ASBMR®
The American Society for Bone and Mineral Research

ASBMR TOPICAL MEETING:

Bone and Skeletal Muscle Interactions

July 17–18, 2012
Kansas City, Missouri, USA

Pre-Meeting Workshop July 16, 2012

Program and Abstracts

This meeting is supported by Grant R13AR063602 from the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Welcome!

CONTENTS

On behalf of the American Society for Bone and Mineral Research, welcome to the first ASBMR Topical Meeting on Bone and Skeletal Muscle Interactions!

Throughout life, from childhood to old age, bone and muscle are intimately connected. Disease or injury in either tissue affects the other. Although muscle loading of bone clearly plays a role in this interaction, little is known about other types of interaction such as cellular and molecular signaling mechanisms or shared genetic control. To address these important questions, it is crucial to bring together muscle and bone researchers to exchange ideas and develop new collaborations, thus accelerating the emerging scientific discoveries in the area of muscle and bone interactions.

We hope you will take advantage of the key networking opportunities available to you, such as the receptions on Monday and Tuesday evening, the Dine-Around with Speakers on Tuesday evening, the panel session on "The Power of Collaborations - A Conversation Starter" during lunch on Tuesday and the poster sessions on Tuesday and Wednesday.

As co-organizers, we extend a special thanks to the organizing committee and to our many colleagues for ideas, recommendations and guidance along the way. We also want to thank the academic institutions and companies that have helped to support this meeting. Finally, we wish to thank the ASBMR staff who provided continuous organizational support.

Sincerely,

Lynda F. Bonewald, Ph.D.
ASBMR President-Elect and Topical Meeting Chair

Roger A. Fielding, Ph.D.
ASBMR Topical Meeting Co-Chair

This meeting is supported by Grant R13AR063602 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Institute of Child Health & Human Development (NICHD), the National Institute of Dental and Craniofacial Research (NIDCR), the National Center for Complementary and Alternative Medicine (NCCAM) and the Office of Disease Prevention (ODP), National Institutes of Health (NIH)

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

American Society for Bone and Mineral Research
Topical Meeting on Bone and Skeletal Muscle Interactions

Organizing Committee

Lynda F. Bonewald, Ph.D., *Chair*
University of Missouri, Kansas City, Missouri, USA

Roger A. Fielding, Ph.D., *Co-Chair*
Tufts University, Boston, Massachusetts, USA

Thomas L. Clemens, Ph.D.
Johns Hopkins University, Baltimore, Maryland, USA

Karyn Esser, Ph.D.
University of Kentucky, Lexington, Kentucky, USA

Douglas P. Kiel, M.D., M.P.H.
Institute for Aging, Hebrew SeniorLife and Harvard Medical School, Boston, Massachusetts, USA

Regis J. O'Keefe, M.D.
University of Rochester Medical Center, Rochester, New York, USA

Eric S. Orwoll, M.D.
Oregon Health and Science University, Portland, Oregon, USA

Charlotte A. Peterson, Ph.D.
University of Kentucky, Lexington, Kentucky, USA

ASBMR Young Investigator Award Recipients

Mai S. Abdel-Ghani
Eduardo L. Abreu, M.D., D.Eng.
Anika Alarakhia, M.D.
Sandra Bermeo, M.Sc., Ph.D.
Rahul Das, M.S.
Melissa Carter
Vanessa Dubois
Joseph T. Garrity, M.S.
Katharina Jähn
Yunpeng Jiang, M.D.
Wonjin Kim, M.D.
Violet Lagari, D.O., M.P.H.
Brya G. Matthews, Ph.D.
Carla McCrave, M.D.
Rachel A. Menegaz
Amber Rath Stern, Ph.D.
Eric S. Rupe
Elena Torreggiani, Ph.D.
Chad D. Touchberry, Ph.D.
Yang Wang, (M.S.)
Zong-Heng Wang
Nick J. Willett, Ph.D.
Joseph P. Wilson, B.S., M.Ed.

Supporters

The American Society for Bone and Mineral Research (ASBMR) recognizes the generous educational grant support provided by the following companies:
Amgen, Inc.
Lilly USA, LLC
Merck & Co., Inc.
Warner Chilcott Company, LLC

ASBMR recognizes the generous support provided by the following universities:
Department of Oral Biology, UMKC School of Dentistry, UMKC Center of Excellence in Mineralized Tissues (CEMT)
Kansas City Area Life Sciences Institute
University of Kansas Medical Center
University of Missouri Columbia
University of Missouri Science & Technology

General Information

REGISTRATION

Registration will take place at the Westin Kansas City at Crown Center in the Century Foyer on the Ballroom Level during the times listed below.

Sunday, July 15, 2012	5:30 pm – 7:30 pm
Monday, July 16, 2012	7:00 am – 8:00 am
	3:00 pm – 7:30 pm
Tuesday, July 17, 2012	7:00 am – 3:00 pm
Wednesday, July 18, 2012	7:00 am – 1:00 pm

SPEAKER READY ROOM

All speakers must check into the Speaker Ready Room, preferably 24 hours before presentation. At that time, speakers are encouraged to review their slides to ensure that all Greek characters and graphs transferred successfully. The Speaker Ready Room is located in the Independence Room on the Ballroom Level of the Westin Kansas City at Crown Center.

Speaker Ready Room Hours

Monday, July 16, 2012	3:00 pm – 7:30 pm
Tuesday, July 17, 2012	7:00 am – 5:00 pm
Wednesday, July 18, 2012	7:00 am – 3:30 pm

POSTER INFORMATION

Posters will be displayed in the Pershing Room on the Ballroom Level of the Westin. Poster presentation is scheduled during the Poster Session on Tuesday, July 17 from 1:45 pm – 2:45 pm. Presenters must be at their posters and available to answer questions during this time.

	Tuesday, July 17, 2012	**Wednesday, July 18, 2012**
Poster Set-Up	7:00 am – 9:00 am	
Poster Dismantle		3:30 pm – 5:00 pm
Poster Viewing Opportunities		
Morning Break	10:15 am – 10:35 am	9:45 am – 10:05 am
Presentation Time	Poster Session 1:45 pm – 2:45 pm	
Lunch Break		11:50 am – 12:50 pm
Afternoon Break	4:30 pm – 4:50 pm	3:05 pm – 3:30 pm
Post-Meeting	6:20 pm – 7:20 pm	

MEETING MEALS

Your registration for the meeting includes a light reception on Monday, July 16, continental breakfast, lunch and a light reception on Tuesday, July 17 and continental breakfast and lunch on Wednesday, July 18. If you have any special dietary needs, please inform the staff at the ASBMR registration desk.

There is no extra fee to participate in Tuesday's Dine-Around with Speakers; however, you are responsible for your own meal expenses at the restaurant. Please let your wait-staff know if you require separate checks. Please inquire at the ASBMR registration desk regarding available seats/times. All participants must sign up in advance by 11:00 am on Tuesday, July 17. Limited space is available.

MEETING OBJECTIVE

Throughout life, from childhood to old age, bone and muscle are intimately connected. Although this coupling clearly exists in both health and disease, little is known about responsible mechanisms other than loading. Therefore, this two-day meeting will draw together leading muscle and bone researchers nationally and internationally along with invited experts to exchange ideas and identify critical research areas that would lead to collaborations to address musculoskeletal disease.

The objectives of this meeting are:

1. To begin to understand the close association between muscle and bone during development and growth and how nutrition and physical activity affect general health.
2. To dissect the association between sarcopenia and osteoporosis and determine what role aging plays in these processes.
3. To identify molecular and cellular mechanisms responsible for the close association between muscle and bone in both health and disease and with aging.
4. To define defective mechanotransduction in both muscle and bone and identify means to treat musculoskeletal disease.
5. To identify means to prevent, treat or reverse muscle and bone loss.
6. To determine if muscle communicates with bone independent of mechanical loading.
7. Based on the proceedings of the meeting, assess the feasibility of establishing a combined research field that integrates muscle and bone physiology in order to generate a better understanding of how these two tissues integrate and crosstalk in both health and disease.

TARGET AUDIENCE

This meeting will bring together national and international investigators currently working in the area of muscle and bone interactions as well as young investigators, NIH-funded investigators, industry scientists, intramural scientists and program staff at the various NIH institutes and clinicians interested in sarcopenia, osteoporosis and muscle and bone interactions.

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 through the joint sponsorship of the Institute for the Advancement of Human Behavior (IAHB) and The American Society for Bone and Mineral Research (ASBMR). The IAHB is accredited by the ACCME to provide continuing medical education for physicians.

The IAHB designates this live activity for a maximum of 22 *AMA PRA Category 1 Credit(s)*^{TM}. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Those attending both the Pre-Meeting Workshop and the Topical Meeting are eligible for a maximum of 22 *AMA PRA Category 1 Credit(s)*^{TM}. Those attending only the Topical Meeting are eligible for a maximum of 13.75 *AMA PRA Category 1 Credit(s)*^{TM}.

You must submit your application for CME credits online at: www.CmeCertificateOnline.com.

1. Scroll down to the ASBMR listing and click on the ASBMR Topical Meeting event.
2. On the site, you will be asked to enter a password, which is **12Topic**, and evaluate various aspects of the program.
3. You may then print your certificate immediately (encouraged), anywhere you have internet access.
4. Additionally, a copy of the certificate will automatically be emailed to you for your records. Your certificate will show the hours you entered.

IMPORTANT!

The online certificate site will be available at the end of the day on July 18, 2012 through August 31, 2012. After that date, the site will be removed and certificates will no longer be available. If you need a CME certificate, you must complete the evaluation and certificate process prior to that date; otherwise you will forfeit your credit for the course.

Questions regarding your continuing education credits should be directed to:

Attention: Jillian Davis
E-mail: Jdavis@smithbucklin.com
Tel: (651) 789-3722

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 Topical Meeting on Bone and Skeletal Muscle Interactions 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
5) Consulting fees or other remuneration
6) Non-remunerative positions of influence such as officer, board member, trustee, spokesperson
7) Receipt of royalties
8) Speakers bureau

COPYRIGHT

DISCLAIMER

All authored abstracts, findings, conclusions, recommendations or oral presentations are those of the author(s) and do not reflect the views of ASBMR or imply any endorsement. No responsibility is assumed, and responsibility is hereby disclaimed, by the ASBMR for any injury and/or damage to persons or property as a matter of product liability, negligence or otherwise, or from any use or operation of methods, products, instructions, or ideas presented in the abstracts or at the ASBMR Topical Meeting on Bone and Skeletal Muscle Interactions. Independent verification of diagnosis and drug dosages should be made. Discussions, views and recommendations regarding medical procedures, choice of drugs, and drug dosages are the responsibility of the authors and presenters.

AUDIO AND VIDEO RECORDING

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, audio recording devices and video recording equipment is strictly prohibited within all Oral Scientific Sessions and the Poster Sessions without the express written permission of the ASBMR. Unauthorized use of recording 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 Century Ballroom Foyer. Stop by and pick up information about the Society, the high-ranking *Journal of Bone and Mineral Research* (*JBMR*) and the upcoming ASBMR 2012 Annual Meeting in Minneapolis, Minnesota, USA, October 12 – 15, 2012.

MEETING EVALUATION

An online evaluation form for the ASBMR Topical Meeting on Bone and Skeletal Muscle Interactions and Pre-Meeting Workshop will be available on the ASBMR website at www.asbmr.org after the meeting. You will also receive an email reminder from ASBMR. Your participation in this evaluation is very 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 Topical Meeting on Bone and Skeletal Muscle Interactions may be released to the press before its official presentation date and time. Press releases must be embargoed until one hour after the presentation.

FUTURE ASBMR MEETING DATES

ASBMR 2012 Annual Meeting
October 12-15, 2012
Minneapolis Convention Center
Minneapolis, Minnesota, USA

ASBMR 2013 Annual Meeting
October 4-8, 2013
Baltimore, Maryland, USA

ASBMR 2014 Annual Meeting
September 12-16, 2014
Houston, Texas, USA

Pre-Meeting Workshop: Techniques in Bone and Muscle Biology

GENERAL INFORMATION

ASBMR is hosting a pre-meeting workshop in conjunction with the University of Missouri – Kansas City School of Dentistry on July 16th. This hands-on workshop, chaired by Mark L. Johnson, Ph.D., Charlotte L. Phillips, Ph.D. and Marco Brotto, M.S.N., Ph.D., will provide participants with an opportunity to learn and practice various techniques related to the analysis of bone and muscle.

Advance registration was required for attendance at this event.

LOCATION

The pre-meeting workshop will be take place at the University of Missouri – Kansas City School of Dentistry. Directions and additional location information will be provided in the Sunday evening Orientation Session/Networking Reception held in the Shawnee/Mission at the Westin Crown Center.

SUPPORTERS

The American Society for Bone and Mineral Research (ASBMR) would like to thank the following for their support of this pre-meeting workshop:

University of Missouri – Kansas City
School of Dentistry, School of Nursing, School of Computing and Engineering

University of Missouri at Columbia
School of Medicine, School of Health Professions, College of Arts and Sciences

WORKSHOP FACULTY

University of Missouri at Columbia Faculty

Dr. Marybeth Brown
Mr. J. Andries Ferreira
Dr. Charlotte L. Phillips
Dr. Dawn DW Cornelison

University of Missouri – Kansas City Faculty

Dr. Yong Wang
Dr. Xiaomei Yao
Dr. Changqi Xu
Ms. Rachel Reed
Dr. Marco Brotto
Dr. Leticia Brotto
Dr. Eduardo Abreu
Mr. ChengLin Mo
Mr. Julian Vallejo
Dr. Mark L. Johnson
Dr. Amber Stern
Dr. Matt Stern
Dr. Nuria Lara
Dr. Sarah Dallas
Mr. Mark Dallas
Ms. Anita Xie
Ms. Carrie Zhao
Dr. Erica Perryn
Dr. Pat Veno
Ms. Jennifer Rosser
Dr. Matt Prideaux
Dr. Katharina Jaehn

ASBMR Topical Meeting: Bone and Skeletal Muscle Interactions
Schedule-at-a-Glance
(* Denotes Workshop Attendees Only)

Sunday, July 15, 2012

Time	Session	Location
5:30 pm – 7:30 pm	Registration Open	Westin - Century Ballroom Foyer
6:00 pm – 7:00 pm	Orientation Session/Networking Reception*	Westin – Shawnee/Mission Room

Monday, July 16, 2012

Time	Session	Location
7:00 am – 8:00 am	Registration Open	Westin – Century Ballroom Foyer
3:00 pm – 7:30 pm	Registration Open	Westin – Century Ballroom Foyer
3:00 pm – 7:30 pm	Speaker Ready Room Open	Westin – Independence Room
7:00 am – 7:45 am	Breakfast	UMKC – SOD
8:00 am – 10:00 am	Concurrent Modules 1, 2, 3, 4*	UMKC – Various
10:00 am – 10:15 am	Break*	UMKC – SOD
10:15 am – 11:45 am	Concurrent Modules 5, 6, 7, 8*	UMKC – Various
11:45 am – 1:00 pm	Lunch and Group Discussion*	UMKC – SOD
1:00 pm – 3:00 pm	Concurrent Modules 1, 2, 3, 4*	UMKC – Various
3:00 pm – 3:15 pm	Break*	UMKC – SOD
3:15 pm – 4:45 pm	Concurrent Modules 5, 6, 7, 8*	UMKC – Various
4:45 pm – 5:00 pm	Closing*	UMKC – SOD
6:00 pm– 7:30 pm	Welcome and Networking Reception	Westin – Century Ballroom B

Tuesday, July 17, 2012

Time	Session	Location
7:00 am – 3:00 pm	Registration Open	Westin – Century Ballroom Foyer
7:00 am – 5:00 pm	Speaker Ready Room Open	Westin – Independence Room
7:00 am – 7:45 am	Breakfast	Westin – Century Ballroom Foyer
7:45 am – 8:00 am	Introduction	Westin – Century Ballroom C
8:00 am – 10:15 am	Session 1 – Bone and Muscle Interactions during Development	Westin – Century Ballroom C
10:15 am – 10:35 am	Break/Poster Viewing	Westin – Pershing Place
10:35 am – 12:50 pm	Session 2 – Aging: Changes in Muscle and Bone, Linkages and Shared Etiologies	Westin – Century Ballroom C
12:50 pm – 1:45 pm	Lunch and Panel Discussion – The Power of Collaborations – A Conversation Starter	Westin – Century Ballroom AB
1:45 pm – 2:45 pm	Poster Session	Westin – Pershing Place
2:45 pm – 4:30 pm	Session 3 – Common Mechanisms Influencing Bone and Muscle Mass-'Pleotropy'	Westin – Century Ballroom C
4:30 pm – 4:50 pm	Break/Poster Viewing	Westin – Pershing Place
4:50 pm – 6:20 pm	Young Investigator Presentations & Awards	Westin – Century Ballroom C
6:20 pm – 7:20 pm	Welcome & Networking Reception/ Poster Viewing	Westin – Pershing Place
7:30 pm/8:00 pm	Dine Around with Speakers	Offsite – Various

Wednesday, July 18, 2012

Time	Session	Location
7:00 am – 1:00 pm	Registration Open	Westin – Century Ballroom Foyer
7:00 am – 3:30 pm	Speaker Ready Room Open	Westin – Independence Room
7:00 am – 8:00 am	Breakfast	Westin – Century Ballroom Foyer
8:00 am – 9:45 am	Session 4 – Defective Mechanotransduction and Repair	Westin – Century Ballroom C
9:45 am – 10:05 am	Break/Poster Viewing	Westin – Pershing Place
10:05 am – 11:50 am	Session 5 – Preventing and Treating Muscle and Bone Loss	Westin – Century Ballroom C
11:50 am – 12:50 pm	Lunch	Westin – Century Ballroom B
11:50 am – 12:50 pm	Poster Viewing	Westin – Pershing Place
12:50 pm – 3:05 pm	Session 6 – Emerging Areas	Westin – Century Ballroom C
3:05 pm – 3:30 pm	Break/Poster Viewing	Westin – Pershing Place
3:30 pm – 4:30 pm	Session 7 – Meeting Wrap Up	Westin – Century Ballroom C
4:30 pm	Meeting Adjourns	

Pre-Meeting Workshop: Techniques in Bone and Muscle Biology Program

Sunday, July 15, 2012

ORIENTATION SESSION/NETWORKING RECEPTION
6:00 pm – 7:00 pm
Location: Westin - Shawnee/Mission Room

Monday, July 16, 2012

BREAKFAST
7:00 am – 7:45 am
UMKC - SOD 4th Floor Faculty Lounge

CONCURRENT MODULES
8:00 am – 10:00 am
Location: UMKC - School of Dentistry (SOD), School of Nursing (SON), LARC
(Faculty in Charge are Indicated, Subject to Change)

Module 1: **Bone and Muscle Histology**
Sarah L. Dallas, Ph.D. and Marybeth Brown, Ph.D.

Module 2: **Muscle Functional Testing**
Marco Brotto BSN, Ph.D., Marybeth Brown, Ph.D. and J. Andries Ferreira

Module 3: **Isolation of Primary Cells**
Amber Stern, Ph.D., Matthew Stern, Ph.D. and Dawn DW Cornelison, Ph.D.

Module 4: **Live Imaging of Bone Cell and ECM Dynamics**
Sarah L. Dallas, Ph.D.

BREAK
10:00 am – 10:15 am
Location: UMKC - SOD 4th Floor Faculty Lounge

CONCURRENT MODULES
10:15 am – 11:45 am
Location: UMKC - School of Dentistry (SOD), School of Nursing (SON), LARC
(Faculty in Charge are Indicated, Subject to Change)

Module 5: **In Vivo Bone Techniques**
Mark L. Johnson, Ph.D.

Module 6: **Ex Vivo Characterization of Bone Biomechanical and Structural Properties**
Amber Stern, Ph.D., Yong Wang, Ph.D. and Charlotte Phillips, Ph.D.

Module 7: **Bone – Muscle Immobilization**
J. Andries Ferreira and Marybeth Brown, Ph.D.

Module 8: **In Vitro Cell Loading**
Amber Stern, Ph.D. and Mark L. Johnson, Ph.D.

LUNCH AND GROUP DISCUSSION
11:45 am – 1:00 pm
Location: UMKC - SOD 4th Floor Faculty Lounge

CONCURRENT MODULES
1:00 pm – 3:00 pm
Location: UMKC - School of Dentistry (SOD), School of Nursing (SON), LARC
(Faculty in Charge are Indicated, Subject to Change)

Module 1: **Bone and Muscle Histology**
Sarah L. Dallas, Ph.D. and Marybeth Brown, Ph.D.

Module 2: **Muscle Functional Testing**
Marco Brotto BSN, Ph.D., Marybeth Brown, Ph.D., and J. Andries Ferreira

Module 3: **Isolation of Primary Cells**
Amber Stern, Ph.D., Matthew Stern, Ph.D. and Dawn DW Cornelison, Ph.D.

