenitor cells over pre-adipocytes. Here, we tested whether the mechanical intervention elevates fuel utilization which could act as co-mechanism of altered fat mass. At 7wk of age, male chow-fed C57BL/6J mice were assigned to control and experimental mice (n=8, each). For 12wk, 15min/d, experimental mice were subjected to low-level whole-body vibrations (90Hz, 0.2g). The animal model and mechanical intervention were identical to those used in previous investigations in which vibrations altered fat and bone mass. During the second and ninth week of the experimental protocol, mice were individually housed in calorimetric cages for 48h, respectively. Prior to returning the mice to their regular cages, they were fasted for 15h and then re-fed. Throughout each 48h period, activity levels of each individual mouse were measured by multiple infrared beams installed in the cages. The respiratory quotient (RQ), the ratio of the amount of carbon dioxide produced to the amount of oxygen consumed was measured in each mouse during the second 48h period. A RQ value of 1.0 indicates exclusive carbohydrate oxidation whereas a RQ value of 0.70 reflects exclusive fatty acid usage. During each of the 12wk, food consumption as well as body mass did not differ between the two groups. Indirect calorimetry showed that during night, mice primarily oxidized carbohydrates (RQ=0.95±0.03 for control, 0.97±0.02 for vibrated) while during the day, a partial fasting period for mice, a mixture of carbohydrates and fatty acids was oxidized (0.91±0.04 vs 0.92±0.04). During any given period, there were neither significant differences in the RQ nor in the activity levels between control and experimental mice. The absence of significant differences in skeletal muscle fatty acid utilization limits its role in the suppression of adiposity by low-level vibrations. The data also suggest that this mechanical intervention may be incapable of reducing already established fat mass and, indirectly, lend further support towards a stem-cell driven mechanism that favors the differentiation of stem cells into osteoprogenitor cells over pre-adipocytes.

***Disclosures**: **S. Judex**, None*

T84

Do Treatments of Osteoporosis Alter the Relation Between Bone and Fat?

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Current FDA approved pharmaceutical treatments of osteoporosis such as anti-resorptive bisphosphonates (e.g., Alendronate) and

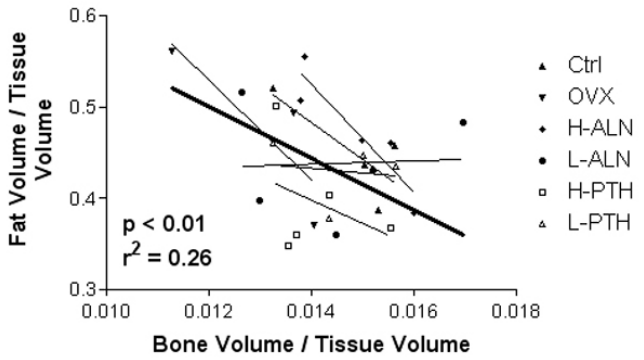
anabolic therapies (e.g., parathyroid hormone) focus on retaining or improving bone mass. However, the regulation of bone and fat formation is clinically relevant to the increasing prevalence and associated cost of osteoporosis. It is unclear how the complex relationship between adiposity and bone may be altered by treatments of osteoporosis. In an effort to identify potential links, we used in vivo micro-computed tomography to relate changes in adiposity, as induced by two popular osteoporosis drug treatments, to changes in skeletal parameters in an ovariectomized (OVX) rat model.

Six-month old female Sprague-Dawley rats were separated into 6 groups (n=5/group): 1) Control, 2) OVX, 3) OVX treated with high-dose (60µg/kg) hPTH(1-34) (H-PTH), 4) OVX treated with low-dose (1µg/kg) hPTH(1-34) (L-PTH), 5) OVX treated with high-dose (100µg/kg/day) Alendronate (H-ALN), or 6) OVX treated with low-dose (1µg/kg/week) Alendronate (L-ALN). All rats were scanned using in vivo micro-CT at 6 and 8 months of age to determine changes in abdominal bone, muscle, and fat.

After 2 months of treatment, there were no significant differences in the change in body weight among all groups. However, there were significant changes in bone, muscle, and fat mass. Both ALN and PTH treatments resulted in significant decreases in the amount of bone and muscle lost and the amount of fat tissue gained compared to OVX ($p<0.05$). However, there were no significant differences between treatments. Interestingly, despite similar changes in bone volume after 2 months, H-ALN and H-PTH showed significant increases in lumbar vertebral bone density (3.4% + 1.0% and 4.3% + 1.4% increase respectively) indicating treatment-related changes in bone tissue density. Overall, there was a negative correlation between changes in adiposity and bone volume ($p<0.05$; $r2=0.26$). However, the same relationship was not observed within treatments indicating that the link between bone and fat was not affected directly by either PTH or ALN treatment.

These data indicate that even short-term treatments of osteoporosis can alter bone quality and adiposity, and that none of the specific treatments and doses modified the general association between changes in fat and bone. Further study is needed to determine how long-term treatment affects the relationship between bone and fat.