Module 4: **Live Imaging of Bone Cell and ECM Dynamics**
Sarah L. Dallas, Ph.D.

BREAK
3:00 pm – 3:15 pm
Location: UMKC - SOD 4th Floor Faculty Lounge

CONCURRENT MODULES
3:15 pm – 4:45 pm
Location: UMKC - School of Dentistry (SOD), School of Nursing (SON), LARC
(Faculty in Charge are Indicated, Subject to Change)

Module 5: **In Vivo Bone Techniques**
Mark L. Johnson, Ph.D.

Module 6: **Ex Vivo Characterization of Bone Biomechanical and Structural Properties**
Amber Stern, Ph.D., Yong Wang, Ph.D. and Charlotte Phillips, PhD.

Module 7: **Bone – Muscle Immobilization**
J. Andries Ferreria and Marybeth Brown, Ph.D.

Module 8: **In Vitro Cell Loading**
Amber Stern, Ph.D. and Mark L. Johnson, Ph.D.

CLOSING
4:45 pm – 5:00 pm
Location: UMKC SOD 4th Floor Faculty Lounge

ASBMR Topical Meeting: Bone and Skeletal Muscle Interactions Program

Monday, July 16, 2012

WELCOME AND NETWORKING RECEPTION
6:00 pm – 7:30 pm
Location: Westin - Century Ballroom B

Tuesday, July 17, 2012

BREAKFAST
7:00 am – 7:45 am
Location: Westin - Century Ballroom Foyer

INTRODUCTION
7:45 am – 8:00 am
Location: Westin - Century Ballroom C

7:45 am **Welcome and Opening Remarks**
Lynda F. Bonewald, Ph.D., University of Missouri, Kansas City, Missouri, USA

SESSION 1
8:00 am – 10:15 am
Location: Westin - Century Ballroom C

Bone and Muscle Interactions during Development
Chairs: Thomas L. Clemens, Ph.D., Johns Hopkins University, Baltimore, Maryland, USA
Dawn DW Cornelison, Ph.D., University of Missouri, Columbia, Missouri, USA

		Presentation Number
8:00 am	**Effects of Muscle Forces on Bone Shape during Embryogenesis** Elazar Zelzer, Ph.D., Weizmann Institute of Science, Rehovot, Israel	**1**
8:30 am	**Coordination of Bone and Skeletal Muscle Tissue Growth during Embryogensis** Bradley B. Olwin, Ph.D., University of Colorado, Boulder, Colorado, USA	**2**
9:00 am	**Normal and Pathological Bone and Muscle Development in Children and Adolescents** Mary B. Leonard, M.D., M.S.C.E., The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA	**3**
9:30 am	**Determinants of Peak Bone and Muscle Mass** Sundeep Khosla, M.D., Mayo Clinic, Rochester, Minnesota, USA	**4**
10:00 am	**Session 1 Panel Discussion** All Session 1 speakers and chairs	

BREAK/ POSTER VIEWING
10:15 am – 10:35 am
Pershing Place

SESSION 2
10:35 am – 12:50 pm
Location: Westin - Century Ballroom C

Aging: Changes in Muscle and Bone, Linkages and Shared Etiologies
Chairs: Eric S. Orwoll, M.D., Oregon Health and Science University, Portland, Oregon, USA
Roger A. Fielding, Ph.D., Tufts University, Boston, Massachusetts, USA

		Presentation Number
10:35 am	**Markers for Sarcopenia: Lessons from Osteoporosis Trials** Steven R. Cummings, M.D., San Francisco Coordinating Center, University of California, San Francisco, California, USA	**5**
11:05 am	**Mobility and Falls** Stephanie A. Studenski, M.D., M.P.H., University of Pittsburgh, Pittsburg, Pennsylvania, USA	**6**
11:35 am	**Etiology of Concomitant Bone and Muscle Loss** Tamara B. Harris, M.D., M.S., National Institute on Aging, NIH, Bethesda, Maryland, USA	**7**
12:05 pm	**Muscle and Bone Interactions: Is Fat the Common Denominator?** Clifford J. Rosen, M.D., Maine Medical Center Research Institute, Scarborough, Maine, USA	**8**
12:35 pm	**Session 2 Panel Discussion** All Session 2 speakers and chairs	

LUNCH AND PANEL DISCUSSION
12:50 – 1:45 pm
Location: Westin - Century Ballroom AB

The Power of Collaborations – A Conversation Starter
Chairs: Lynda F. Bonewald, Ph.D., University of Missouri, Kansas City, Missouri, USA
Roger A. Fielding, Ph.D., Tufts University, Boston, Massachusetts, USA

Panelists:
Steven R. Cummings, M.D., San Francisco Coordinating Center, San Francisco, California, USA
Glen Nuckols, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland, USA
Nathan K. LeBrasseur, Ph.D., Mayo Clinic, Rochester, Minnesota, USA
Mark A. Febbraio, Ph.D., Baker IDI Heart & Diabetes Institute, Melbourne, Australia

---

POSTER SESSION
1:45 – 2:45 pm
Location: Westin - Pershing Place

SESSION 3
2:45 pm – 4:30 pm
Location: Westin - Century Ballroom C

Common Mechanisms Influencing Bone and Muscle Mass-'Pleotropy'
Chairs: Karyn Esser Ph.D., University of Kentucky, Lexington, Kentucky, USA
Douglas P. Kiel, M.D., M.P.H., Institute for Aging Research, Hebrew SeniorLife and Harvard Medical School, Boston, Massachusetts, USA

		Presentation Number
2:45 pm	**Genetic Determinants** Douglas P. Kiel, M.D., M.P.H., Institute for Aging Research, Hebrew SeniorLife and Harvard Medical School, Boston, Massachusetts, USA	**9**
3:15 pm	**Signaling Pathways Mediating Skeletal Muscle Mass and Function** David Glass, M.D., Novartis Institutes for BioMedical Research Inc, Cambridge, Massachusetts, USA	**10**
3:45 pm	**The Growth Hormone IGF-1 Axis in Bone and Muscle** Thomas L. Clemens, Ph.D., Johns Hopkins University, Baltimore, Maryland, USA	**11**
4:15 pm	**Session 3 Panel Discuss** All Session 3 chairs and speakers	

BREAK/ POSTER VIEWING
4:30 pm – 4:50 pm
Location: Westin - Pershing Place

YOUNG INVESTIGATOR PRESENTATIONS
4:50 pm – 6:20 pm
Location: Westin - Century Ballroom C

Chair: Lynda F. Bonewald, Ph.D., University of Missouri, Kansas City, Missouri, USA

		Presentation Number
4:50 pm	**Introductions and Award Presentations** Lynda F. Bonewald, Ph.D., University of Missouri, Kansas City, Missouri, USA	
5:05 pm	**A Muscle Specific Factor Increases Survival of Dexamethasone-Stressed Osteocytes** Katharina Jähn, University of Missouri School of Medicine, Kansas City, Missouri, USA	**P82**
5:20 pm	**Effect of Orchidectomy on the Musculoskeletal System of Myostatin Knock Out Mice** Vanessa Dubois, University of Leuven, Leuven, Belgium	**P28**
5:35 pm	**Acute Exposure to Fibroblast Growth Factor 23 Increases Cardiac Contractility** Chad D. Touchberry, Ph.D., University of Missouri School of Medicine, Kansas City, Missouri, USA	**P62**

5:50 pm	**Concomitant Volumetric Muscle Loss Impairs an Early Vascular Response in a Composite Bone-Muscle Defect Model** Nick J. Willett, Ph.D., Georgia Institute of Technology, Atlanta, Georgia, USA	**P41**
6:05 pm	**The Identification and Characterization of New Proteins Essential for *Drosophila* Myotendinous Junction Formation** Zong-Heng Wang, University of Missouri School of Medicine, Kansas City, Missouri, USA	**P66**

WELCOME AND NETWORKING RECEPTION/POSTER VIEWING
6:20 pm – 7:20 pm
Location: Westin - Pershing Place

DINE AROUND WITH SPEAKERS
Staggered reservations are available from 7:30 pm through 8:00 pm
Please check your confirmation card for specific seating times and locations/directions.
See the ASBMR registration desk by 11:00 am for more details.

Wednesday, July 18, 2012

BREAKFAST
7:00 am – 8:00 am
Location: Westin - Century Ballroom Foyer

SESSION 4
8:00 am – 9:45 am
Location: Westin - Century Ballroom C

Defective Mechanotransduction and Repair
Chairs: Regis J. O' Keefe, M.D., University of Rochester Medical Center, Rochester, New York, USA
Joseph A. Houmard, Ph.D., East Carolina University, Greenville, North Carolina, USA

		Presentation Number
8:00 am	**Disuse-Induced Loss of Muscle/Bone Mass: Always Predictive of Loss of Function?** Susan A. Bloomfield, Ph.D., Texas A&M University, College Station, Texas, USA	**12**
8:30 am	**Exercise and Mechanical Signals: Surprises Regarding the Effect of Vibration on Bone and Muscle** Clinton T. Rubin, Ph.D., Stony Brook University, Stony Brook, New York, USA	**13**
9:00 am	**Orthopaedic Applications and Repair: Gaps in Our Knowledge of the Role of Muscle on Bone Repair** Regis J. O' Keefe, M.D., University of Rochester Medical Center, Rochester, New York, USA	**14**
9:30 am	**Session 4 Panel Discussion** All Session 4 speakers and chairs	

BREAK/POSTER VIEWING
9:45 am – 10:05 am
Location: Westin - Pershing Place

SESSION 5
10:05 am – 11:50 am
Location: Westin - Century Ballroom C

Preventing and Treating Muscle and Bone Loss
Chairs: Roger A. Fielding, Ph.D., Tufts University, Boston, Massachusetts, USA
Nathan K. LeBrasseur, Ph.D., Mayo Clinic, Rochester, Minnesota, USA

		Presentation Number
10:05 am	**Nutrition: Effect of Vitamin D in Muscle and Relationship to Bone** Bess Dawson-Hughes, M.D., Tufts University, Boston, Massachusetts, USA	**15**
10:35 am	**Activin Type II Receptor Biologics for Preventing and Treating Muscle and Bone Loss** Teresa A. Zimmers, Ph.D., Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania, USA	**16**
11:05 am	**A Regenerative Medicine Approach to Musculotendinous Tissue Reconstruction** Stephen F. Badylak, D.V.M., Ph.D., M.D., University of Pittsburgh, Pittsburgh, Pennsylvania, USA	**17**
11:35 am	**Session 5 Panel Discussion** All Session 5 speakers and chairs	

LUNCH AND POSTER VIEWING
11:50 am – 12:50 pm
Location: Westin - Lunch in Century Ballroom B. Posters in Pershing Place

SESSION 6
12:50 pm – 3:05 pm
Century Ballroom C

Emerging Areas
Chairs: Robert Marcus, M.D. Stanford University, Stanford, California, USA
Vincent J. Caiozzo, Ph.D., University of California, Irvine, California, USA

		Presentation Number
12:50 pm	**Osteocalcin and the Regulation of Muscle Mass** Gerard Karsenty, M.D., Ph.D., Columbia University Medical Center, New York, New York, USA	**18**
1:20 pm	**Muscle as an Endocrine Organ** Mark A. Febbraio, Ph.D., Cellular & Molecular Metabolism Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Australia	**19**

1:50 pm **The Wnts in Muscle-Bone Cross Talk** **20**
Mark L. Johnson, Ph.D., University of Missouri, Kansas City, Missouri, USA

2:20 pm **Regulatory Interactions between Myeloid Cells and Skeletal Muscle** **21**
James G. Tidball, Ph.D., University of California, Los Angeles, California, USA

2:50 pm **Session 6 Panel Discussion**
All Session 6 speakers and chairs

BREAK/POSTER VIEWING
3:05 pm – 3:30 pm
Location: Westin - Pershing Place

SESSION 7
3:30 pm – 4:30 pm
Location: Westin - Century Ballroom C

Meeting Wrap Up
Chair: Lynda F. Bonewald, Ph.D., University of Missouri, Kansas City, Missouri, USA

3:30 pm **Panel Discussion - Where Do We Go From Here?**

- Lyndon Joseph, Ph.D., National Institute on Aging, NIH, Bethesda, Maryland, USA
- Joan A. McGowan, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland, USA
- Glen Nuckols, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland, USA
- John Williams, Ph.D., National Institute on Aging, NIH, Bethesda, Maryland, USA
- Karen Winer, M.D., National Institute of Child Health and Human Development, NIH, Bethesda, Maryland, USA
- All Session Chairs

Meeting Adjourns
4:30 pm

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

Session 1: Bone and Muscle Interactions during Development

1

Effects of Muscle Forces on Bone Shape during Embryogenesis

Elazar Zelzer, Weizmann Institute of Science, Israel

The involvement of embryonic movement and muscle contraction in skeletogenesis has long been recognized. Nevertheless, several important questions about this effect are yet to be elucidated. In our studies, we seek to determine the scope of this involvement and to identify mechanical and molecular signals that mediate it. The third question regards the integration of these two types of signal into one developmental program.

Synovial joints develop from a pool of progenitor cells that differentiate into various cell types. Using several murine models that lack either limb musculature or its contractility, we show that muscle contraction is required to maintain joint progenitors committed to their fate. In its absence, the differentiation sequence was disrupted, resulting in impaired cavitation and morphogenesis. We then show that contraction-dependent activation of β-catenin is the mediating molecular mechanism. Our findings link between cell fate determination and embryonic movement during organogenesis.

In another work, we study the role of intrauterine muscle-induced mechanical loads in bone morphogenesis. Analysis revealed that developing mouse bones are subjected to significant and increasing mechanical challenges. Using daily micro-CT scans of appendicular long bones, we identified a developmental program we name preferential bone growth, which determines the specific circumferential shape of each bone by employing asymmetric mineral deposition and transient cortical thickening. Computer models demonstrate that the resulting bone structure has optimal load-bearing capacity. Finally, we used muscular dysgenesis mice to show that in the absence of muscle contractions, the typical circumference of each bone is lost, leading to development of mechanically inferior bones. This study identifies muscle force regulation of bone preferential growth as a common module that shapes the distinctive outline of long bones and optimizes their load baring capacity.

A third study explores the involvement of muscle contraction in zebrafish skeletal morphogenesis, demonstrating its role in regulation of chondrocyte intercalation. Analysis of chemically and genetically paralyzed embryos revealed shortening of pharyngeal cartilage elements, accompanied by marked changes in cell morphology and organization. Chondrocytes in paralyzed zebrafish were smaller and more rounded and exhibited abnormal stacking patterns, indicating aberrant intercalation. Impaired chondrocyte intercalation in growth plates of "muscle-less" mouse embryos implied evolutionary conservation of this effect. These findings uncover a new role for muscle-induced mechanical loads in skeletal morphogenesis by regulating chondrocyte intercalation in two different vertebrate models.

Disclosures: Elazar Zelzer, *None.*

2

Coordination of Bone and Skeletal Muscle Tissue Growth during Embryogenesis

Bradley B. Olwin*, Yvette Bren-Mattison, Melissa Hausburg, University of Colorado, USA

During embryogenesis, muscle and bone develop in close temporal and spatial proximity. Indian Hedgehog, a bone-derived signaling molecule, participates in growth of skeletal muscle. In Ihh (Indian Hegdehog) null embryos, skeletal muscle development appears abnormal at embryonic day 14.5 and at later ages through embryonic day 20.5, dramatic losses of limb muscle occur. To further examine the role of Ihh in myogenesis, we manipulated Ihh expression in the developing chick hind limb. Reduction of Ihh in chicken embryo hind limbs reduced skeletal muscle mass similar to that seen in *Ihh*$^{-/-}$ mouse embryos. The reduction in muscle mass appears to be a direct effect of Ihh since ectopic expression of Ihh restores muscle mass. These effects are independent of bone length, and occur when Shh(Sonic Hedgehog) is not expressed, suggesting Ihh acts directly on fetal myoblasts to regulate secondary myogenesis. Loss of muscle mass in Ihh null mouse embryos is accompanied by a dramatic increase in myoblast apoptosis accompanied by a loss of p21 protein and changes in TGF beta signaling. Our data suggest that Ihh promotes fetal myoblast survival during their differentiation into secondary myofibers by maintaining p21 protein levels, which promotes cell cycle exit and prevents apoptosis.

Disclosures: Bradley B. Olwin, *None.*

3

Normal and Pathological Bone and Muscle Development in Children and Adolescents

Mary B. Leonard, Children's Hospital of Philadelphia, USA

Skeletal development is characterized by sex-, race- and maturation- specific increases in trabecular and cortical bone mineral density and cortical dimensions. Modeling on the periosteal and endosteal surfaces produce changes in cortical geometry that impact life-long fracture risk. As muscle size and strength increase during growth, bones adapt by increasing cortical dimensions and strength. The capacity of bone to respond to mechanical loading is greatest during childhood. We have conducted a series of studies using tibia peripheral quantitative CT to examine associations between muscle and cortical dimensions. We reported that adjustment for sex and race differences in muscle size attenuated, but did not eliminate sex and race differences in cortical dimensions in children and young adults. The associations between muscle and bone outcomes did not differ according to sex or race, suggesting similar mechanostat set-points.

Given the strong associations between muscle mass and bone strength, investigators have advocated for the assessment of bone relative to muscle in children with chronic diseases. We demonstrated that chronic glucocorticoid therapy was associated with increased fat mass, increased muscle mass and greater cortical dimensions in children with nephrotic syndrome. In contrast, chronic inflammatory diseases (Crohn disease, juvenile inflammatory arthritis

and bone marrow transplantation) were associated with significant deficits in muscle mass and cortical dimensions. In these cross-sectional studies, adjustment for muscle mass significantly attenuated the diseases effects on cortical dimensions, compared with healthy controls. More recent longitudinal studies suggested that the relations between changes in muscle mass and changes in cortical dimensions varied across diseases. For example, in pediatric renal transplant recipients, muscle deficits resolved completely within months after transplantation; however, cortical bone deficits persisted. Similar evidence of an impaired muscle-bone until was observed in children and adolescents with Crohn disease. In contrast, children with acute lymphoblastic leukemia demonstrated significant increases in muscle mass following completion of chemotherapy and these increases in muscle mass were associated with the expected gains in cortical dimensions, compared with longitudinal data in healthy controls.

Differences in the skeletal response to loading may be due to disease effects on muscle force relative to muscle mass (muscle-specific effects), decreased physical activity, adverse cytokine effects on the mechanosensing osteocytes, abnormalities in insulin-like growth factor 1 (IGF-1), or glucocorticoid effects to inhibit bone formation. Future studies are needed to determine if physical activity or biomechanical interventions will increase muscle mass and bone density and dimensions in children with chronic diseases, and to identify diseases with the greatest potential to respond.

Disclosures: Mary B. Leonard, *None.*

4

Determinants of Peak Bone and Muscle Mass

Sundeep Khosla, Mayo Clinic, USA

Although a number of studies have demonstrated associations between total lean body mass (LBM) and bone mass, significant questions remain regarding the nature of this relationship. An important issue is whether this association is simply due to covariance with body size – for example, during growth there is an even greater correlation between bone mass and body surface area than between bone mass and LBM. Thus, in a recent study (n = 375 women and 325 men, 21-97 yrs), we examined the relationship of bone mass and geometry (by QCT) and microstructure (by HRpQCT) to relative appendicular skeletal mass (ASM) obtained by dividing lean mass of the arms and legs by height, thereby circumventing the confounder that bigger individuals will simply have more muscle and bone mass. Our data demonstrated that relative ASM was significantly associated with cortical and trabecular bone geometry and microstructure, respectively, at multiple skeletal sites. The associations of relative ASM with trabecular microstructure raise the possibility that the muscle-bone relationship may be driven, in part, by more than mechanotransduction, specifically by secreted osteo/myokines.

The traditional theoretical construct for the muscle-bone relationship, however, was formulated by Frost and invokes a skeletal "mechanostat" – most likely, the osteocyte – as sensing mechanical strains from muscle loading and transducing the strain signals into alterations in bone mass. Consistent with (but not proving) the mechanostat hypothesis, longitudinal studies in children have shown that the peak velocity for acquisition of LBM precedes the peak velocity for acquisition of bone mass, suggesting that muscle development may drive acquisition of bone mass. In addition, there is correlative evidence that the rise in circulating IGF-I levels during growth may promote periosteal apposition indirectly, primarily through stimulating muscle growth. Twin pair studies using mono- and dizygotic twins indicate that the proportion of the covariance between LBM and bone mass attributable to genetic factors is between 55% and 85%.

Frost also postulated that estrogen (E) modulated the sensitivity of the mechanostat. Consistent with this, ulnar bones from ERα or β knockout mice have smaller anabolic responses to mechanical loading as compared to bones from wild-type mice. Observational studies in humans also suggest that E may modulate the skeletal response to loading.

Disclosures: Sundeep Khosla, *None.*

Session 2: Aging: Changes in Muscle and Bone, Linkages and Shared Etiologies

5

Markers for Sarcopenia: Lessons from Osteoporosis Trials

Steven R. Cummings, San Franciso Coordinating Center, University of California - San Francisco, USA

Research in "Sarcopenia" is at a stage resembling clinical research and practice in "Osteoporosis" about 20 years ago. This new field can learn several lessons from the evolution of research for osteoporosis.

As bone densitometry moved into clinical research and practice, cut-points were needed to define normal vs. disease. The association between decreasing BMD and increasing risk of fracture is continuous with no natural break points. Therefore, the T-score cut-points for 'osteoporosis' and 'osteopenia' had to be somewhat arbitrarily defined by a committee, the WHO Working Group. The definitions had unintended applications (entry criteria for trials and indications for treatments) and consequences (more than half of postmenopausal women got a disease, "osteopenia" often with unnecessary drug treatment; drug development and FDA approvals too narrowly focused on BMD – often a poor surrogate for treatment efficacy). Development of FRAX has begun to make treatment more rationally based on absolute risk of fracture. Development of markers of bone turnover and more sophisticated measurements of bone may provide better "surrogate" markers for drug development.

Like BMD, measurements of muscle (such as muscle mass and gait speed) also have a continuous association with clinically important endpoints such as mobility disability. There are no natural break points. Therefore, the definitions of 'normal' and 'sarcopenia' will necessarily be somewhat arbitrary decisions of a committee. However, these cut-points should not automatically be used to define "diseases" that cause unnecessary anxiety. They should not necessarily become entry criteria for trials, or indications for treatment. Rather, an index of absolute risk of important endpoints may better define entry criteria for trials and indications for drug treatment. Therapeutic research in muscle needs validated "surrogate markers" for treatment efficacy for drug development and patient care. Markers are under development and coordinated efforts across sponsors' trials will be necessary to validate their use as "surrogate markers." Lessons from the history of "Osteoporosis" could thereby improve the development and use of treatments for "Sarcopenia."

Disclosures: Steven R. Cummings, *None.*

6

Mobility and Falls

Stephanie A. Studenski, University of Pittsburgh, USA

Altered mobility and falls are common in later life and interact with osteoporosis to contribute to fracture and injury. The aim of this presentation is to introduce the audience to key measures of mobility and falls in older adults and to consider the potential roles and interactions of muscle and bone with mobility, balance and falls.

Disclosures: Stephanie A. Studenski, *None.*

7

Etiology of Concomitant Bone and Muscle Loss

Tamara B. Harris, National Institute on Aging, National Institutes of Health, USA

This talk will focus primarily on epidemiologic data showing relationships between bone and muscle in cross-sectional large population studies and longitudinal studies. Until recently, it was thought that this relationship was primarily mechanical, but over recent years, studies have shown that hormones and other factors circulating between tissues and the brain may regulate bone and muscle. In addition, both muscle and bone appear to become infiltrated with adipose at the same time that the structural integrity of the bone and muscle is lost. Evidence will be discussed that suggests the extent to which these changes are dependent or independent and whether interventions for muscle loss, in particular, are likely to be successful in altering the trajectory of loss in bone. An example is found in data on women aged 75 and older from one large population study, the Age, Gene/Environment Susceptibility-Reykjavik Study, a population-based study with quantitative computerized tomography of the femur and computerized tomography scans of the mid-thigh. While 42 percent of the women's values for bone and muscle fell into matching tertile values (i.e. low-low, mid-mid, etc), 16 percent were skewed into a low/high category suggesting that there is substantial mismatching between bone and muscle. Whether similar findings are present in men and what factors might account for these differences will be presented at the meeting.

Disclosures: Tamara B. Harris, *None.*

8

Muscle-Bone Interactions: Is Fat the Common Denominator?

Clifford J. Rosen, Maine Medical Center Research Institute, USA

Bone, muscle and fat arise from the same multipotent mesenchymal progenitor cell (MSC). During mammalian aging adipocytes infiltrate skeletal and muscle tissue. Although adipocytes were considered inert fillers one type of fat cell, the brown or "brown like" adipocyte, may have a distinct influence on bone and muscle. There are structural and functional differences between white (WAT) and brown adipose tissue (BAT); the former contains adipocytes that are storage vesicles for fatty acids, whereas the latter is characterized by adipocytes that are small and thermogenic and have a rich vascular supply. Brown adipocytes arise from a mesenchymal progenitor cell that expresses myf5, a transcription factor necessary for muscle differentiation. Functionally brown adipocytes resemble skeletal muscle and are the major mechanism for non-shivering thermogenesis. "Brown-like" or "bright" pre-adipocytes arise from WAT cells and lack myf5 expression but express genes such as UCP-1 Cidea and DiO2, markers characteristic of brown fat. These "bright" cells can be induced by sympathetic innervation or overexpression of FoxC2, and PDRM16. In mice that have defective pre-formed BAT, bone mass is very low, sympathetic tone is heightened as a compensatory response, and there are nests of "bright" cells throughout fat depots. Bright cells are also found in heterotopic ossification when BMP2 overexpressing cells are transplanted onto muscle cells, and during the early phases of fracture healing. In genetically engineered mice over-expressing FoxC2 in fat tissue only, BMD is markedly increased as is lean body mass. Conditioned media from these mice have a pro-differentiation effect on pre-osteoblasts in co-culture. Similarly, myostatin-/- mice with increased muscle mass also have more "brown-like" adipocytes and markedly higher bone mass compared to WT. These lines of evidence, plus human data showing a positive correlation between volume of pre-formed BAT and BMD suggest that metabolic homeostasis dictates important hormonal interactions among bone muscle and fat.

Disclosures: Clifford J. Rosen, *None.*

Session 3: Common Mechanisms Influencing Bone and Muscle Mass: "Pleotropy"

9

Genetic Determinants

Douglas P. Kiel, Hebrew SeniorLife and Harvard Medical School, USA

Bone and muscle tissue are derived from a common mesenchymal precursor and accumulate peak tissue mass in close association, according to genetic information and environmental stimuli. Aging results in the progressive and parallel loss of bone (osteopenia) and skeletal muscle (sarcopenia) with profound consequences to quality of life. A better understanding of the mechanisms underlying the synchronized development and involution is critical to developing new and more effective means to combat osteoporosis and sarcopenia in our increasingly aged population. Given the close association between muscle and bone, there are likely to be genes that influence both phenotypes, which is known as "pleiotropy." There is evidence of such pleiotropy based on the observation of genetic correlation between muscle mass and various bone phenotypes such as bone mineral density and bone geometry. Furthermore, linkage studies have identified quantitative trait loci that suggest shared genetic determinants of bone and muscle phenotypes. There are at least two candidate genes, *GDF8* and *PPARGC1A*, that have been shown in animal models and in humans to have associations with muscle mass and bone mineral density. In addition to linkage studies and candidate gene studies, genome wide association studies (GWAS) offer an agnostic approach to gene discovery and can be leveraged to explore the possibility of genetic pleiotropy. Recently large meta-analyses of GWAS have been done for bone mineral density, hip geometry, and lean body mass. The summary statistics from these meta-analyses can be combined statistically to identify potentially pleiotropic genes for bone and

muscle. Results of several such analyses will be reviewed. Following these analyses, bioinformatic approaches can be used to confirm the biologic significance of the findings and to discover enrichment of gene networks underlying the findings. To ultimately confirm pleiotropy, basic laboratory and animal models can then be tested. These methods hold promise for the identification of pleiotropic genes associated with bone and muscle, which may suggest new biologic pathways that can lead to a better understanding of the shared biology of bone and muscle and the development of therapeutic interventions to enhance both tissues.

Disclosures: Douglas P. Kiel, *Eli Lilly 2, 6; Merck 2, 6; Amgen 2, 6; Novartis 6.*

10

Signaling Pathways Mediating Skeletal Muscle Mass and Function

David Glass, Novartis Institutes for Biomedical Research Inc., USA

Skeletal muscle atrophy is a significant co-morbidity seen in a variety of diseases, including Congestive Heart Failure, renal failure, cancer, and AIDS. Even during simple muscle inactivity, such as when a cast is put on a limb, the affected muscle can decrease by as much as 50% in mass, with a coincident decrease in strength. The loss of muscle during aging is referred to as "sarcopenia" and may occur due to distinct mechanisms. Two E3 ubiquitin ligases – MuRF1 (Muscle RING Finger 1) and MAFbx (Muscle Atrophy Fbox protein, which is also known as Atrogin), are transcriptionally upregulated in all physiological settings of muscle atrophy that have been analyzed. The pathways which regulate these ligases will be discussed. Also, more recently, new pathways perturbing muscle mass have been found - one pathway downstream of a GPCR mediating effects from a phospholipid derivative called Lysophosphatidic acid (LPA), involves Protein Kinase C signaling. Furthermore, muscle specific nodes into defined pathways such as the mTOR pathway continue to be found - here the role of MNK2 in perturbing protein translation in settings of atrophy will also be discussed, in addition to a newly identified E3 ligase that mediates IGF1 signaling in muscle, by degrading IGF1R phosphorylated IRS1.

Disclosures: David Glass, *None.*

11

The Growth Hormone-IGF-1 Axis in Bone and Muscle

Thomas L. Clemens *[1], Douglas J. DiGirolamo [1], Karyn Esser [2], [1]Johns Hopkins University, USA, [2]University of Kentucky, USA

Body size in mammals varies exponentially, even within members of the same species. Both linear and circumferential growth is controlled by the actions of growth factors, among which growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are central players. Humans with genetic alterations in the GH/IGF-1 axis exhibit profound disturbances of both bone and muscle growth. Precisely how GH and IGF-1 might act independently to influence growth has remained unclear, due to the that fact that GH stimulates IGF-1 production. To circumvent this problem, we devised a genetic approach to selectively inactivate individual components of the GH/IGF-1 axis in bone and skeletal muscle. Surprisingly, mice completely lacking GHR in their cartilaginous skeleton developed normally with evidence of compensation due to upregulation of the IGF-1R in GHR null chondrocytes. By contrast, loss of IGF-1R in chondrocytes resulted on postnatal death with profound delays in mineralization of endochondral bone. When GHR or IGF-1R was selectively eliminated in osteoblasts, mice exhibited reduced osteoblast numbers and defects in mineralization in both mutants. Interestingly, exogenous infusion of GH consistently increased osteoblasts numbers in wild type mice but not in mice lacking IGF-1R. These findings indicate that the skeletal actions of GH require the presence of IGF-1R. Finally, we generated mice lacking either GHR or IGF-1R specifically in skeletal muscle. Both models exhibited impaired skeletal muscle development characterized by reductions in myonuclei number and myofiber cross-sectional area that were accompanied by deficiencies in functional performance. Despite the striking similarity of muscle phenotypes, mice lacking GHR developed metabolic features that were not observed in the IGF-1R mutants; including marked peripheral adiposity, insulin resistance, and glucose intolerance. Insulin resistance in GHR-deficient myotubes derived from reduced insulin receptor protein abundance and increased inhibitory phosphorylation of IRS-1 on Ser 1101. These results identify distinct signaling pathways through which GHR regulates bone and skeletal muscle development and modulates nutrient metabolism.

Disclosures: Thomas L. Clemens, *None.*

Session 4: Defective Mechanotransduction and Repair

12

Disuse-Induced Loss of Muscle/Bone Mass: Always Predictive of Loss of Function?

Susan A. Bloomfield, Texas A&M University - College Station, USA

Disuse is a potent down-regulator of bone and muscle mass; both tissues respond in most species with an increase in catabolic activity and a decrease in anabolic functions. This is an appropriate biological response to large changes in the local strain environment (in bone) and to reduced contractile activity (in muscle). The time course of disuse changes is dramatically different between the two tissues as are the consequences for tissue function. Changes in protein synthesis in skeletal muscle can be measured within hours of immobilization or unloading, Muscle mass will decline quite rapidly thereafter, although magnitude and rate of mass loss varies among fiber types. The ability to generate tension (strength) and to do this rapidly (power) declines as well, but not always in parallel with muscle mass. Deficits in bone formation (and/or increased resorption) become evident early, and ultimately lead to alterations in bone mass, geometry, and/or microarchitecture with prolonged exposure. Recovery of strength of these two intimately related tissues after a period of disuse also follows different time courses, producing a prolonged mismatch between bone and muscle strength both early in the recovery period and again after full recovery of muscle integrity. Both tissues show evidence of continued decline in strength or mass early during a period of resumed weight bearing (measured in days for muscle but weeks in bone) before anabolic functions regain prominence and a net gain in mass and/or strength is realized. Exercise involving vigorous muscle contraction can alter these dynamics of loss/regain during a period of disuse as well as during recovery. It proves difficult, however, to define an optimal

protocol that benefits both tissues equally; specific modalities proven adequate to preserve either muscle or bone are not always successful in maintaining the integrity of the musculoskeletal unit as a whole. Intriguing early findings with innovative cell culture models may or may not be predictive of *in vivo* function.

Disclosures: Susan A. Bloomfield, *None.*

13

Exercise and Mechanical Signals: Surprises Regarding the Effect of Vibration on Bone and Muscle

Clinton T. Rubin*[1], Ete Chan [1], Ben Adler [1], Danielle Green[1], Gabriel Pagnotti[1], Stefan Judex [1], Janet Rubin [2], [1]Stony Brook University, USA, [2] University of North Carolina, USA

Exercise is perhaps the single "intervention" recognized as a deterrent to systemic diseases such as osteoporosis, sarcopenia, diabetes and obesity, yet the manner in which mechanical signals inhibit their pathogenesis remains unknown. In an effort to understand what aspects of exercise are critical to the achievement and retention of musculoskeletal wellness, our work has evolved to a focus on how brief daily periods of high frequency (30-90 Hz), low intensity (<0.4g) vibration (LIV) is anabolic to both bone[(1)] and muscle,[(2)] an adaptive response achieved – in part – by the biasing of mesenchymal stem cell fate selection towards forming these higher order connective tissues.[(3)]

When subjected to LIV for 20 minutes a day (0.3g magnitude, 30Hz) over the course of a year, skeletally mature female sheep demonstrated an increase in trabecular bone density and volume of 30% compared to controls.[(4)] Moving from sheep to mouse, and from bone to muscle, the LIV signal increased muscle area within a six week period (Fig. 1).[(5)] Although these mechanical signals are orders of magnitude below those which are generated during strenuous activity,[(6)] we believe these signals represent a physiologic surrogate for the spectrum of low-level mechanical signals provided by muscle activity,[(7)] which decays with aging.[(8)]

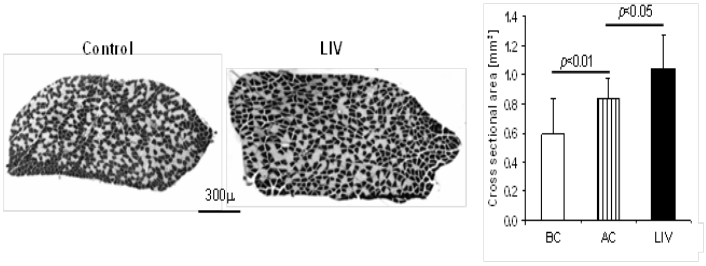


Figure 2. *Effect of LIV on the soleus muscle. Left: photo-micrographs of ATPase stained cross section of the soleus muscle from the LIV vs. an age-matched control (AC). The white color represents type I muscle fiber, and dark represents type II muscle fiber. Right: Mean soleus cross sectional area of Baseline Control (BC), Age matched control (AC) and LIV mice.*

While the sensitivity of bone and muscle to mechanical signals is readily recognized, in an unexpected finding, these extremely low magnitude, high frequency signals also suppress adipogenesis in the growing animal,[(9)] with reductions in total volume of both subcutaneous and visceral fat in mice subject to 12w of LIV. The starkly distinct response of these tissues (↑bone & muscle; ↓fat) to LIV suggests that these signals influence the differentiation pathway of mesenchymal stem cells (MSCs). Over the course of 15 weeks, 15 minutes per day of a 0.2g, 90Hz mechanical stimulus, a signal barely perceptible to human touch, resulted in 27% less total fat (neck to distal tibia) in the "buzzed" mice as compared to sham handled controls. Key risk factors in the onset of type II diabetes were also reduced in these animals; free fatty acid and triglyceride content in the liver were 43% and 39% lower than the controls, respectively.

Translated to the human, this would help explain why a sedentary lifestyle is permissive to both osteoporosis and obesity, seemingly distinct diseases, and could suggest that LIV reduces adipogenesis and strengthen the musculoskeletal system as much by defining the fate of MSCs as influencing the resident cell population within bone, muscle, or fat. The biasing of MSC differentiation by mechanical signals represents a unique means by which adiposity can be inhibited while simultaneously promoting a better musculoskeletal system, and may provide the basis for a safe, non-invasive, non-pharmacologic strategy to prevent systemic disorders such as obesity, sarcopenia osteoporosis, yet uniquely – without targeting the resident fat or bone cell. *This work kindly supported by grants AR 43498, AR 56655 & AR 42360.*

Disclosures: Clinton T. Rubin, *Marodyne Medical 6.*

14

Orthopaedic Applications and Repair: Gaps in Our Knowledge of the Role of Muscle on Bone Repair

Regis J. O'Keefe, University of Rochester Medical Center, USA

Bone regeneration is critical for the maintenance of mobility since injury to skeleton affects the majority of people at some point during their lives. Additionally, many current treatments for degenerative diseases of the musculoskeletal system, including spine fusions and total joint arthroplasty, require regeneration of bone tissue. Although scar is a common aspect of the healing of many tissues, the unique mechanical properties of bone make it essential that the process is faithfully completed for the reconstitution of skeletal integrity.

While most bone fractures heal, approximately 10-15% have delayed union or non-union. Impaired healing is associated with aging, certain diseases (diabetes, obesity), environmental factors (smoking), and yet to be defined genetic factors. Local factors are also critically important. Fracture healing is more often compromised at skeletal sites with a reduced soft tissue envelope. Increasing severity of injury to the surrounding muscle and soft tissues is highly associated with the development of non-union. Given the clear importance of the soft tissue envelope, it is essential that an improved understanding of the role of muscle tissues in skeletal regeneration be gained.

The primary event in the process of bone repair involves stem cell recruitment, proliferation, expansion, and accumulation at the fracture site. Given, the importance of these initial events, a more complete understanding of the sources of stem cells, their relative contributions, and the signals regulating their expansion and differentiation is essential. Potential sources of stem cell progenitors include bone marrow stem cells (BMSCs), periosteum-derived stem cells (PDSCs), systemic circulation-derived stem cells (CDSCs), Vascular endothelium-derived pericytes (VEDPs), and muscle-derived stem cells (MDSCs). While evidence suggests that stem cells from all of these sources contribute to repair, periosteal tissues appear to be the primary source of cells. Injury to the periosteum results in non-union in pre-clinical models of fracture healing. Furthermore, cell lineage tracing studies demonstrate an essential role for PDSCs, or bone lining cells in the initial accumulation of the precursor cell

pool necessary to initiate the healing response. BMSCs appear to have a lesser role in the development of the external callus tissue, but are likely involved in the endosteal repair process. SDSCs are recruited to sites of injury but represent a relatively small population of cells. SDSCs likely influence bone repair by secretion of paracrine factors, rather than by direct contribution of cells to the tissue healing response. VEDP are likely highly involved in the more organized bone formation that secondarily occurs during the remodeling process. Less is known about the direct role of MDSCs in bone repair.

Muscle contains pluripotent stem cell progenitors and is the site of ectopic ossification whereby muscle injury leads to the formation of ectopic bone in the muscle bed. MDSCs undergo differentiation into cartilage and bone tissues in vitro and tissue engineering approaches using these cells in vivo to promote bone repair has been effective in animal models. While less has been done with lineage tracing of MDSCs in fracture healing, available data suggest that MDSC populations are not typically involved in the bone repair except in the setting of extensive injury to the periosteum where they may become an important, or possibly a primary source of progenitor cells.

In addition to a potential role as a source of progenitor cells for regeneration, muscle also likely influences fracture healing by 1) acting as a highly vascular tissue that enables angiogenesis and revascularization of the bone injury site; and 2) by providing local growth factors and cytokines that regulate the repair process. The role of revascularization in fracture healing is well established. Revascularization prevents infection but also delivers the VEDP that are essential for the secondary bone remodeling process. However work in animal models also suggests that the source of vascular tissues is important. While fascia-cutaneous tissues restore vascularity to fractures as effectively as muscle tissues, the bone regenerative process is enhanced with the presence of muscle tissues adjacent to the fracture site. This suggests that muscle, similar to bone, secretes factors or cytokines that regulate the repair process. However, the factors secreted by muscle potentially involved in this process remain to be determined.