Figure 1.



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

T86

Osteoprotegerin and adipocytokine levels in relation to bone mineral density and visceral fat content in young men

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Recent evidence suggests that adiponectin may play a role in bone metabolism. The aim of this study was to determinate the relationship between visceral adiposity and bone mineral density (BMD) as well as adipocytokines in younger men. Main outcome measures include adiponectin, osteoprotegerin, IL-6 levels estimated by immunoassay and body composition analysis and BMD at lumbar spine and femoral neck using dual energy X-ray absorptiometry. This is an observational study and the study population includes 112 men aged 49 ± 6 years. The mean percentage of the total body fat is34.5 with visceral fat of 35.8. Correlations between visceral adiposity ($r = 0.24$, $p < 0.001$) and BMD at whole-body were greater than the whole body fat content ($r = 0.18$, $p < 0.01$). Visceral fat content correlated negatively with osteoprotegerin levels ($r = 0.26$, $p < 0.001$); adiponectin levels negatively correlated with Total hip ($r = 0.32$, $p < 0.0001$) and the Total BMD($r = 0.29$, $p < 0.0001$). Similarly Osteoprotegerin levels and IL-6 levels ($r = 0.24$, $p < 0.001$) negatively correlated with Total hip ($r = 0.24$, $p < 0.001$; $r = 0.23$, $p < 0.001$) and the total BMD($r = 0.24$, $p < 0.001$; $r = 0.18$, $p < 0.001$)) respectively.

Conclusion: BMD at total body and hip is negatively associated with percentage of visceral fat, adiponectin, IL-6 and osteoprotegerin levels in younger men. These correlations suggest a plausible connection between bone and abdominal fat with increased inflammatory markers.

***Disclosures**: **S. Yaturu**, None*

T88

Magnetic Resonance Imaging of Bone Marrow Fat in Osteoarthritis

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The purpose of this study was to use Magnetic Resonance Imaging (MRI) to measure the fractional fat content in tissues affected by osteoarthritis (OA). We have hypothesized that Generalised OA is a systemic disorder affecting the whole musculoskeletal system and that increased adiposity may be a part of the disease process. In previous laboratory studies we found an increased bone marrow fat content and a change in the fatty acid composition in OA patients. In this study we have used MRI to measure the fractional lipid content within the proximal femur in patients with known advanced OA of the hip awaiting a total hip replacement (THR) and compared this with measurements from a group of age matched volunteers with no evidence of OA.

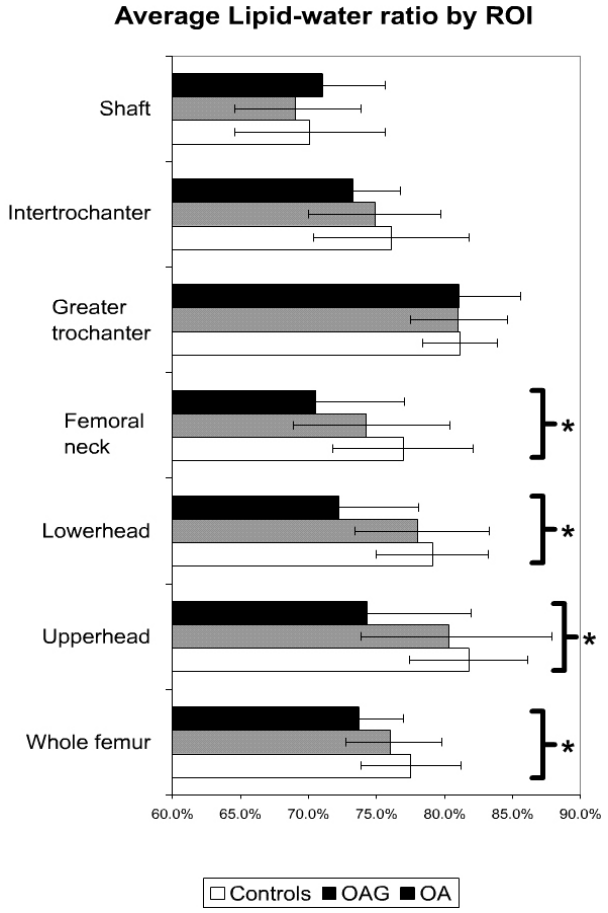
15 patients with end-stage hip OA were recruited prior to THR and imaged using MRI, in a Siemens Magnetom Impact 1T scanner, on the day they were admitted for surgery. An age-matched cohort was recruited from the general population using the criteria that they must be over 60 years old, have no reported arthritic disease or had joint replacement surgery. The Dixon 2-point technique was used to acquire “sum” and “difference” images (where fat and water signals are in and out of phase respectively) and the fractional lipid content was calculated at selected sites throughout the proximal femur.