Finally there is a strong connection between muscle and bone health. Increased muscle mass and strength improve balance and reduce the potential for fall-related fractures in the elderly. Anabolic agents are being investigated for both bone and muscle tissues and data shows that that some agents may target genes or pathways that simultaneously result in improvement in muscle mass, bone density, and fracture healing.

Ongoing work to further define the inter-relationship between bone and muscle metabolism is critical and will help to prevent skeletal injury and improve the regeneration of injured muscle and bone.

Disclosures: Regis J. O'Keefe, *None.*

Session 5: Preventing and Treating Muscle and Bone Loss

15

Nutrition: Effects of Vitamin D in Muscle and Relationship to Bone

Bess Dawson-Hughes, Tufts University, USA

It has been recognized for over 35 years that bone mass and muscle mass are linked across age and race groups. Bone and muscle are also linked functionally, with muscle weakness increasing risk of falling and falls being the main trigger of fractures. Vitamin D is important for muscle, but the mechanisms are not yet clearly defined and may be both direct and indirect. Evidence for a direct effect on muscle includes the extreme muscle weakness often seen in severe vitamin D deficiency and the identification of vitamin D receptors (VDRs) in the nuclei of human myocytes. The active metabolite of vitamin D, 1, 25-dihydroxyvitamin D, activates these receptors to initiate genomic effects; it also triggers rapid non-genomic actions through activation of membrane VDRs. Support for indirect effects on muscle includes the lack of consistency in the identification of VDRs in the myocyte and the finding in the mouse that genetic deletion of the VDR in intestinal tissue results in changes in muscle tissue. Indirect effects are also supported by the observation that not all humans with very low 25OHD levels have myopathy and that high dose calcium can reverse the clinical syndrome of osteomalacia. Many clinical studies have examined the effect of supplemental vitamin D on muscle strength and performance. The evidence for improvements in muscle performance is mixed with some findings positive for vitamin D or others null. A recent meta-analysis of 17 such trials revealed no significant effect overall; but, a large effect was observed in the two trials in which the mean starting level of 25OHD was 25 nmol/L or below. The diverse set of testing procedures used in the trials likely contributes to this variability. There is also the possibility that at least some of the effect of vitamin D on performance is due to its effect on balance, which was not assessed in all of the trials. There is general agreement that supplementation of vitamin D can lower risk of falling by up to 15 or 20% in elders. The effect of vitamin D on fall risk appears to be inversely related to the starting 25OHD level and directly related to the dose administered, but the optimal levels are not certain. Concurrent calcium supplement may enhance the effectiveness of vitamin D in lowering fall risk, but this has not been clearly demonstrated.

Disclosures: Bess Dawson-Hughes, *None.*

16

Activin Type II Receptor Biologics for Preventing and Treating Muscle and Bone Loss

Teresa A. Zimmers, Thomas Jefferson University, USA

This abstract summarizes work to date by various groups using Activin Type II receptor fusion proteins, developed to sequester their biologically important ligands, including Activin A, Activin B, Myostatin, GDF-11 and GDF-2/BMP-9. A soluble ACVR2B was first tested in the context of inhibiting Myostatin, a tonic muscle growth inhibitor. Indeed, in preclinical studies ACVR2B/Fc results in

muscle hypertrophy in normal animals and stems muscle wasting in mouse models of cancer cachexia, muscular dystrophy, hindlimb unloading, sepsis, and obesity. Other ACVR2B ligands also inhibit muscle growth, as ACVR2B/Fc also promotes muscle growth in Myostatin null mice. Effects of ACVR2B/Fc on bone preservation have been observed. Initial trials of a human ACVR2B/Fc in postmenopausal women showed promise, but a trial in boys with muscular dystrophy was recently suspended due to development of spontaneous nosebleed, gum bleeds and telangiectasias. These complications along with pericardial and pleural effusions were separately shown in non-human primates treated with a similar ACVR2B/Fc. The same group showed that more specific targeting of myostatin using a neutralizing monoclonal antibody resulted in muscle hypertrophy without these side effects, suggesting that other ACVR2B ligands are responsible for the hematological/vascular abnormalities. ACVR2A fusion proteins have also been developed and are in testing to enhance erythropoiesis, increase bone formation and slow tumor growth. Activin A is a target of particular interest, known to be expressed in bone and to be increased in sera of mice and patients with breast cancer and multiple myeloma, rising further in the presence of metastases. Modulation of Activin A in mice has complex effects on bone. Administration increases bone mineral density, but its inhibitor inhibin-A increases bone mass. In preclinical studies, ACVR2A/Fc stimulates osteoblastogenesis and bone formation. Moreover, it prevents osteolytic bone lesions in a myeloma model and inhibits bone destruction and metastasis in a breast cancer model. A phase I trial of the human version showed effects on markers of bone formation without apparent serious adverse events. Results of Phase II trials in multiple myeloma and Phase II/III trials for anemia have not yet been reported. While these approaches show promise, optimizing the clinical utility of such decoy receptors while minimizing undesirable effects will hinge upon understanding the biological activities, binding specificities and interactions of individual ligands.

Disclosures: Teresa A. Zimmers, *None.*

17

A Regenerative Medicine Approach to Musculotendinous Tissue Reconstruction

Stephen F. Badylak, University of Pittsburgh, USA

Volumetric muscle loss has limited treatment options. In spite of the excellent regenerative capacity of skeletal muscle, loss of greater than 20% of muscle mass (within a given muscle group) invariably results in loss of function, extensive deposition of scar tissue, and lifelong morbidity.

A regenerative medicine approach to functional skeletal muscle tissue regeneration has been investigated in both preclinical studies and in early human clinical trials. The approach is based upon a bioinductive scaffold that has shown the ability to promote restoration of structure and function in several anatomic soft tissue locations. The approach is based upon the concept of endogenous stem cell recruitment (i.e. “homing”), combined with provision of an appropriate microenvironmental niche and local modulation of the innate immune response. Recruited stem and progenitor cells are stimulated to form site appropriate musculotendinous tissue in response to the above mentioned factors. Results of preclinical studies and early clinical trial results will be presented.

Disclosures: Stephen F. Badylak, *None.*

Session 6: Emerging Areas

18

Osteocalcin and the Regulation of Muscle Mass

Gerard Karsenty, Paula A. Marks, Columbia University Medical Center, USA

The undercarboxylated form of osteocalcin acts as a hormone regulating insulin secretion and sensitivity, metabolism, energy expenditure and male fertility. The fact that these functions decline with age suggests the hypothesis that bone, via osteocalcin, may be an endocrine determinant of aging rather than a mere victim of it. In the logic of this hypothesis osteocalcin would be needed to maintain at an optimum level multiple physiological functions. One hallmark of aging is a decrease of muscle mass, or sarcopenia, that occurs at the same time bone mass decreases. We therefore asked whether osteocalcin is a bone derived signal affecting muscle mass. We will present evidence at the meeting that osteocalcin favors the maintenance of muscle mass. Indeed in the absence of osteocalcin the weight of all muscles tested decreases overtime. This decrease in muscle mass is secondary to a decrease in the diameter of the fibers while the number of fibers is unaffected by osteocalcin. A functional consequence of the sarcopenia phenotype observed in the *Osteocalcin-/-* mice is that their ability to run, the time they can run and the speed at which they run, is significantly decreased compared to wild type littermates. We will also show that osteocalcin is required for muscle regeneration if muscles are injured. At the molecular level osteocalcin favors myoblast proliferation and this appears to be the main mechanism by which it regulates muscle mass. Experiments are ongoing to determine if this novel function of osteocalcin occurs following its signaling through the G protein coupled receptor Gprc6a.

Disclosures: Gerard Karsenty, *None.*

19

Muscle as an Endocrine Organ

Mark A. Febbraio, Cellular & Molecular Metabolism Laboratory, Baker IDI, Heart and Diabetes Institute, Australia

Skeletal muscle is primarily known as an organ of locomotion. However, almost 50 years ago Goldstein[1] proposed the hypothesis that muscle cells possess a “humoral” component that contributes to the maintenaince of glucose homeostasis during exercise. Several years ago, we identified skeletal muscle as a cytokine-producing organ, demonstrating that the metabolic and physiologic effects of exercise may be mediated by muscle derived humoral factors (fore review see[2]). We have demonstrated that interleukin-6 (IL-6) was the prototypical “myokine”, up-regulated by muscle contraction and released from contracting skeletal muscle, to play important roles in lipid and glucose metabolism in metabolically active tissues[3-5], while others have demonstrated that the exercise induced incretin response is entirely dependent upon IL-6 release from skeletal muscle[6]. Since the identification of IL-6 as the prototypical myokine, other candidate myokines such as IL-8, IL-15, leukemia inhibitory factor (LIF), brain derived neurotropic factor (BDNF), follistatin like 1 (FST-1) and fibroblast growth factor-21 (FGF-21) have been discovered[7]. Recently, work from the Speigelman laboratory has generated great

renewed excitement in the the area of myokine identification. This group identified FNDC5 as a potential mediator of exercise–induced increases in thermogenic genes such as UCP-1 in the adipose tissue[8]. Clearly, the role of skeletal muscle as an endocrine organ is in its infancy, but rapidly expanding.

References
1. Goldstein, M.S. *Diabetes* 10, 232-234 (1961).
2. Pedersen, B.K. & Febbraio, M.A. *Physiol Rev* 88, 1379-1406 (2008).
3. van Hall, G., *et al. J Clin Endocrinol Metab* 88, 3005-3010 (2003).
4. Febbraio, M.A., et al *Diabetes* 53, 1643-1648 (2004).
5. Carey, A.L., *et al. Diabetes* 55, 2688-2697 (2006).
6. Ellingsgaard, H., *et al.* I *Nat Med* 17, 1481-1489 (2011).
7. Pedersen, B.K. & Febbraio, M.A. *Nat Rev Endocrinol* (2012).
8. Bostrom, P., *et al. Nature* 481, 463-468 (2012).

Disclosures: Mark A. Febbraio, *None.*

20

The Wnts in Muscle-Bone Cross Talk

Mark L. Johnson, University of Missouri-Kansas City, School of Dentistry, USA

Long standing theory regarding skeletal muscle and bone has been that skeletal muscle applies load to bone and bone provides an attachment site for skeletal muscles. However, this solely mechanical relationship does not fully explain the loss of muscle mass/function with aging that occurs in sarcopenia and changes in bone that occurs in osteoporosis. Besides mechanical coupling, skeletal muscle produces factors called myokines and conversely, it is known that osteocytes in bone produce factors that act on other tissues.

The Wnt/β-catenin pathway plays a crucial role in bone function. Deletion of β-catenin in the osteocyte compromises its viability and ability to communicate with other cells, bone's response to mechanical loading, and results in increased bone fragility. Recent evidence supports an important role for the Wnt/β-catenin pathway in muscle development and function. We have examined the role of this pathway in muscle-bone crosstalk and if this crosstalk is independent or synergistic with mechanical loading.

We have used a combination of in vivo and in vitro models to test skeletal muscle-bone crosstalk. Specific animal models with altered bone mass or muscle function have concomitant changes in the other tissue. Both cell line and primary cell culture and ex vivo tissue studies demonstrate the production of factors that alters the function of the reciprocal cell type. Skeletal muscle cells and specific muscle types produce factors that enhance osteocyte viability and the response of the osteocyte to mechanical loading. Osteocytes produce factors that improve muscle contractility and other functions. Our data also suggest a central role of Wnt/β-catenin signaling in mediating the effects of these factors. Furthermore, preliminary data suggests that the effects of these factors are diminished with aging.

These data support our hypotheses that beyond the mechanical coupling of these two tissues, there is a molecular coupling that involves the Wnt/β-catenin signaling pathway. Several key questions remain: What is the identity of these factors? How are these factors regulated and does the production of these factors change with age? What is the mechanism by which these factors target muscle or bone and/or do they also target other tissues? Clearly, the identification of these factors and understanding the mechanism through which they act could lead to novel targets for the development of new compounds to treat both sarcopenia and osteoporosis.

Disclosures: Mark L. Johnson, *None.*

21

Regulatory Interactions between Myeloid Cells and Skeletal Muscle

James G. Tidball, University of California-Los Angeles, USA

Bone marrow-derived, hematopoietic cells have the capacity to contribute to muscle growth and regeneration that result from modified muscle use, muscle injury or disease. For example, previous studies (Carmago et al., 2003) demonstrated that transplantation of CD45+; Sca-1+ bone marrow cells into injured muscle produced healthy muscle fibers that contained donor-derived nuclei that were transcriptionally-active, indicating that bone-marrow derived cells could enter the myogenic compartment and influence muscle repair. Although the precise developmental state of the bone marrow-derived cells that are fusion competent with muscle cells is uncertain, most observations indicate that hematopoietic cells that enter the myeloid lineage are more fusion-competent than cells that enter the lymphoid lineage, and indicate that relatively undifferentiated myeloid cells are more fusion competent than differentiated myeloid cells. However, myeloid cells that are terminally-differentiated are also capable of strongly influencing muscle growth and regeneration through the release of soluble factors. For example, differentiated macrophages that are activated to the M2 phenotype by interleukin-10 (IL-10) can induce shifts in the patterns of expression of muscle transcription factors that regulate muscle differentiation, they can promote the proliferation of muscle stem cells and they accelerate the differentiation of post-mitotic myogenic cells. Furthermore, removal of M2 macrophages *in vivo* or the ablation of IL-10 in animals experiencing muscle injury or disease greatly reduces the regenerative capacity of the muscle.

Disclosures: James G. Tidball, *None.*

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

POSTERS

Aging: Changes in Muscle and Bone, Linkages and Shared Etiologies

P1

The Effects of Weight Loss and Changes in Fat and Lean Tissue on Bone Mineral Density in Women and Men – Results of a Randomized Controlled Trial.

Amir Tirosh[1], Russell de Souza[1], Frank Sacks[1], George Bray[2], Steven Smith[2], Meryl Leboff*[1]. [1]Brigham and Women's Hospital, USA, [2]Pennington Biomedical Research Center of the Louisiana State University System, USA

The Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) is a randomized controlled trial of 811 overweight and obese adults assigned to one of 4 weight loss diets differing in fat, protein, and carbohydrates (NEJM.360:859, 2009). We recently showed that participants on these weight loss diets lost more fat than lean mass, without differences among the 4 diets (AJCN. 95:614, 2012). The impact of these changes on bone mineral density (BMD), however, is uncertain. In 424 participants (mean age 52±9 years, 57% females) from 2 centers (Boston and Baton Rouge), we measured BMD at the spine, total hip (TH) and femoral neck (FN), and quantified fat and lean mass by DXA (QDR 4500A, Hologic, Bedford MA) at baseline, and at 6 months and 2 years following the dietary interventions. These studies showed that at baseline, there was a stronger correlation between BMD and body composition measurements in women than in men, with the lean body mass compartment exhibiting the strongest correlation with BMD (r=0.419, 0.507 and 0.523 for spine, FN and TH, respectively, all $p<0.001$). In men, only lean body mass correlated with hip BMD (r=0.298 between lean body mass and TH BMD, $p<0.001$). The overall average weight loss was 7.9% after 6 months and 6.9% after 2 years. Following this significant weight loss, the change in BMD from baseline to 6 months was 0.0005 gm/cm2 (p=0.07) at the spine, -0.001 gm/cm2 ($p<0.001$) at the TH and -0.0007 gm/cm2 (p=0.016) at the FN. From baseline to 2 years, these changes were 0.005 (p=0.04), -0.014 ($p<0.001$), and -0.014 gm/cm2 ($p<0.001$), respectively. Loss to follow-up was 22% at 6 months and 44% at 2 years. Changes in spine, FN and TH BMD following 2 years of dietary intervention, directly correlated with changes in lean body mass in women (r=0.200, 0.324 and 0.260 for spine, FN and TH, respectively), whereas changes in fat mass correlated only with changes in TH BMD (0.274, $p<0.001$) but not with change in spine or FN BMD. In men, changes in lean or fat mass were not associated with changes in FN and TH BMD, whereas the changes in lean body mass (-0.323, $p<0.001$) and in fat mass (-0.213, p=0.027) were negatively correlated with change in BMD at the spine. In conclusion, weight loss results in gender-specific effects on BMD. While men exhibited an increase in spine BMD and no change in hip BMD, women tended to decrease BMD at all sites regardless of the diet to which they were assigned.

***Disclosures: Meryl Leboff**, None.*

P2

Characteristics of Initiators of Different Osteoporosis (OP) Medications among Women with Postmenopausal Osteoporosis (PMO) in The Health Improvement Network (THIN) in the UK.

Fei Xue[1], Chuck Wentworth[2], Victor Gastanaga[1], Cathy Critchlow*[1]. [1]Amgen Inc., USA, [2]Analytic Consulting Solutions, Inc, USA

Background: Observational studies have suggested that different OP medications for the treatment of PMO, e.g., bisphosphonates (BPs) vs. other medications, may have dissimilar effectiveness and safety profiles. It is unclear to what extent such discrepancies are attributable to confounding by indication due to different baseline patient characteristics, such as age, comorbidities and concomitant medications.

Objectives: Describe PMO patients who initiated treatment with BPs or other OP medications.

Methods: Women ⩾55 years with ⩾12 months of data in the THIN database were included as PMO patients if they had received a diagnosis of OP, a diagnosis of osteoporotic fracture or treatment with OP medications, and did not have a cancer diagnosis in the period up to 5 years prior to cohort entry from 1995 to 2008. A woman with PMO was included in the BP initiator cohort if she received a BP and did not receive any OP medication in the prior 12 months. Women in the initiator cohort of other OP medications (calcitonin, parathyroid hormone and SERMs) were identified in the same manner. Each cohort was described with regard to demographic factors, comorbidities and concomitant medications based on the 12-month baseline period prior to drug initiation.

Results: In 114,760 women with PMO, 60,954 (53.1%) initiated treatment with BPs and 2,648 (2.3%) with other OP medications. Relative to initiators of other OP medications, initiators of BPs were older (mean age=73.1 vs. 66.7 yrs), and tended to have a higher prevalence of OP (17.4% vs. 15.4%), osteoporotic fracture (11.4% vs. 4.5%), hypertension (14.0% vs. 8.8%), diabetes (10.7% vs. 6.5%), heart failure (1.4% vs. 0.7%), atrial fibrillation (1.8% vs. 0.6%), coronary heart disease (5.1% vs. 3.6%), asthma (7.9% vs. 4.4%), COPD (5.0% vs. 2.0%), hypercholesterolemia (1.1% vs. 0.9%), kidney failure (0.5% vs. 0.2%), stroke (2.1% vs. 0.7%), obesity (0.5% vs. 0.3%), and anti-diabetics treatment (5.7% vs. 2.5%).

Conclusion: Our results suggest patients who initiated treatment with BPs tended to be older and have more baseline comorbidities than initiators of other OP medications, likely reflecting physician preferences when deciding whom to treat with BP, the standard of care for PMO during the study period. Such discrepancies should be taken into consideration when comparing the post-treatment efficacy and safety profile of these medications.

***Disclosures: Cathy Critchlow**, None.*

P3

Age Effects on Osteocyte Lacunar and Canalicular Microarchitecture in Non-Human Primate Cortical Bone.

Amber Stern*[2], Branson Billings[1], Matthew Stern[2], Christopher Bergman[3], Thomas Register[3]. [1]University of Missouri-Kansas City School of Dentistry, USA, [2]University of Missouri - Kansas City, USA, [3]Wake Forest University School of Medicine, USA

Throughout our lifetime bone is constantly remodeling. Osteocytes are thought to play an important role in the equilibrium of bone remodeling through their complex network of extracellular and intracellular communication. However, with age, our bone becomes less responsive to strain which results in a reduced bone mass and increased risk of fracture.

The purpose of this study was to use quantitative analysis to assess the changes of bones' microarchitecture that accompany the aging process utilizing scanning electron microscopy (SEM). These data will be used in the creation of more anatomically accurate finite element models to predict strain perceived by young and old osteocytes.

SEM imaging was used to examine the osteocyte lacuna-canalicular system of young (n=5) and old (n=5) non-human primate femurs. Images obtained at 300x magnification were collected to assess lacunar density of the samples, while osteocyte lacunar cross sectional area and number of canaliculi per osteocyte lacuna were measured using a magnification of 3000x. All images were quantitatively examined using ImageJ software and the samples were blinded to the investigator.

A significant increase (Student's T-test, $p<0.05$) was observed between the young (n=51) and old (n=52) samples with regard to osteocyte lacunar cross sectional area (119.4 ± 3.3 μm^2 vs. $131.7 \pm 3.9 \mu m^2$). The increase in cross sectional area was observed along the minor axis of the osteocyte lacuna. A significant decrease in lacunar density was observed between samples from young (n=25) and old (n=25) bone (729.3 ± 18.0 lacunae/mm^2 vs. 603.7 ± 15.2 lacunae/mm^2), while, no significant difference was observed between young and old samples in the number of canaliculi per osteocyte that were visable (37.1 ± 1.1 vs. 36.8 ± 1.2).

These results reiterate the microstructural changes of bone that occur with age. The values found in this study are currently being used to create more anatomically accurate finite element models to estimate changes in osteocyte strain transduction with age.

***Disclosures: Amber Stern**, None.*

P4

The Role of Uncoupling Protein 3 on Oxidative Stress-related Muscle Atrophy.

Katsuya Hirasaka*[1], Hironori Yamamoto[1], Edward Mills[2], Shohei Kohno[3], Tomoki Abe[3], Chika Ikeda[3], Tasuku Maeda[3], Shigetada Kondo[3], Ayako Ohno[3], Yuushi Okumura[3], Takeshi Nikawa[3]. [1]University of Tokushima, Japan, [2]University of Texas at Austin, USA, [3]The University of Tokushima Graduate School, Japan

Oxidative stress contributes to progression of age-associated muscle atrophy (sarcopenia). Mitochondria are major source of reactive oxygen species (ROS) and exceed ROS production is due to disturbed mitochondrial function. Uncoupling protein 3 (UCP3) is primarily expressed in the inner membrane of skeletal muscle mitochondria. It has been proposed that UCP3, which decreases in aged fast twitch muscle, reduces production of ROS and oxidative damage. However, the mechanisms by which UCP3 attenuates ROS production are not well understood. Here we report that UCP3 interacts with the non-processed form of thioredoxin 2 (Trx2), a redox protein that is localized in mitochondria, but not processed Trx2, which is involved in cellular responses to ROS. The hydrophilic sequences within the N-terminal tail of UCP3, which faces the intermembrane space, are necessary for binding to Trx2. In addition, Trx2 directly associated with UCP3 through a mitochondrial targeting signaling sequence, was processed in the intermembrane space, and thereby allowing redox reactions. Furthermore, C2C12 myoblast cell lines stably overexpressing UCP3 within the physiological range (~2.5 fold) of induction (approximately 2.5-fold) over mock infected controls significantly attenuated ROS production in isolated mitochondrial without effects on membrane potential, however this effect is lost by Trx2 knock down. Combined, these results imply that Trx2 plays a mechanistic role in UCP3-dependent mitochondrial ROS suppression. Thus, our data also suggest that the antioxidant efficacy of Trx2-mediated UCP3 may provide a novel therapeutic approach against oxidative stress-related disease containing sarcopenia.

***Disclosures: Katsuya Hirasaka**, None.*

P5

Is the Relationship between Whole Body Bone and Soft Tissue Seen in Childhood Maintained in Adulthood?

Tom Sanchez[1], Jingmei Wang[2], Felix Rajan[3], Terry Schwalenberg[1], Kathy Dudzek*[1]. [1]Norland - A Cooper Surgical Company, USA, [2]Norland–a CooperSurgical Company, China, [3]Siemens Healthcare, USA

Reports indicate a strong relationship between total body bone and lean mass in children and a moderate relationship between total body bone and fat mass in girls but not in boys. The current study explores if those relationships are continued in adult men and women.

A population of 150 subjects (75 males between 20 and 51 years old and 75 females between 20 and 67 years) underwent whole body studies to assess total body bone, lean and fat using the same DXA system (Norland XR-46 fitted with Illuminatus software) used earlier to assess total body bone, lean and fat mass in a population of children. Analysis of the relationship between bone and lean or fat in male and female subjects was by regression with covariance analysis.

Analysis shows strong significant relationships between bone and lean mass in adult males (BMC = 0.0387X + 1124; r = 0.6853; $P<0.0001$; RMSE = 303) and females (BMC = 0.03078X + 1577; r = 0.5456 $P<0.0001$; RMSE = 289). Examining the relationship between bone and fat mass reveals a relatively strong relationship in adult females (BMC = 0.0175X + 2336; r = 0.4742; $P<0.0001$; RMSE = 304) and a more moderate relationship in adult males (BMC = 0.0146X + 3207; r = 0.3603; $P<0.001$; RMSE = 388).

The data indicate that–as observed in children–bone mass is significantly related to lean mass in men and women and that a significant relationship also exists between bone and fat in the women.

***Disclosures: Kathy Dudzek**, None.*

P6

The Relation between Age-related Declines in Hand Grip Strength and Arterial Stiffness in Korean Men.

Sang Hyeon Je*[1], Duck Joo Lee[2]. [1]Ajou University Hospital, Department of Family Medicine, South Korea, [2]Ajou University School of Medicine, South Korea

Background: The hand grip strength (HGS) is an indicator of overall muscle strength as well as aging. We evaluate the associations between HGS and other clinical markers of aging in Korean men

Methods: We collected data from 5412 men who visited the Health Promotion Center in Geoje city, Korea for health examination in 2003-2009. Tests for fasting blood sample, HGS, lipid profile,

homocysteine, blood pressure and routine blood chemistry and spirometry were performed. Pulse wave velocity was used at the carotid artery level to assess arterial stiffness. Men were categorized into three HGS groups (low, medium, and high). The men also were grouped into three aged groups; young, middle and old aged groups. Odds ratio was estimated using low HGS as a reference group.

Results: Age, arterial stiffness, uric acid, homocysteine and FEV1/FVC were statistically significantly different across three HGS and aged groups ($P<0.05$). Compared to low HGS group, odds ratio for the arterial stiffness in medium group was 0.72 and 0.23 in high group. This finding was statistically significant in the middle aged gropu ($P<0.05$). Other clinical markers of aging in each aged group did not differ by HGS.

Conclusions: In middle aged Korean men, 40-59 years old, HGS may be related to arterial stiffness.

***Disclosures: Sang Hyeon Je**, None.*

P7

Chewing on Something New: Dietary Variability and Craniofacial Plasticity.

Rachel Menegaz*[1], Ashley Szczodroski[2], Tammy Rold[2], Timothy Hoffman[2], Matthew Ravosa[3]. [1]University of Missouri, USA, [2]Harry S. Truman Memorial Veterans' Hospital, USA, [3]University of Notre Dame, USA

The effects of dietary variability on craniofacial growth and the health of aging individuals is poorly understood. Prior research has described the influence of dietary composition, which determines the intensity of masticatory forces and mechanical loading on bone formation, cranial morphology and tissue adaptations. However, these studies have not addressed the significance of dietary variability within an organism's lifespan. The lack of such dynamic data hinders our ability to synthesize the experimental aspect of skeletal biology with natural behaviors, which often involve the use of a wide variety of dietary resources and may change postnatally. Our goal is to detail the soft- and hard-tissue responses that maintain the overall integrity of the cranial skeleton in response to routine chewing/biting forces, and to provide unique analyses of the relationship between aging processes and an individual's ability to respond dynamically to environmental changes.

In order to model skull growth as affected by temporal shifts in dietary properties, four dietary cohorts (n=10/cohort) of male Sprague-Dawley rat were raised from weaning to skeletal maturity. Two cohorts were fed a stable diet of either solid or powdered pellets. The other two cohorts were fed a variable diet of either solid/powdered pellets for the first half of the study, followed by a shift to the opposite diet. Longitudinal *in-vivo* microCT imaging was used to quantify ontogenetic changes in craniofacial morphology. Mineral apposition rates (MAR) were assessed via fluorochrome labeling, and three serological markers related to skeletal physiology were analyzed across the growth period.

Principal component analyses of 3D models constructed from longitudinal microCT scans reveal significant shape differences both between powdered and whole pellet diet cohorts, and between stable and variable diet cohorts. Animals on the variable powered-to-solid pellet diet (cohort 4) showed a significant increase (protected $P = 0.008$) inMAR in the mandible following the dietary shift. Serum markers of bone physiology tended to track total skeletal growth as quantified by linear postcranial measurements. However, cohort 4 showed elevated levels of serum osteocalcin following the dietary shift, as well as decreased levels of PINP 5b at the time points both before and after the dietary shift.

These results demonstrate the capacity of individuals experiencing dietary variability to adapt to increasing biomechanical demands during the course of ontogeny. Furthermore, this functional adaptation can be detected at several hierarchical levels. A complete understanding of craniofacial function and plasticity is hindered by the traditionally static approach to experimental and natural studies. This study emphasizes the need for an increased awareness of the role of environmental variability on morphology, particularly in growing organisms.

***Disclosures: Rachel Menegaz**, None.*

P8

The Role of the Proteins of the Nuclear Envelope in the Pathophysiology of Osteosarcopenia.

Sandra Bermeo-Serrato*[1], Christopher Vidal[1], Wei Li[2], Diane Fatkin[3], Gustavo Duque[2]. [1]University of Sydney, Australia, [2]Aging Bone Research Program, The University of Sydney, Australia, [3]Victor Chang Institute, Australia

Introduction: Sarcopenia and osteopenia are two components of the frailty syndrome that could share common underlying mechanisms. Recently, we have reported that lamin A/C deficient mice ($Lmna^{-/-}$) show both osteo and sarcopenia together with fat infiltration of muscle and bone (*Tong et al, Mech Ageing and Develop, 2011*). This evidence suggests that the proteins of the nuclear envelope, particularly in the inner nuclear membrane (INM), could play a role in the pathogenesis of osteosarcopenia and frailty. Since all the proteins of the INM are interconnected, in this study we assessed whether changes in lamin A/C expression in $Lmna^{-/-}$ mice also affect other proteins of the INM in muscle and bone.

Methods: Sections of the middle of the thigh and total proteins from femoral bone marrow and muscle were obtained from wild type (WT) and $Lmna^{-/-}$ mice tested by western blotting and immunofluorescence respectively. Expression of the three major proteins of the INM (Emerin, MAN1 and nesprin-1) were compared between wild type and $Lmna^{-/-}$ mice using immunofluorescence and western blotting. RESULTS: Our results showed that $Lmna^{-/-}$ mice had a decreased MAN1 expression in both bone and muscle (71 and 83%, respectively) as compared to WT controls ($p<0.01$). Conversely, there was an increase (~2 fold) of both nesprin-1 and emerin in bone of $Lmna^{-/-}$ mice, whereas in muscle we observed no change in the expression of emerin with nesprin-1 being significantly lower in $Lmna^{-/-}$ mice (- 49%) as compared with their WT controls ($p<0.01$).

Conclusion: Absence of lamin A/C is associated with major changes in the expression of other proteins of the INM in muscle and bone. Whereas MAN1 expression is decreased in both tissues, an increase in nesprin-1 and emerin in bone happened concomitantly with stable emerin and lower nesprin-1 in muscle. In conclusion, our results suggest that both, lamin A/C and MAN-1, could play an important role in the pathogenesis of osteosarcopenia while the role of high emerin and nesprin-1 in osteopenia and low nesprin-1 in sarcopenia remains to be elucidated. In conclusion, we report that the proteins of the INM could play different roles in the pathogenesis of osteosarcopenia. The characterization of their specific role and targeted interventions to increase their expression and function could become an effective therapy for frailty in the near future.

***Disclosures: Sandra Bermeo-Serrato**, None.*

P9

Does Vitamin D Supplementation Affect Body Composition and Strength?.

Violet Lagari*[1], Orlando Gomez-Marin[2], Silvina Levis[2]. [1]University of Miami, USA, [2]University of Miami School of Medicine, USA

Purpose: Few prospective studies have examined the association between vitamin D and muscle mass or strength. The aim of this study is to investigate if vitamin D supplementation is associated with improvements in lean mass, fat mass, and strength.

Methods: Body composition [BC] measurements were obtained on 86 subjects at baseline and 6 months using dual-energy x-ray absorptiometry as part of a single site, double-blind, randomized clinical trial enrolling subjects aged 65 and older to receive 400 (low dose) or 2,000 (high dose) IU vitamin D_3 daily, regardless of their current 25(OH) vitamin D [25(OH)D] level. Preliminary multivariate analyses revealed a significant gender x treatment interaction. Thus, within gender, separate correlation and regression analyses were performed to assess different interrelationships and the effect of treatment on relative changes in 25(OH)D, total lean mass, total fat mass and grip strength, an indicator of muscle strength.

Results: Gender-specific comparisons yielded no significant differences between treatment groups with respect to baseline characteristics such as age, BMI, race, ethnicity, smoking status, alcohol use, grip strength, weight, height, 25(OH)D, calcium and vitamin D supplements, grip strength, and total lean and fat mass. Among women, some relative changes (%) for the low and high dose groups, respectively, included: total lean mass, 0.56 $\pm$ 2.6 and 0.36 $\pm$ 2.9; grip strength, -5.6 $\pm$ 11.0 and -3.2 $\pm$ 12.6; total fat mass, 0.08 $\pm$ 5.6 and -0.85 $\pm$ 5.4; 25(OH)D, -6.3 $\pm$ 28.1 and 24.6 $\pm$ 51.2. Also among women, significant inverse correlations were found between relative changes in total lean mass and relative change in grip strength ($p = 0.035$); and between relative change in total fat mass and relative change in 25(OH)D ($p=0.014$). The latter relationship remained significant after adjusting simultaneously for treatment, age, and BMI. Among men, there were no significant correlations between relative changes in total lean, fat mass, grip strength, and 25(OH)D.

Conclusions: The significant inverse correlation between relative changes in total fat and 25(OH)D, support the evidence that higher supplemental doses are needed in persons who are overweight. The results of this clinical trial assessing the effects of vitamin D supplementation on BC and muscle strength do not show an effect of vitamin D supplementation on changes in total lean mass or grip strength.

***Disclosures: Violet Lagari**, None.*

This study received funding from: Merck & Co. and the Marjorie Cowan Family Foundation

P10

Bone Mineral Density (BMD) Combined with the Trabecular Bone Score (TBS) Significantly Improves the Identification of Women at High Risk of Fracture: The SEMOF Cohort Study.

Albrecht Popp*[1], Salome Meer[1], Marc-Antoine Krieg[2], Romain Perrelet[1], Didier Hans[2], Kurt Lippuner[1]. [1]Osteoporosis Policlinic, University of Bern, Switzerland, [2]Lausanne University Hospital, Switzerland

Introduction: Trabecular Bone Score (TBS) is a novel grey-level texture measurement reflecting bone micro-architecture (MA) based on the use of experimental variograms of 2D projection images. The aim of this analysis was to investigate whether combining quantitative and qualitative (i.e. BMD and TBS) information obtained from DXA scans performed on a single device contributed to better identification of women at high fracture risk.

Methods: In the prospective SEMOF study, women were randomly selected from official state registries between January 1998 and April 2000. Only women from the Center fo Bern, Switzerland, with no history of hip fracture, between 70 and 80 years of age, independent for their daily activities were included in the present analysis. Lumbar spine and hip BMD assessed by DXA (Hologic, USA) and MA evaluation by TBS (Medimaps, France) were recorded. After checking for normal distribution, results of all parameters were expressed as means and SD. The hazard of the first clinical fracture was calculated by using the age and BMI adjusted proportional hazards model of Cox.

Results: The necessary information was available for 557 out of 701 women (79%) with the following baseline characteristics (mean $\pm$ SD): age 76.1 $\pm$ 3.0 years, BMI 25.6 $\pm$ 3.9 kg/m^2, lumbar spine and hip BMD, 0.863 $\pm$ 0.174 and 0.771 $\pm$ 0.121 g/cm^2, respectively, and TBS 1.195 $\pm$ 0.115. As expected, correlation between lumbar spine BMD and site matched TBS was low (r^2=0.25). After 2.72 $\pm$ 0.77 years of follow-up, the incidence of fragility fracture was 9.4%. Age- and BMI-adjusted ORs (per SD decrease) were 1.6 (1.1-2.1) (AUC = 0.68), 1.8 (1.4-2.3) (AUC = 0.66), 1.7 (1.2-2.3) (AUC = 0.61) for spine, total hip, and femoral neck BMD, respectively, and 1.9 (1.4-2.5) (AUC = 0.73) for TBS. TBS remained significant after adjustment of any of the BMD values. When using a triage approach, 57% of fragility fractures had a BMD T-score below -2.5 and 75% of fractures had a TBS $<$ 1.200. Combining BMD$<$ -2.5 SD at any site or TBS $<$ 1.200 identified 85% of all women with an osteoporotic fracture.

Conclusion: As in the already published studies, these preliminary results confirm the partial independence between BMD and TBS. More importantly, combining TBS and BMD values improved fracture prediction. Thus TBS added to BMD information may become an important parameter for further refining individual fracture risk.

***Disclosures: Albrecht Popp**, None.*

P11

Osteocyte Apoptosis Induced by Glucocorticoids Is Prevented and Reversed by Anti-sclerostin Antibody in a Male Rat Model.

Zahra Achiou[1], Delphine Benaitreau[2], Carine Tournier[2], Eric Dolleans[2], Eric Lespessailles[3], Michael Ominsky[4], Stephane Pallu[5], Claude Laurent Benhamou[1], Geal Rochefort*[1]. [1]CHR ORLEANS, France, [2]University of Orléans, France, [3]Centre Hospitalier Regional, France, [4]Amgen Inc., USA, [5]EA 4708 - I3MTO Orléans, France

Introduction: Long-term glucocorticoid therapy leads to osteoporosis and is associated with increased osteocyte and osteoblast apoptosis. Administration of antibodies anti-sclerostin, a product of the mature osteocyte, has been shown to increase bone formation and bone mass in various animal models, including in a mouse model of glucocorticoid mediated bone loss. In the current study, the effects of a sclerostin neutralizing monoclonal antibody (Scl-AbVI) on bone mass, bone strength, and osteocyte apoptosis were examined in a glucocorticoid-induced osteoporosis model in rats.

Methods: Forty five male Wistar rats, 4 month-old, were randomly assigned to 3 groups. Two groups were subcutaneously injected 5 days a week with vehicle (C) or 5 mg/kg methylprednisolone (M), with a third group injected with both methylprednisolone and Scl-Ab VI (25 mg/kg/day, 2 days a week) (M+S). After 9 weeks of treatment, femoral BMD was analyzed by DXA, microarchitecture of the femoral trabecular and cortical bone was evaluated by micro-CT, and mechanical strength of the femur midshaft was determined by 3 point bending test. In addition, the percentage of apoptotic osteocytes in the cortical bone of the tibia was assessed by immunostaining of cleaved caspase-3.

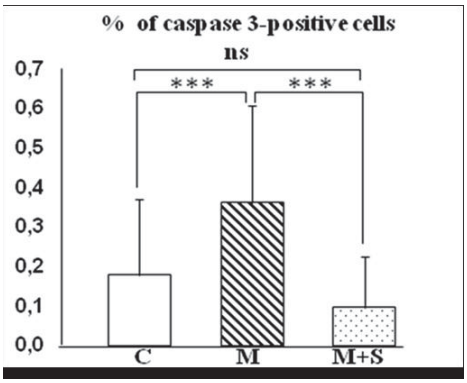
Results: Methylprednisolone treatment induced a significant decrease in femoral BMD, Bone Volume/Tissue Volume, trabecular thickness, Trabecular number, and cortical thickness (all

$p<0.05$ vs C). Scl-Ab VI injections restored these parameters to levels higher than those observed in the control group (%BV/TV: + 180% M+S vs C; + 264% M+S vs M), with increased cortical thickness and decreased endocortical circumference.

Osteocyte apoptosis was increased by glucocorticoid treatment (+100% M vs C group) and was restored to a level statistically similar to controls, after Scl-Ab VI treatment (Figure). Moreover, the fractional number of apoptotic osteocytes was inversely correlated to femoral BMD ($r= -0.40$; $p< 0.04$) across all groups. Bone strength was significantly increased by Scl-Ab VI treatment at the femur midshaft versus glucocorticoid treatment (+16% in maximum load; $p<0.05$).

Conclusion: These data have shown for the first time that Scl-Ab VI limits osteocyte apoptosis induced by glucocorticoids, the magnitude of which is negatively correlated with BMD. Moreover, Scl-Ab VI prevented glucocorticoid-induced changes in trabecular and cortical bone microarchitecture and bone strength.

Percentage of caspase-3 positive cells. Means ± SD. ***: $p<0.006$; ns: non significant



***Disclosures: Geal Rochefort**, None.*
This study received funding from: Amgen

P12

Occurrence of Previous Depression in Patients with Femoral Fracture in Pre-Operative Phase.