There was a significantly lower fractional lipid content in the tissue from the THR group (OA1) 73.7 ± 3.3% compared with the controls 77.5 ± 3.7% ($P < 0.01$, Student’s t-test) (Figure 1). Comparing the sub-regions, this difference was consistent and significant in the most proximal regions of the femur. The contralateral hip in the THR group (OA2) showed intermediate values between the age matched group and the pre-THR hip indicating changes remote from the affected joint. We also recorded an increase in the fractional lipid content of muscle tissue surrounding the femur of the OA patients, although dietary and exercise-related factors complicate the interpretation of these data.

In this study we have shown a small but significant difference between the fractional lipid contents measured in the bone marrow of the proximal femur in OA patients and an age matched cohort. Surprisingly, these results showed that OA bone marrow had a lower fractional fat content than age-matched controls. Together with our

previous laboratory studies, these data suggest that both fat and water contents are increased in bone marrow in OA.

Figure 1: Mean and SD percentage lipid levels in each ROI for each group. * P<0.05.



***Disclosures**: **R. M. Aspden**, Wyeth 2*

T90

Increased expression of lipocalin-type prostaglandin D2 synthase in osteoarthritic cartilage

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Lipocalin-type prostaglandin D synthase (L-PGDS) is responsible for the biosynthesis of prostaglandin (PG) D and J series, which have been shown to exhibit anti-inflammatory and anti-catabolic effects. However, the expression and regulation of L-PGDS in articular cartilage are unknown. The aim of this study was to investigate the expression of L-PGDS in cartilage from normal donors and from patients with osteoarthritis (OA), and to characterize its regulation by interleukin-1β (IL-1) in cultured OA chondrocytes. Using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry, we found that the expression levels of L-PGDS mRNA and protein were increased in OA compared with normal cartilage. Treatment of chondrocytes with IL-1 up-regulated L-PGDS mRNA and protein expression as well as PGD2 production in a dose- and time-dependent manner. The up-regulation of L-PGDS by IL-1 was blocked by the translational inhibitor cycloheximide, indicating that this effect is indirect requiring de novo protein synthesis. Specific inhibitors of the mitogen-activated protein kinases (MAPK) p38 (SB 203580) and the c-jun N-terminal kinase (JNK) (SP600125), and of the NF-□B signalling pathway (SN-50) suppressed IL-1-induced up-regulation of L-PGDS expression. In contrast, an inhibitor of the extracellular signal-regulated kinase (ERK/MAPK) (PD98059) demonstrated no significant influence. We also found that PGD2, the end product of L-PGDS, prevented IL-1-induced up-regulation of L-PGDS expression. This is the first report demonstrating increased levels of L-PGDS in OA cartilage. IL-1 may be responsible for this up-regulation through activation of the JNK and p38 MAPK and NF-□B signalling pathways. These data suggest that L-PGDS might have an important role in the pathophysiology of OA.

***Disclosures**: **N. Zayed**, None*

T92

A Functional Role for Mast Cells in the Production of Brown Adipocytes from Peripheral Nerves in Early Heterotopic Ossification

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The purpose of this study is to delineate the earliest events that are critical to heterotopic ossification (HO), including progenitor cell recruitment, vascular extravasation, and tissue homing. We have developed a murine-based in vivo bone morphogenetic protein-2 (BMP-2) model that rapidly induces HO within 7 days. In this model, heterotopic bone is induced via the injection of Ad5BMP-2-transduced cells into the mouse quadriceps muscle. Importantly, the BMP-2-expressing cells are not incorporated into the site of new bone. In our model, HO ensues along a specific temporal pathway similar to endochondral ossification. Significantly, immunohistochemical analyses indicate that cells appear to be trafficking from the peripheral nerve sheath towards the site of cells expressing BMP-2, and moreover, there is a pronounced accumulation of tissue mast cells, both lining and infiltrating the nerve immediately following delivery of BMP-2. This observation has been corroborated by FACS analyses, which indicate an elevated number of c-Kit+ mononuclear cells isolated from the skeletal muscle tissue in the BMP-2 model versus control. The subsequent progression of HO includes the migration of uncoupling protein-1 (UCP-1)-positive brown adipocytes off of the nerve sheath as early as 48 hours after BMP-2 induction. These brown adipocytes stain positive for the vascular growth factor VEGFD and create a hypoxic microenvironment that contributes to the subsequent formation of new vessels and the trafficking of Sox9-positive pre-chondrocytes and osterix-expressing cells from local peripheral nerves at Day 5 post-injection of BMP-2-transduced cells. In contrast, mice that have had a portion of the sciatic nerve removed and the hind limb denervated revealed a complete attenuation of the HO process at the earliest stages. Therefore, the preliminary evidence from our model suggests that peripheral nerves and mast cells are intimately associated with one another at the earliest stages of the HO process. We postulate that ectopic expression of BMP-2 elicits an acute inflammatory response that drives the homing of mast cells to the site, and that molecular cross-talk between mast cells and local peripheral nerves orchestrates the process of HO. Current studies are ongoing to investigate the potential signaling mechanisms between mast cells and peripheral nerves that drive HO. These findings will guide future efforts aimed at blocking HO at the earliest stages.

***Disclosures**: **E. Rodenberg**, None*

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