Fabiana Fonseca*, Ana Elisa Sena Klein Rosa, Segantin Bianca Isis, Priscila Primo Cáo, Thaise Arruda. Pontifical Catholic University of São Paulo, Brazil

The relationship of the body in old age is very particular to each be. the objective of this study was to highlight the prevalence of depression in elderly 40 (thirty-one females and nine males) with fracture of femur in indication of surgical treatment in pre-operative stage, diagnosed with depression pregresso serviced by the Hospital's Orthopedic Service of the São Paulo State Public Server. This is an observational study of cross-section. Data collection occurred with visit to patient records files and interviews structured way during the period October 2010 to February 2011. All statistical analyses were made by software Statistical Package for the Social Sciences for Windows, version 15.0. The survey was submitted to the Committee of ethics in research of Hospital Sao Paulo State civil servant and approved in April 2010. The age varied from 62 to 97 years, averaging equal to 78.9 years, dp of 9.5 years and median of 79 years, with the intention of crossing factors that concern the process of human aging, we got six seniors (15%) with a diagnosis of depression. For Gerontology, old age should be understood under different looks. With this study, we were able to note that a policy of Public Health is required to develop actions that are intended to identify situations that may be harmful to the elderly in relation to the increased risk of falls.

***Disclosures: Fabiana Fonseca**, None.*

P13

Identification of Trabecular Excrescences in Aging Bone.

Adam Taylor*[1], Chris Platt[2], Jonathan Jarvis[2], Lakshminarayan Ranganath[2], James Gallagher[2], Alan Boyde[3]. [1]Lancaster University, United Kingdom, [2]University of Liverpool, United Kingdom, [3]Barts & The London School of Medicine & Dentistry

Purpose: We have previously described the non-coupled formation of bone in trabecular excrescences, recognized firstly in bone samples from patients with osteoarthopathy of alkaptonuria and subsequently in patients with osteoarthritis (Taylor, 2012). Three types of these structures have been identified, all of which are laid down by osteoblasts without prior preparation by osteoclasts. We aimed to determine if these novel microanatomical structures were also present in normal aged human bone samples.

Methods: Three cadaveric knees (mean = 88yrs) lacking macroscopic OA were obtained with ethical approval. Distal femur and proximal tibia were sectioned in the coronal plane. Decalcified wax embedded sections were used for analysis by routine histology (brightfield, fluorescence and polarized) and slabs for scanning electron microscopy - macerated for topographic and PMMA embedded polished block faces for compositional backscattered electron SEM imaging.

Results: All three types of previously described excrescences were found in all three samples analyzed. These structures appeared to show poor integration with the existing trabeculae. Many of the identified structures demonstrated little morphology typically of osteoclastic or osteoblastic action. Pre-existing trabeculae and the centers of excrescences showed normal levels of fluorescence but the periphery and the surfaces of contact between the excrescences and the prior trabeculae showed low levels of fluorescence. Examination by polarized light revealed normal lamellar structure throughout the existing trabeculae. This contrasted with a lack of lamellar structure seen in the excrescences. A small number of excrescences demonstrated a poorly developed lamellar structure at the centre of the excrescences but no evidence of lamellar structure at the interface with the prior trabeculae.

Conclusions: We have demonstrated that trabecular excrescences are not just present in overtly pathological bone but also in apparently normal aged human bone samples. These structures arise without previous osteoclastic action, supporting the theory that coupled remodeling is not the sole mechanism of bone internal restructuring. Ref: Taylor et al, 2012, European Cells & Materials

***Disclosures: Adam Taylor**, None.*

P14

CT-assisted Balloon Sacroplasty for the Treatment of Insufficiency Fractures Considering Individual Approaches Adapted to the Course of the Fracture Type Denis I, II and III.

Reimer Andresen*[1], Sebastian Radmer[2], Peter Kamusella[3], Christian Wissgott[3], Jan Banzer[4], Hans-Christof Schober[5]. [1]Westküstenklinikum Heide, Germany, [2]Center of Orthopedics, University Hospital, Berlin, Germany, [3]Institute of Diagnostic & Interventional Radiology/Neuroradiology, Westküstenklinikum Heide, Germany, [4]Charité Universitätsmedizin Berlin, Germany, [5]Klinikum Südstadt RostockKlinik Für Innere Medizin I, Germany

Introduction: In elderly patients with reduced bone quality, insufficiency fractures of the sacrum are relatively common and are typically associated with intense, debilitating pain. The objective of our study was to determine the practicability of cement augmentation using a balloon catheter via individual approaches taking into consideration the complex anatomy of the sacrum and the course

of the fracture, as well as the postinterventional determination of leakages and representation of the outcome pain.

Material and Methods: In 30 patients with severe osteoporosis (23 women with an average age of 72.4 years, 7 men with an average age of 68.7 years), a sacral fracture was detected by CT and MRT. This fracture was unilateral in 17 women and bilateral in the other patients. In order to achieve a cement distribution longitudinally in relation to the fracture, the balloon catheter was inserted into the sacrum via a hollow needle either from caudal to cranial, from dorsal to ventral or from lateral transiliac to medial. The balloon catheter was then inflated and deflated 1-3 times along the fracture in the respective direction, and the hollow space created was then filled with PMMA cement using a low-pressure procedure. A conventional radiograph in two planes and a control CT were then performed. Pain intensity was determined pre-intervention, on the 2nd day post-intervention and 6 and 12 months post-intervention, using a visual analogue scale (VAS).

Results: The balloon sacroplasty was performed successfully from a technical point of view in all patients. The radiographic and CT control showed sufficient cement distribution in the sacrum along the course of the fracture, whereby leakage could be ruled out. According to the VAS, the mean value for pain was 8.8 pre-intervention, there was a significant reduction in pain on the 2nd postoperative day, with an average value of 2.7 ($p<0.001$), which was stable at 2.5 after 6 months and 2.3 after 12 months.

Discussion: Approaches that take into account the anatomy of the sacrum and the course of the sacral fracture enable reliable augmentation with an optimum amount of cement. This makes balloon sacroplasty an effective treatment that has few complications for rapid and significant pain reduction in patients with a sacral fracture.

***Disclosures: Reimer Andresen**, None.*

P15

Cortical Porosity in Humans with Type 1 Diabetes Mellitus and Fractures: A Preliminary Study.

Laura Armas*[1], Mohammed Akhter[2], Robert Recker[2]. [1]Creighton University, USA, [2]Creighton University Osteoporosis Research Center, USA

Patients with Type 1 diabetes mellitus (T1DM) have a 7-12 times greater fracture risk than healthy adults. While lower BMD accounts for a small percentage of this increased risk, other factors related to bone quality also increase fracture risk. We report here preliminary results of 5 patients with T1DM (3 females, 2 males, ages 21-46), who have sustained a fragility fracture while diabetic, and 2 healthy controls (1M, 1 F, ages 37, 45 yr). The subjects were healthy without diabetic complications and the females were premenopausal. A transilial bone biopsy was obtained after tetracycline labeling. The biopsy specimens were fixed, embedded and the intact specimens were scanned (16 micron resolution) using compact cone-beam type tomography in a desktop micro-CT (micro-CT-40, Scanco Medical AG, Bassersdorf, Switzerland). The measured BV/TV (bone volume to total volume ratio) variable from the cortex of each bone biopsy was used to calculate the cortical porosity (1-BV/TV) %. Calculated cortical porosity was 19% greater in the fracturing diabetic group compared to the control group (Table). While cortical BV/TV parallels the cortical porosity, it also agrees with the trabecular BV/TV (Table) which was 13% lower in the fracturing diabetics..

Table

(Mean ± SD)	Trab-BV/TV (%)	Cort-BV/TV (%)	Cort-Porosity (%)
Control	20.9 ± 0.1 *	80.4 ± 5.0	19.5 ± 5.0
Fracture T1DM	18.3 ± 0.1 *	76.8 ± 4.5	23.2 ± 4.5
Trab- Trabecular bone; Cort- Cortical bone; *Armas et al. Bone, 2012			

***Disclosures: Laura Armas**, None.*
This study received funding from: NIH - grant 1K23AR055542 - 01A1

P16

Caloric Restriction Attenuates Bone Loss during Hypothalamic Suppression.

Kathryn Mitchell, Megan Lunny, Vanessa Yingling*. Temple University, USA

Low energy availability and hypothalamic amenorrhea are both risk factors for developing insufficient bone mineral density in an estimated 22-50% of young physically active women. However, caloric restriction has also been associated with increased life span and increased mitochondrial biogenesis. We set out to determine the effect of both calorie restriction and hypothalamic suppression on bone strength and geometry. 30 female Sprague-Dawley rats, age day 23, were randomly assigned to a control (C, n=8) group that received daily saline injections (.2cc), or two experimental groups; delayed puberty (GnRH-a, n=14) or food restricted and delayed puberty (FR-G, n=8). Delayed puberty was achieved through daily injections of gonadotropin releasing hormone antagonist (GnRH-a, .2cc, dosage .2mg*kg^{-1}) and the food restriction was a 30% caloric restriction (no micronutrient deficit) based on the C group's average daily consumption. All protocols lasted 27 days and animals were sacrifice at 50 days of age. Body weight at sacrifice for the FR-G was significantly lower than C (15%, $p<0.001$) while GnRH-a was higher than C (8%, $p=0.013$). The GnRH-a treatment successfully suppressed hypothalamic function as evidenced by lower uterine and ovary weights in both the FR-G and GnRH-a groups ($p<0.001$). FR-G animals lost primarily body fat indicated by the higher percent muscle per fat compared to control (64%, $p=0.038$). Bone strength was similar between groups, however when normalized by body weight the FR-G and GnRH-a groups had higher peak moments than control (19%, $p=0.004$ and 20%, $p<0.001$ respectively). Micro CT analysis of the femur indicate a lower polar moment of inertia in FR-G compared to control and GnRH-a, however there was an increase in average cortical thickness in the FR-G group. The BV/TV of the distal femur was lower in both FR-G and GnRH-a compared to control. When normalized by body weight, FR-G had significantly higher BV/TV compared to GnRH-a. Caloric restriction combined with hypothalamic suppression increased femoral strength relative to body weight compared to control, potentially due to an increased cortical thickness. Caloric restriction seemed to provide a protective effect on trabecular bone from the significant decrease in bone volume resulting from the GnRH-a injections. The maintenance of lean body mass, slower growth rate or increased mitochondrial efficiency associated with caloric restriction may benefit bone strength and structure.

***Disclosures: Vanessa Yingling**, None.*

P17

Evaluation of 42 Cases of Subtrochanteric Fractures using the ASBMR Taskforce Criteria for Atypical Femoral Fractures.

Angela Juby*, Sean Crowther. University of Alberta, Canada

Purpose: The purpose of this study was to evaluate the radiographs of cases of subtrochanteric fracture identified in a database search, and see whether they fulfilled the ASBMR taskforce criteria for atypical femoral fracture.

Methods: Data was obtained from a retrospective chart review of all cases with an ICD 10 code for subtrochanteric fracture or unspecified hip or femur fracture, referred to two tertiary care hospitals over a seven year period (2002-2009), in Edmonton, Alberta, Canada. 50 cases of isolated subtrochanteric fracture or femoral shaft fracture were identified after chart review, out of 232 probable cases suggested by coding alone. A radiologist independently reviewed all 50 cases using the ASBMR Taskforce criteria to assess for atypical femoral fracture. Ethics approval was obtained from the regional ethics review board.

Results: Radiographic films were available for review in 42 cases. In 8 cases (4 each from 2002 and 2003) films were not available. Of those 42, 19 fulfilled the criteria for atypical fracture, that is, they all had the five major features: location; appropriate history; transverse or short oblique; non-comminuted; and a possible medial spike. 7 of these cases also had radiological minor features (cortical thickening, delayed healing or bilaterality). Clinical data review identified a further 11 cases with positive minor features, bringing 18/19 cases having all five major and some minor features. The clinical minor features included prodromal symptoms (5 cases), comorbidities (4), bisphosphonate use (13), glucocorticoid use (4), anticonvulsant use(1). Of interest, the 5 previously reported cases based on general X ray reports and prodromal symptoms, 3 did not meet the new criteria for atypical femoral fracture.

Conclusions: Atypical femoral fractures are rare fractures. Over a seven year period, from two major referral hospitals servicing Northern Alberta (a catchment area of approximately 2 million people), only 50 possible cases were identified from general radiographic reports. However, only 19/42 (45%) of these were true atypical femoral fractures based on the ASBMR criteria. During the study period, approximately 21 000 typical osteoporotic hip fractures would have occurred, highlighting the much greater risk of typical versus atypical fractures in osteoporotic patients.

***Disclosures: Angela Juby**, None.*

P18

Vitamin D status and knee pain severity in functionally intact older adults: The Health ABC Study.

Laura Tosi*[1], Robert Boudreau[2], Kent Kwoh[3], Tanushree Prasad[3], Hilsa Ayonayon[4], Tamara Harris[5], Denise Houston[6], Stephen Kritchevsky[6], Kushang Patel[7], Eleanor Simonsick[5], Jane Cauley[8]. [1]Children's National Medical Center, USA, [2]University of Pittsburgh - Dept of Epidemiology, [3]University of Pittsburgh, [4]University of California - San Francisco, [5]National Institute of Aging, [6]Wake Forest University, [7]National Institutes of Health, [8]University of Pittsburgh Graduate School of Public Health, USA

Background: Clinical evidence suggests that 25-hydroxyvitamin D (25(OH)D) may be important in multiple biologic processes including pain reporting, yet there exists no convincing evidence that levels of 25(OH)D are lower in individuals with chronic pain vs those pain free. The study examines the association between 25(OH)D status and reported joint pain severity in functionally intact persons aged 70-79.

Methods: Of 3,075 participants in the Health, Aging and Body Composition study, 2,793 (mean age 74.7 $\pm$ 2.9 yrs, 51.2% women, 39.7% black) had serum 25(OH)D measures at year 2 and concurrent responses on the number of painful joints and the Modified WOMAC scale for knee pain. Quartiles of 25(OH)D were calculated based on the combined sample. Number of painful joints (0-20), joint groups (0-8: hand/wrist, hip, knee, foot/toe; left+right) and WOMAC knee pain were compared across quartiles using ANOVA and ANCOVA to adjust for covariates. The worst knee was used in the WOMAC analyses.

Results: The 25(OH)D quartile cutoffs were 18.0 ng/ml, 24.7 ng/ml and 32.3 ng/ml (min=5.0, max=186.9). The most frequently reported painful joint groups were hand/wrists (50.2%) and knees (36.2%). For both sexes, higher physical activity and dietary Vitamin-D intake were associated with higher 25(OH)D. In men, 25(OH)D status was not associated with any of the three pain measures in unadjusted or adjusted models ($p > 0.261$). For women, the highest quartile of 25(OH)D was associated with lower numbers of painful joint groups adjusted for age, race, field site, and season of serum draw (p=0.030), but became no longer significant (p=0.347) when further adjusted for education level, BMI and depressive symptoms. For WOMAC knee pain, the highest 25(OH)D quartile was strongly associated with lower knee pain in unadjusted ($p<0.001$) and all adjusted models ($p<0.041$).The lower three quartiles had similar WOMAC knee pain (p=0.980), with adjusted WOMAC knee pain of 4.05 for the lower quartiles combined vs 3.19 for the highest quartile (p=0.004). Dietary vitamin D attenuated this difference by 8.1%.

Conclusions: The association of 25(OH)D status with joint pain reporting is sexually dimorphic and joint specific. 25(OH)D status does not predict joint pain in men. In women, however, 25(OH)D status and WOMAC knee pain are strongly associated across all adjusted models. Clinical trials using 25(OH)D therapy to prevent/treat knee pain in women should be considered.

Tables 1 and 2

Table 1: Mean WOMAC across 25-hydroxyvitamin D status and sex

	25-hydroxyvitamin D					
	Q1	Q2	Q3	Q4	Overall F p-value	p-trend
Men	(n = 271)	(n = 369)	(n = 371)	(n = 353)		
Unadjusted	1.90	2.11	1.91	2.17	0.7393	0.5658
Model 1	1.85	2.13	1.94	2.16	0.7250	0.5300
Model 2a	2.86	3.20	3.07	3.43	0.3625	0.1544
Model 3a	2.75	3.15	2.91	3.35	0.2604	0.1748
Women	(n = 427)	(n = 329)	(n = 328)	(n = 345)		
Unadjusted	3.77	3.49	3.17	2.10	< 0.0001‡	< 0.0001†
Model 1	3.58	3.51	3.25	2.20	0.0007‡	0.0003†
Model 2b	4.03	4.09	4.03	3.19	0.0406‡	0.0324†
Model 3b	4.03	4.10	4.01	3.25	0.0843	0.0564

* 25-hydroxyvitamin D quartiles: Q1 (< 18.02 ng/ml), Q2 (≥ 18.02 - < 24.71 ng/ml), Q3 (≥ 24.71 - < 32.3 ng/ml), Q4 (≥ 32.30 ng/ml)

Model 1: adjusted for age, race, field site, season of HABC visit

Men:

Model 2a: adjusted for age, race, field site, season of HABC visit, education level, BMI, depressive symptoms

Model 3a: Model 2a + vitamin-D containing supplement use, vitamin D intake

Women:

Model 2b: adjusted for age, race, field site, season of HABC visit, BMI, depressive symptoms

Model 3b: Model 2b + vitamin-D containing supplement use, vitamin D intake

‡ p-value for overall F-test < 0.05

† p-value for trend < 0.05

Table 2: Mean WOMAC across 25-hydroxyvitamin D status – WOMEN only

Women	Q123 (n = 1084)	Q4 (n = 345)	p-value
Unadjusted	3.50	2.10	< 0.0001†
Model 1	3.45	2.22	< 0.0001†
Model 2	4.05	3.19	0.0041†
Model 3	4.04	3.25	0.0103†

* 25-hydroxyvitamin D quartiles: Q1 (< 18.02 ng/ml), Q2 (≥ 18.02 - < 24.71 ng/ml), Q3 (≥ 24.71 - < 32.3 ng/ml), Q4 (≥ 32.30 ng/ml)

Model 1: adjusted for age, race, field site, season of HABC visit

Model 2: adjusted for age, race, field site, season of HABC visit, BMI, depressive symptoms

Model 3: Model 2b + vitamin-D containing supplement use and vitamin D intake

† p-value < 0.05

***Disclosures: Laura Tosi**, None.*

P19

Muscle Assessment by HRpQCT: A Preliminary Assessment of its Potential Utility.

Marta Erlandson*[1], Andy Kin On Wong[2], Eva Szabo[3], Martin Zulliger[4], Aakash Bhargava[2], Karen Beattie[2], Jonathan Adachi[5], Angela Cheung[3]. [1]University of Toronto, Canada, [2]McMaster University, Canada, [3]University Health Network, Canada, [4]Scanco Medical AG, [5]St. Joseph's Hospital, Canada

Purpose: To determine how well HRpQCT muscle measures obtained at the distal tibia using an experimental algorithm compare to standard pQCT measures at the commonly reported 66% calf site.

Methods: Women $\geqslant$50 years from the Hamilton cohort of the Canadian Multicenter Osteoporosis Study (CaMos) completed a single slice (2.3$\pm$0.3mm thick) pQCT scan (XCT 2000, Stratec) at the 66% site of the tibia as measured from the distal malleolus to the medial tibial plateau. Total bone area was segmented using contour mode 1 and peel mode 2 with a threshold of 280 mg/cm^3 and 400 mg/cm^3. Limb area was analyzed using contour mode 3 and peel mode 2 with a threshold of 40 mg/ cm^3. A filter was applied to smooth the segmented limb area. pQCT-derived muscle cross-sectional area (MCSA) was calculated by subtracting total bone area from limb area. Muscle density (MD) was computed by taking the quotient of the corresponding muscle mass and MCSA. Standard HRpQCT scans (Xtreme CT, Scanco) were also acquired at the distal tibia and bone was segmented from the images using standard contouring. HRpQCT-derived MD and MCSA were then calculated using a newly developed algorithm in which tight thresholding limits (34.22-194.32 mg HA/cm^3) were used to identify muscle seed volumes which were then iteratively expanded. A short band of grayscale values between the fat and muscle was left open as undetermined into which the seed volumes expand to encompass the entire muscle tissue volume. Bland-Altman plots were used to assess the agreement between pQCT and HRpQCT MD and MCSA. Pearson correlation coefficients were calculated to assess the relationship between pQCT and HRpQCT measurements.

Results: 45 women had a pQCT-derived mean MD at the mid-calf (69.9$\pm$4.9 mg/cm^3) that was significantly lower than the HRpQCT-derived MD at the more distal site (71.2$\pm$4.0 mgHA/cm^3) (p=0.025). The Bland-Altman analyses revealed no evidence of directional bias for MD; however, MCSA varied according to magnitude such that a larger MCSA increased the discrepancy between the two modalities. MD was moderately correlated between the two modalities (r=0.64, p<0.01); there was no relationship between MCSA measured by pQCT at the mid-calf and the MCSA measured at the distal site (r=0.24, p=0.12).

Conclusions:These preliminarily findings suggest that lower leg MD obtained from HRpQCT images may provide 41% of the information obtained from pQCT. Further testing and optimization of the HRpQCT algorithm may improve these correlations.

***Disclosures: Marta Erlandson**, None.*

P20

Is There an Increased Risk of Hip Fracture in Multiple Sclerosis (MS)? Analysis of the Nationwide Inpatient Sample (NIS).

Rajib Bhattacharya*[1], Richard Dubinsky[2]. [1]KU Medical Center, USA, [2]University of Kansas, USA

Objective: To determine if people with MS have a higher rate of hip fractures and to explore the discharge disposition of MS patients with acute hip fracture.

Background: Impaired ambulation, frequent falls, and prolonged immobilization combined with the high rate of vitamin D deficiency in people with MS could lead to an increased risk of hip fracture.

Methods: Retrospective cohort analysis of 20 years of the NIS (HCUP, AGRQ.gov), a 20% stratified sample of US hospital admissions. Admissions with a primary diagnosis of acute hip fracture were identified, as was the subset with a secondary diagnosis of MS. Indirect adjustment was used to compare the prevalence of MS in this population, to that of the US (Noonan, Neurology, 2002). Because of the large number of records and multiple comparisons, p was set a priori at $<$ .0001.

Results: 0.25% of 1,063,726 hip fracture admissions were for MS. Over the 20 years of this dataset the proportion with MS increased from 0.21% to 0.31%. There was a trend for lesser mortality for MS (0.25 vs. 2.97%, p $<$ .0001) yet discharge to nursing home or rehabilitation was less for MS (69.25% vs. 72.17%, p $<$ .0001). When compared to the population prevalence the prevalence of MS among the patients with hip fracture was 2.844 (95% confidence interval; 2.841, 2.852) predicted when age adjusted and 2.505 (2.499, 2.512) when adjusted for gender and age. When adjusted for race (white, black) the prevalence was 2.175 (2.168,2.182). Race was specified for 65% of the sample.

Conclusions: In this nationwide sample of 20 years of US hospital admissions the rate of hip fracture for people with MS was more than twice predicted. While the risk of falls can be partially mitigated through physical therapy and fractures with vitamin D, bisphosphonates are less effective pre-menopause.

***Disclosures: Rajib Bhattacharya**, None.*

P21

Effects of Age and Vitamin D on Parathyroid Hormone Levels.

Frank Blocki*[1], Sudhaker D. Rao[2], Andre Valcour[3]. [1]DiaSorin Incorporated, USA, [2]Bone & Mineral Research Laboratory, Henry Ford Hospital, USA, [3]Center for Esoteric Testing, LabCorp, USA

Osteoporosis & primary hyperparathyroidism (PHPT) are two common bone & mineral disorders whose clinical expression is affected by prevailing 25OHD & calcium status. Numerous studies suggest that serum 25OHD levels supporting optimal skeletal health be defined as those where PTH declines to a minimum: results, however, are widely disparate with sufficient levels posited anywhere from 12 to 44ng/mL. Limitations common to these studies were small sample sizes ($<$100 to ~30,000). Here, nearly 313,000 subjects were tested for 25OHD & PTH from July 2010 to June 2011. Median PTH values & % patients exceeding the clinical PTH threshold of 65pg/mL from groups of 6259 patients each plotted against 25OHD provided smooth, exceptionally well-fitted curves (Mean PTH=11.9+140.6(25OHD)$^{-0.46}$; R^2=0.994 & (%High=57.2 +166.7(25OHD)$^{-0.21}$; R^2=0.995) & evidenced no inflection points or horizontal asymptotes. Parsed across four levels ($<$20, 20-40, 40-60, $>$60 years), the same groups disclose a striking dependency upon age. Data presented here provides cause for a closer contemplation of the Institute of Medicine's recent guidance of 20ng/mL as that level assuring 25OHD sufficiency for the majority of Americans: 85,000 (27%) subjects fell below this conservative threshold. Of greater clinical relevance, 40% (27,950/70,000) & 51% (7,650/15,000) of subjects with 25OHD $<$20 & 10ng/mL, respectively, had biochemical hyperparathyroidism with PTH levels $>$65pg/mL. Despite significant limitations, several important clinical observations are germane. 1st, median PTH levels of a very large sample set plotted across the continuum of patient 25OHD levels reveals NO threshold above which increasing 25OHD fails to further suppress PTH. 2nd is the sheer magnitude of subjects $>$60 years old with frank deficiency whose PTH levels were above the reference range UL. This preponderance of abnormal results reinforces the 3rd International Workshop on Asymptomatic PHPT's call for 25OHD repletion (to levels minimally above 20ng/mL) of suspected PHPT

subjects before consideration of elective surgery. Finally, the strong age dependency of PTH levels with 25OHD likely reflects the composite of depleted calcium stores, calcium malabsorption &/or renal malfunction. This population based study may well move the clinical community away from fixed values of 20 vs 30 vs 40ng/mL as defining vitamin D sufficiency toward patient management based upon an age dependent, PTH-25OHD continuum.

***Disclosures: Frank Blocki**, None.*

P22

The Influence of Mechanical Stress to the Osteoporotic Pain-related Property in Osteoporotic Rats with Compressed Caudal Vertebrae.

Miyako Suzuki*[1], Gen Inoue[2], Seiji Ohtori[2], Sumihisa Orita[2], Masayuki Miyagi[2], Tetsuhiro Ishikawa[2], Hiroto Kamoda[2], Yoshihiro Sakuma[2], Yasuhiro Oikawa[2], Go Kubota[2], Kazuyo Yamauchi[2], Kazuhisa Takahashi[2]. [1]Chiba University, Japan, [2]Department of Orthopaedic Surgery, Graduate School of Medicine, Chiba University

Introduction: We have previously reported that the expression of calcitonin gene-related peptide (CGRP), a marker of inflammatory pain, is increased in dorsal root ganglia (DRG) neurons innervating lumbar vertebrae of osteoporotic rats. In addition, we have sought to determine whether osteoporotic pain is simply derived from inflammatory pain or includes any other pain-related factors. We focused on the possibility that osteoporotic pain is exacerbated by mechanical stress on the vertebral bodies. The purpose of this study was to examine the effect of mechanical stress on osteoporotic pain using a caudal vertebrae compression model in rats.

Materials and Methods: As an osteoporosis model, we used female rats ovariectomized (OVX) at 5 weeks (n = 24). Fluoro-Gold (FG), retrograde neurotracer, was applied on the periosteal surface of a caudal vertebra to detect DRG neuronal cells innervating the caudal vertebra. After the FG-labeling, they were divided into two groups: OVX+CMP group (n=12) with longitudinally-compressed caudal vertebra using wires and rubber bands and OVX group (n =12) without compression. One, 2, 4 and 8 weeks after the FG-labeling, the vertebra applied FG and bilateral S1 to S3 DRGs were resected. In the caudal vertebra and FG-labeled DRG neurons, expression of CGRP and activating transcription factor 3 (ATF3), a marker for neuronal injury, were compared between the two groups using immunohistochemistry.

Results: In the vertebrae, ATF3 immunoreactive(-ir) nerve fibers were observed only in the OVX+CMP group at 8 weeks. In DRGs, the proportions of FG-labeled CGRP-ir DRG neurons in the OVX+CMP group were significantly elevated at 4 and 8 weeks compared with the OVX group ($p < 0.05$). The proportion of FG-labeled ATF3-ir DRG neurons was also significantly increased at 8 weeks ($p < 0.05$).

Discussion: More ATF3 immunoreactivity was observed in both DRG neurons and the caudal vertebra in the 8-weeks OVX+CMP group besides the increased CGRP expression. The result of this study implies that some factors of nerve injury might be involved in sensory neurons innervating caudal osteoporotic vertebra, which suggests that the osteoporotic pain might include an element of neuropathic pain in addition to one of inflammatory pain. Our results demonstrated that the compression stress onto the vertebrae increased the biomarker for neuronal injury. These findings imply that mechanical compression stress onto vertebrae may exacerbate osteoporotic pain.

***Disclosures: Miyako Suzuki**, None.*

P23

Dual Energy X-ray Absorptiometry Body Composition: A New Phantom for Clinical Trials.

Colin Miller[1], Blaine Horvath[2], Hui Jing Yu[2], Stuart Jackson[3], Neil Binkley*[4]. [1]BioClinica, Inc., USA, [2]BioClinica, [3]University of Alberta, [4]University of Wisconsin, Madison, USA

Background: Dual energy X-ray absorptiometry (DXA) instruments are established as a method for body composition measurement because of their ease of use and low radiation dose. However, longitudinal variation is observed in DXA measurements and a phantom is needed to monitor and calibrate such drift. Phantoms are available to monitor bone mineral density. However, there has been no phantom available for the monitoring of body composition in clinical trials. A BioClinica Body Composition (BBC) phantom was developed for quality control and monitoring machine drift within major DXA manufacturers. The unique phantom design measures 36 cm in width and 61 cm in length and weighs 29 kg. The phantom contains high-density polyethylene, polyvinyl chloride and aluminum plates for simulation of bone. The goal of this study is to evaluate the precision of this new BBC Phantom.

Methods: Part of the development process, a beta version of the phantom was evaluated by GE Lunar and Hologic. To examine the stability of this new phantom, a total of 10 BBCs were scanned on a GE Lunar Prodigy: each was scanned 5 times on separate days. To compare measurement variability between two manufacturers, 1 of these 10 BBCs was also scanned 5 times on a Hologic Discovery A. Multiple additional scans will be performed on a GE Lunar Prodigy and GE Lunar iDXA densitometer.

Results: Precision of 5 consecutive phantom scans, expressed as the coefficient of variation, for all 10 phantoms ranged from 0.14% to 0.31% for total body BMC, 0.30% to 0.92% for total body fat, 0.13% to 0.59% for total body lean tissue, and 0.31% to 0.93% for total percent fat. For a given phantom, the average (SD) values for total body BMC, total body fat, total body lean and total percent fat were estimated as 610g (3), 7000g (22), 10619g (44) and 38% (0.15) from Hologic scans, and as 616g (1), 6263g (35), 10913g (20), and 36% (0.13) from GE Lunar scans. All preliminary data are summarized in Table 1.

Conclusion: Data from total body DXA scans of this new phantom showed high precision for lean, fat and bone mineral compartments. This semi-portable phantom may prove to be appropriate in clinical trials for the evaluation of body composition.

Table 1

Table 1. Phantom Body Composition Analysis Summary (%CV)

Manufacturer	BBC #	WBTOT_BMC [g]	WBTOT_FAT [g]	WBTOT_LEAN [g]	WBTOT_PFAT [%]
GE (Lunar)	1	0.25%	0.39%	0.22%	0.40%
GE (Lunar)	2	0.22%	0.40%	0.31%	0.46%
GE (Lunar)	3	0.14%	0.84%	0.45%	0.85%
GE (Lunar)	4	0.31%	0.92%	0.52%	0.93%
GE (Lunar)	5	0.19%	0.30%	0.13%	0.31%
GE (Lunar)	6	0.29%	0.83%	0.59%	0.87%
GE (Lunar)	7	0.22%	0.69%	0.40%	0.66%
GE (Lunar)	8	0.14%	0.85%	0.48%	0.80%
GE (Lunar)	9	0.20%	0.66%	0.40%	0.74%
GE (Lunar)	10	0.21%	0.56%	0.18%	0.38%
Hologic	10	0.50%	0.31%	0.42%	0.39%

*WBTOT = Whole Body Total

***Disclosures: Neil Binkley**, None.*
This study received funding from: BioClinica

Bone and Muscle Interactions During Development

P24

Muscle LRP4 Regulates Osteoclastogenesis.

Ji-Ung Jung*, Hai-Tao Wu, Cheng-Yong Shen, Lin Mei, Wen-Cheng Xiong. Georgia Health Sciences University, USA

Low-density lipoprotein receptor-related protein 4 (LRP4), a member of the LDLR family expressed in various organs, regulates extracellular cell signaling pathways in animal development. Recent studies have demonstrated that mutations in Lrp4 gene cause bone dysplasia, an abnormal overgrowth of bone accompanying increased skeletal density. However, molecular mechanisms underlying LRP4 regulating osteogenesis remain poorly understood. In this study, we provide evidence for differential regulation of osteoclastogenesis by muscle- and osteoblast-derived LRP4. We have generated transgenic mouse lines expressing mouse tgLrp4-FL and tgLrp4ΔICD in muscle and crossed them onto the Lrp4-mitten mutant background to study LRP4' function in adult bone remodeling, as Lrp4 mitten mutant results in early embryonic lethality. Interestingly, LRP4ΔICD expression in muscle in both wild type and Lrp4-mitt background resulted in marked increase of both cortical and trabeculae bone volumes and decreased bone marrow cavity. These deficits appeared to be due in large the decreased osteoclastic bone resorption, as the number of osteoclasts is marked decreased, with little change, if there is any, of bone formation. Further mechanical studies showed that LRP4 is expressed in multiple types of cells, including cultured osteoblast-lineage cells and osteoclast precursors, and muscle cells. LRP4ΔICD expression in muscle or other cells (e.g., HEK293 cells) could be secreted as a soluble form of Lrp4, which attenuated RANKL-induced osteoclastogenesis. Taken together, these observations suggest that muscle-derived soluble Lrp4 appears to inhibit osteoclast differentiation, demonstrating an interaction between muscle and bone and revealing a potential cellular mechanism underlying Lrp4 regulating bone remodeling.

***Disclosures: Ji-Ung Jung**, None.*

P25

Regulation of Myogenic Differentiation in C2C12 Cells by Cyclooxygenase-1.

Chenglin Mo*, Orisa Igwe, Marco Brotto. University of Missouri-Kansas City, USA

In previous studies, we have demonstrated that PGE_2 signaling is essential for myogenic differentiation in C2C12 myoblasts. Treatment with 50nM PGE_2 considerably increased myotube area, whereas treatment with AH6809, an EP1 and EP2 inhibitor, significantly attenuated myotube development. PGE_2 is synthesized through cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) pathways, but their roles in myogenesis have not been defined. In this study, we first determined the expression of COX-1 and COX-2 by Real-Time(RT) PCR and western blot (WB) in C2C12 myoblasts. Both PCR and WB results indicated that COX-1 had a higher expression than COX-2. Next, we followed the mRNA expression of COX-1 and COX-2 during myogenic differentiation at 0, 2, 6, 12, 24, 48h. COX-1 expression was upregulated by 2-fold at 6 and 12h, and returned to baseline levels (time 0) at 24h, followed by a 2-fold downregulation at 48h. On the other hand, COX-2 expression was downregulated over this period, reaching a 5-fold downregulation at 48h. The WB results showed similar trends to those observed with RT-PCR; COX-1, but not COX-2, which was upregulated at 12h, and returned to baseline levels at 48h. Furthermore, the effects of specific COX-1 inhibitor (FR122047), specific COX-2 inhibitor (NS-398), and non-specific COX-1/2 inhibitor (Indomethacin) on myogenic differentiation were tested. FR122047 (7.5 μM) and indomethacin (100 μM) significantly decreased myotube area after 96h of differentiation (Myotube area was 45% and 53% of control, respectively). However, NS-398 at up to 50 μM did not exert a similar inhibitory effect (myotube area did not show any change compared with control). Our results suggest that COX-1 may play a more important role in myogenic differentiation than COX-2.

Acknowledgements: This work was supported by NIDCR DE021888(OJI), NIH RC2 AR058962, Missouri Life Sciences Research Board(MB).

***Disclosures: Chenglin Mo**, None.*

P26

Characterisation of Musculoskeletal Phenotype in Pre-pubertal Gambian Children.

Kate Ward*[1], Landing Jarjou[2], Gail Goldberg[3], Yankuba Sawo[2], Ann Prentice[1]. [1]MRC Human Nutrition Research, United Kingdom, [2]MRC Keneba, Gambia, [3]MRC Human Nutrition Research; MRC Keneba The Gambia, United Kingdom

Gender differences in muscle and bone during development are well described in countries with moderate to high calcium intakes; there are few data from countries where children have delayed puberty and low habitual calcium intakes. This study aimed to determine whether gender differences exist in muscle and bone in pre-pubertal Gambian children and whether muscle area predicts bone phenotype.

Children aged 7.8-12.0y were recruited. Measurements were made using peripheral QCT. Volumetric total(To) and trabecular(Tb) BMD and total area were measured at 8% radius (DR) and tibia(DT). Total and medullary area, cortical content(BMC), stress-strain index (SSI) and muscle area were measured at 66% radius(R66) and 50% tibia(T50). Gender effects were tested using univariate and multiple regression adjusting for age, object length and muscle area. Data are presented as % differences *[95% CI]*.

447 children (216M) were recruited. Mean ± SD age M=9.3 ± 0.1y, F=9.2 ± 0.1y; height M=127 ± 6cm, F=128 ± 7cm; weight M=23.8 ± 0.2kg, F=24.0 ± 0.3kg. Unadjusted analyses showed M had larger bones and higher ToBMD and TbBMD at DR and DT. At R66 and T50 M had larger bones, greater muscle area and SSI, and higher BMC.

After adjustment gender differences at DR and DT remained (% value, M compared to F): ToBMD (DR 2.8*[-0.2, 5.7]*, DT 2.8*[0.7, 5.0]*), TbBMD (DR 8.4*[3.4,13.4]*, DT 4.4*[1.1,7.6]*), total area (DR 9.7*[6.6, 12.9]*, DT 4.9*[2.6,7.3]*) were higher in M. BMC was higher at T50 in M (2.3*[-0.2, 4.8]*. However after adjustment there were no significant gender differences in medullary area(R66 2.5*[-3.2,8.1]*, T50 -0.8*[-4.9,3.4]*), total area (R66 0.9*[-1.8,3.7]*, T50 1.0*[-1.2,3.1]*), SSI (R66 1.9*[-1.4,5.3]* or T50 1.7*[-1.1,4.6]*). In the multiple regression models, muscle area was a significant predictor for bone geometry, co-efficients (%) for muscle area: total area DR 9.7*[6.6,12.9]*, DT 4.9*[2.6,7.3]*, R66 54.0*[42.6,65.3]*, T50 36.3*[29.8,42.9]*; medullary area R66 69.6*[46.7,92.6]*, T50 37.8*[25.0,50.5]*; SSI R66 76.2*[62.6,89.9]* and T50 52.9*[44.1,61.7]*.

Gender differences in BMD and size exist at DR and DT. After adjustment for muscle area, a proxy for muscle force, gender differences in bone shape and strength disappeared indicating the importance of muscle in developing bone. Determining the contribution of muscle function and other factors to adolescent bone growth, and the timing and magnitude of peak bone strength is important in this population.

***Disclosures: Kate Ward**, None.*

P27

Fluorescent Tracking of Myogenic and Vascular Cells during Orthopaedic Repair.

Aaron Schindeler*, Justin Bobyn, Mille Kolind, David Little. The Children's Hospital at Westmead, Australia

Purpose: The origin of the cell types able to contribute to bone repair is controversial, with periosteal, marrow, soft tissue, and circulating progenitors all implicated to varying degrees. Using a genetically modified mouse model, we have previously shown that myogenic cells are able to contribute to open but not closed fracture repair (BMC Musculosk Disord 12:288, 2011). Previous work used a enzymatic hAP reporter, but we now employ a fluorescent GFP reporter. This system, which utilizes Cre-Lox genetics to permanently label all cells of a particular lineage, allowed us to dissect the cellular contributors to repair.

Methods: MyoD-cre Z/EG and Tie2-cre Z/EG mice were generated by breeding established Cre and reporter mouse lines. Open fractures were surgically made in the tibiae of double transgenic mice and samples harvested at 7d, 10d and 14d. Tissues were cryo-embedded and sectioned, and myogenic and vascular endothelial lineage cells (GFP+) were visualized by confocal microscopy. Cells were co-labelled by immunofluorescence to label current lineage expression markers for muscle (MyoD), cartilage (Sox9), bone (Runx2), and vascular endothelial cells (Tie2, CD31).

Results: GFP+ cells were present in the calluses of MyoD-cre Z/EG and Tie2-cre Z/EG mice, indicating both cell lineages could contribute to open fracture repair. It was observed that GFP+ Tie2 lineage cells partially contribute to the CD31+ and CD31- cell populations in the fracture callus. Thus vascular endothelial progenitors contribute to both vascular and non-vascular components of healing fractures, but other as yet unidentified cell lineages are also involved.

Conclusions: This system provides a unique method for tracking the contribution of different cell lineages to bone repair. Using a fluorescent conditional reporter with immunofluorescent labelling allows for current and historical lineage markers to be tracked in the same sample.

***Disclosures: Aaron Schindeler**, None.*

P28

Effect of Orchidectomy on the Musculoskeletal System of Myostatin Knock Out Mice.

Vanessa Dubois*[1], Michaël Laurent[2], Mieke Sinnesael[2], Steven Boonen[3], Dirk Vanderschueren[2], Frank Claessens[2]. [1]University of Leuven, Belgium, [2]Catholic University of Leuven, Belgium, [3]Center for Metabolic Bone Disease & Division of Geriatric Medicine, Belgium

Purpose: Androgens play crucial physiological roles in establishing and maintaining the male reproductive phenotype, but have also anabolic effects on several extragenital structures including muscle and bone. Myostatin (Mstn), a member of the transforming growth factor-beta (TGF-β) superfamily, is a strong negative regulator of skeletal muscle mass. In this study, we wanted to determine the interaction between androgens and Mstn in the growth and maintenance of male skeletal muscle, and to understand the relative contribution, if any, of Mstn in mediating androgen anabolic action in muscle. In addition, we examined whether bone parameters of Mstn-deficient mice correlate with muscle hypertrophy.

Methods: At the start of puberty (3 weeks of age), male Mstn KO (Mstn-/-) and WT littermates (Mstn+/+) were randomly divided into two groups and were either sham-operated or orchidectomized. Body weight was measured weekly. At 12 weeks of age, body composition was evaluated by whole-body dual-energy X-ray absorptiometry (DXA) and grip strength of the limbs was measured. Tibial bone parameters as well as muscle cross-sectional area were recorded by in vivo microcomputed tomography (μCT). Gastrocnemius, soleus, extensor digitorum longus, tibialis anterior, levator ani and bulbocavernosus muscles were dissected and weighed. Efficacy of orchidectomy was verified by measurement of seminal vesicle wet weight immediately after euthanasia.

Results: In the WT group, orchidectomy reduced body weight, lean body mass and grip strength. The mass of levator ani and bulbocavernosus was also decreased, while the limb muscles showed a tendency towards a lower mass. In addition, orchidectomy of WT mice resulted in a decrease in trabecular bone volume, trabecular number and cortical thickness. In Mstn KO mice, orchidectomy reduced the above-mentioned parameters to the same extend as in the WT group. Muscle cross-sectional area of Mstn KO mice was larger compared to that of WT mice, while no difference was observed in cortical cross-sectional area.

Conclusions: From these data, we can conclude that Mstn signaling is not essential in mediating androgen effects on muscle. In addition, our findings indicate that the increased muscle mass in Mstn-deficient mice does not induce additional cortical bone deposition, undermining the statement of muscle mass as a major determinant of bone morphology. These observations have to be confirmed with larger groups of animals.

***Disclosures: Vanessa Dubois**, None.*

P29

Connexin 43 Deficiency in Osteoblasts/Osteocytes Impairs Tendon and Muscle Formation in the Shoulder.

Hua Shen*[1], Susan Grimston[2], Roberto Civitelli[2], Stavros Thomopoulos[1]. [1]Washington University School of Medicine, USA, [2]Washington University in St. Louis School of Medicine, USA

Introduction: It has recently been suggested that bone acts as an endocrine organ that can influence muscle formation and function[1]. Connexin 43 (Cx43), a gap junction protein, has been implicated in osteoblast differentiation and mechano-chemical signaling in osteocytes[2-3]. The objective of this study was to use an osteoblast/osteocyte specific conditional knockout mouse model to investigate the role of Cx43 in postnatal shoulder development. We hypothesized that Cx43 deficiency in osteoblasts/osteocytes would impair the formation of the humeral head as well as the associated rotator cuff tendons and muscles.

Methods: Conditional knockout mice depleted in Cx43 in osteoblasts/osteocytes (ColCre;Cx43-/flox) and their WT equivalent (Cx43+/flox) were sacrificed at postnatal day 14, 28, and 56. Mouse shoulders were dissected to obtain the entire supraspinatus (SS) muscle and tendon attached to the humerus. The resulting specimens were scanned using micro computed tomography for SS muscle volume, SS tendon cross sectional area (CSA), humeral length, and indices for trabecular and cortical bone architecture. Paraffin sections of these specimens were then prepared and stained with H&E, Masson Trichrome, and Toluidine blue.

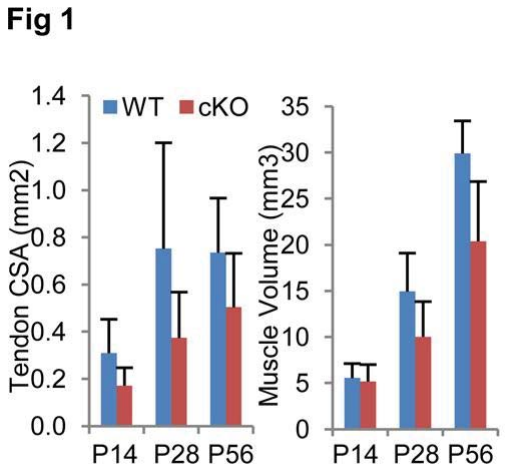
Results: Cx43 deficiency in osteoblasts/osteocytes significantly reduced cortical bone thickness, bone area/total area ratio, bone mineral density and increased total area and marrow area as described previously[4]. Cx43 deficiency also hindered humeral growth. Both humeral length and growth plate of cKO mice were significantly shorter than those of WT. In agreement with our hypothesis, loss of Cx43 in osteoblasts/osteocytes affected tendon and muscle development, leading to a smaller SS tendon and muscle (Fig1). No pathological changes were found histologically in SS muscle and tendon of Cx43 cKO mice.

Conclusion: Cx43 deficiency in osteoblasts/osteocytes alters cortical bone architecture and growth plate morphology in the postnatal humerus. Furthermore, Cx43 deficiency in osteoblasts/osteocytes affects the development of the adjacent tendon and muscle. We speculate that Cx43 deficiency in osteocytes might have impaired bone-to-muscle endocrine signaling and are currently investigating the possibility.

This study was funded by NIH AR055580.

1, Bonewald L. ORS Meeting 2012. 2, Civitelli R. Arch Biochem Biophys 2008;473:188-92. 3, Siller-Jackson AJ, et al. J Biol Chem 2008;283:26374-82. 4, Grimston SK, et al. J Bone Miner Res 2008;23:879-86.

Fig1, Changes in SS tendon and muscle of WT and Cx43 cKO mice during postnatal development



***Disclosures: Hua Shen**, None.*

P30

Low-Amplitude, High-Frequency Vibration and Musculoskeletal Health in the mdx Mouse Model of Duchenne Muscular Dystrophy.

Susan Novotny*[1], David Nuckley[1], Gordon Warren[2], Dawn Lowe[1]. [1]University of Minnesota, USA, [2]Georgia State University, USA

Low-amplitude, high-frequency vibration appears to be most efficacious in skeletons with low bone mass. Duchenne muscular dystrophy (DMD) causes such a condition. Our previous work showed that acute vibration at 45Hz and 0.6*g* best amplified genes associated with osteogenesis in the *mdx* mouse model of DMD. However it was unknown if chronic exposure would translate to improved bone health. Thus, the present study aimed to determine if an 8-wk vibration regimen improved skeletal health and that vibration does not further compromise muscle function or accelerate disease progression. *Mdx* mice, aged 3wk, received 8-wk of vibration (15min/d at 0.6*g* and 45Hz) and were compared to non-vibrated *mdx* mice. Anterior crural muscle function was assessed *in vivo* and contractility of extensor digitorum longus (EDL) muscle was analyzed *ex vivo*. Visceral and subcutaneous fat pads were isolated and weighted. μCT of the tibia was done at the proximal metaphysis and mid-diaphysis. Dynamic histomorphometry was performed to indicate bone growth using fluorochrome labels injected 5 and 1d before sacrifice. Bone strength was assessed by 3-point bending and ultimate load and stiffness were measured. Paired t-tests were used to compare vibrated and non-vibrated *mdx* mice. Chronically vibrated *mdx* mice had up to 9% lower body and EDL masses ($p<0.05$), as well as a trend toward lower visceral fat ($p=0.09$) compared to non-vibrated mice. Muscle function was not compromised by vibration; isometric torque, tetanic force, specific force, and peak eccentric force were not different between groups ($p \geqslant 0.25$). Vibration did not improve any measure of tibial bone geometry at the midshaft or proximal metaphysis ($p \geqslant 0.19$). The lack of bone geometry findings was confirmed by 3-point bending and dynamic histomorphometry in which tibial strength and bone formation did not differ ($p \geqslant 0.19$). Despite the lack of improvements in bone health, vibration was not injurious to *mdx* muscle. Further, the reduction in body mass and trends toward reduced fat mass with vibration are potentially desirable in DMD. Further work is needed to determine if other vibration parameters have the capacity to improve musculoskeletal health in DMD populations.

Acknowledgements: Muscular Dystrophy Association; Biomaterials Characterization & Quantitative Histomorphometry Core, Mayo Clinic, Rochester, MN; Institute for Bioengineering and Bioscience, Georgia Tech, Atlanta, GA

***Disclosures: Susan Novotny**, None.*

Common Mechanisms Influencing Bone and Muscle Mass-'Pleotropy'

P31

Genetic Determinants of Sarcopenic-Obesity Are Different from the Genetic Determinants of Obesity or Sarcopenia Alone. A Genome-Wide Association Study in Framingham Cohorts.

Yi-Hsiang Hsu*[1], Elizabeth Newton[2], Robert McLean[1], Marian Hannan[1], L Adrienne Cupples[3], Douglas Kiel[1]. [1]Hebrew SeniorLife Institute for Aging Research & Harvard Medical School, USA, [2]Hebrew SeniorLife Institute for Aging Research, USA, [3]Dept Biostatistics, School of Public Health, Boston University, USA

Sarcopenic-obesity (SO) is characterized by excess body fat and decreased muscle mass and strength. It is common and affecting > 12% of U.S. adults aged 60 years and older. With a growing number of US seniors and an epidemic of obesity, SO have become major public health burden. Although obesity is a known risk factor for major adverse health outcomes, emerging evidence shows that older adults with SO have an even greater risk on cardiovascular diseases, metabolic disorders, mobility impairment and disability compared to those with obesity or sarcopenia alone. Identifying genetic risk factors for SO may help to understand the underlying biology and identify new molecular targets for treatments. We performed a GWAS in 3,763 Framingham participants (1,578 men and 2,185 women aged > 50 years) with 2.6 million SNPs under an additive model adjusted for potential confounders. To identify unique genetic determinants associated with SO only, we applied a mixed-effect multinomial-logit model, which accounts for the relations among obesity alone, sarcopenia alone and SO when estimating the association between SNPs and SO. We defined SO using the two most common definitions: (1) muscle mass (DXA appendicular lean mass divided by height^2) and (2) muscle strength (lowest sex-specific tertile of grip strength). Obesity was as total body fat % (DXA) >30 in men and >40 in women. Using the mass-based definition, we classified 11%, 48% and 10% of participants with SO, obesity alone and sarcopenia alone, respectively. Using the strength-based definition, we classified 10%, 47% and 7% of participants with SO, obesity alone and sarcopenia alone, respectively. We found several SNPs significantly associated with SO, but not obesity or sarcopenia alone, i.e, SNPs located in or near *MANBA, CENPE, PGK2* (mass-based SO definition) and *FN1* (strength-based definition) gene. Of note, a few of these SNPs were also reported to be

associated with diabetes, restless leg syndrome or subclinical atherosclerosis from NHGRI GWAS catalog. In our multinomial-logit analyses, SNPs significantly associated with obesity alone were not found to be associated with SO. In conclusion, our results reveal novel candidate genes associated with SO, but not with obesity or sarcopenia alone, which suggests that underlying pathophysiology of SO may be different from fatness alone and/or muscle weakness alone. The top findings (p-value $< 5x10^{-5}$) are being further evaluated in independent studies.

***Disclosures: Yi-Hsiang Hsu**, None.*

P32

Bivariate Genome-wide Association Analysis Identifies Novel Candidate Genes for Appendicular Lean Mass and Cross-sectional Bone Geometry: The CHARGE and GEFOS Consortia.

Xing Chen[1], Yi-Hsiang Hsu[2], Karol Estrada[3], Serkalem Demisie[4], Maria Zillikens[5], Tamara Harris[6], Thomas Beck[7], L. Adrienne Cupples[4], Alireza Moayyeri[8], Andre Uitterlinden[9], Tim Spector[8], Fernando Rivadeneira[3], Douglas Kiel[1], David Karasik*[1]. [1]Hebrew SeniorLife, USA, [2]Hebrew SeniorLife Institute for Aging Research & Harvard Medical School, USA, [3]Erasmus University Medical Center, The Netherlands, [4]BU School Pub Health, USA, [5]Erasmus MC, Netherlands, [6]Intramural Research Program, National Institute on Aging, USA, [7]Quantum Medical Metrics, LLC, USA, [8]King's College London, United Kingdom, [9]Rm Ee 575, Genetic Laboratory, The Netherlands

Previously, we demonstrated significant genetic correlations between lean (muscle) mass and hip geometry in the Framingham Study, indicating that shared genetic determinants may regulate both muscle and bone. To identify genes with pleiotropic effects on muscle and bone traits, we performed a bivariate genome-wide association analysis using data from two consortia.

Appendicular lean mass (aLM), combining upper and lower extremities, was measured by DXA in participants of the CHARGE Musculoskeletal Consortium (22,360 Caucasian men and women from 15 cohorts). The narrowest width of the femoral neck (NNW) and its section modulus (NNZ) were calculated using the Hip Structural Analysis program on DXA scans of the GEFOS consortium participants (17,528 men and women from 10 mostly Caucasian studies).

We performed bivariate GWAS analysis using a novel linear combination of test statistics which takes into account the correlation among phenotypes. We first performed univariate GWAS meta-analyses of 2.5 million autosomal SNPs (imputed based on CEU HapMap phase II panel) on hip geometry and aLM, separately. An additive genetic effect model was applied with adjustment for age, sex, height, ancestral genetic background, as well as age^2 and fat mass (for aLM only). We then combined the test statistics (Z-scores) directly from each of the univariate GWAS analyses, using a variance-covariance structure for weighting. We considered as potentially pleiotropic those SNPs that (a) achieved genome-wide significance (bivariate p-value $\leqslant 5x10^{-8}$) and (b) whose bivariate p-value is an order of magnitude lower than both univariate p-values.

Genome-wide significant SNPs with potential pleiotropic effects on aLM and hip geometry included SNPs mapping to *SAPS3 (a.k.a. PPP6R3,* on 11q13 near *LRP5)* for aLM-NNZ phenotype pair and *ADAMTSL3* (on 15q25.2) for aLM-NNW pair. *LRP5* is a major gene in the Wnt signaling pathway that plays a role in mechanosensitivity; *ADAMTSL3*, whose function is unclear, has been found associated with adult height. Several suggestive loci (bivariate p $< 5x10^{-6}$ and smaller than both univariate p-values) included *ZNF804A, TMSL3, LOC196541,* and *SMG6* for aLM-NNZ phenotype pair; and *LYPLAL1, CCDC66, RIMS1, PARD3,* and *NTRK3* for aLM-NNW pair.

In conclusion, using the bivariate GWAS approach, we discovered loci with effects on the regulation of muscle and bone. Some of the newly discovered genes are involved in processes influencing skeletal metabolism and may have pleiotropic effects on muscles. Functional follow-up of these associated loci is warranted together with additional replication of variants associated at suggestive level.

***Disclosures: David Karasik**, None.*

P33

Role of Tribbles in *Drosophila* larval skeletal Muscle Development.

Rahul Das*[1], Leonard Dobens[2], Venessa Masoner[2]. [1]University of Missouri Kansas City, USA, [2]School of Biological Sciences, University of Missouri - Kansas City, USA

Insulin/Insulin like growth factor (IGF) signaling pathway is an evolutionarily conserved pathway. This pathway regulates vital aspects of metabolism, growth, cell division and nutrient homeostasis in multicellular organisms. The *Drosophila melanogaster* protein Tribbles (Trbl) is the founding member of the Trb family of kinase-like proteins. Trbl acts as an important regulator of mitosis, cell adhesion and protein degradation during different stages of *Drosophila* development. There are three Mammalian Trbl homologs: TRB1, TRB2 and TRB3. Several studies demonstrated the regulation of insulin signaling pathway by mammalian TRBs[1], especially TRB3 and TRB2. For example, in skeletal muscle, TRB3 level is significantly upregulated in patients with type 2 diabetes, diabetic (db/db) mouse and fatty rat model. Over-expression of TRB3 in muscle cells blocks insulin stimulated activation of Akt kinase (a major downstream effector of Insulin signaling) and translocation of GLUT4 glucose transporter to the cell membrane [2]. Surprisingly, deletion of *trb3* or knockdown of *trb2* gene has no effect on insulin signaling (including Akt inhibition) and glucose homeostasis in animal models [3, 4]. This raises the possibility that Trb family members exert at least some function through their conserved domains.

In this study, we used *Drosophila* larval skeletal muscle as a model system to test the function of Trbl. Using a Trbl specific antisera, we observed that Trbl protein is expressed in the muscle. Overexpression of Trbl in muscle using UAS/GAL4 system results in muscle cells with comparatively smaller nuclei and shorter myofiber area, which indicates impaired insulin signaling. We also constructed a mutated form of *trbl* bearing a single amino acid substitution (D/NLK) in the conserved kinase-like domain. Muscle specific overexpression of this protein results in severe reduction of the overall size of larvae. Moreover, Trbl is able to rescue the lethality that resulted from misexpression of *Drosophila* Akt kinase in the muscle. These data suggest that the role of Trb family members in Insulin signaling pathway is evolutionarily conserved and all mammalian Trb proteins may exert redundant function(s) in this pathway.

1. Yokoyama, T. and T. Nakamura, Cancer Sci, 2011.102(6)
2. Liu, J., et al., Am J Physiol Endocrinol Metab, 2009.298(3)
3. Okamoto, H., et al., Diabetes, 2007.56(5)
4. Takasato, M., et al., Biochem Biophys Res Commun, 2008.373(4)

***Disclosures: Rahul Das**, None.*

P34

Polymorphisms Associated with Physical Activity and Body Composition.

Eric Rupe*[1], Laura Tosi[2], Todd Spock[3], Karin Kuhn[3], Mai Abdel-Ghani[4], Clare Griffis[5], Heather Gordish-Dressman[2], Eric Hoffman[2], Joseph Devaney[2]. [1]The George Washington University, USA, [2]Children's National Medical Center, USA, [3]The George Washington University School of Medicine & Health Sciences, USA, [4]George Mason University, USA, [5]Uniformed Services University of the Health Sciences, USA

Objective: To explore whether six single nucleotide polymorphisms (SNPs) previously associated with leisure-time exercise behavior in an older population (mean 45.9 years) – rs12405556 (LEPR gene), rs10946904 (PRSS16 gene), rs1766581 (SIPA1L2 gene), rs2762527 (PAPSS2 gene), rs9633417 (SGIP1 gene), and rs667923 (DNASE2 gene) – are associated with body and bone composition, strength, change in strength after resistance training, and physical activity in two healthy, college-aged populations of men and women.

Subjects and methods: We genotyped six SNPs in two populations. First we studied individuals enrolled in a resistance-training program of the non-dominant arm (n = 753, mean 24 years) from the FAMUSS cohort. We measured associations of the SNPs with these phenotypes: whole arm muscle (MRI), subcutaneous arm fat (MRI), bone volumes of the arm (MRI), 1-repetition max (1RM) and elbow flexion strength, before and after 12 weeks of resistance training.

Second, we examined the influence of the SNPs on physical activity levels and body measurements in the MB-UMASS cohort (n = 136, mean 22.5 years). In this study, subjects completed the International Physical Activity Questionnaire, and were measured for four criteria (by DEXA): bone mineral density, lean mass, fat mass, and percent tissue fat.

Hardy-Weinberg equilibrium was validated for each SNP. Associations and phenotypes were tested using ANCOVA. For those with a significant F-test, pair-wise comparisons were performed and p-values adjusted for multiple comparisons using the Sidak method, including appropriate covariates.

Results: We found two associations with strength/muscle measurements in the FAMUSS cohort: rs10946904 with baseline and change in 1RM in men, and rs12405556 with change in muscle volume in women. We found three SNPs to be associated with body measurements: rs12405556 with baseline total bone volume, total fat, and total lean mass, all in men; rs10946904 with baseline subcutaneous fat in women; and rs1766581 with bone mineral content in women. Finally, we found two SNPs to be associated with total physical activity – rs10946904 for males and rs2762527 for females.

Discussion: We found that numerous SNPs, previously associated with physical activity in an older population, effect body composition in young individuals. We also found associations with muscle strength and response to resistance training. The same genotypes that were associated with response to training were associated with physical activity. These variants may influence skeletal muscle response to resistance training and may respond to aerobic intervention. This needs further exploration. Finally, being able to corroborate previous findings for two SNPs regarding their association with exercise behavior further supports their significance.

***Disclosures: Eric Rupe**, None.*

P35

Effects of Ti, PMMA, UHMWPE, and Co-Cr Particles on Differentiation and Functions of Bone Marrow Stromal Cells.

Yunpeng Jiang*[1], Zheng Song[2], Paul Wooley[2], Shang-You Yang[1]. [1]Wichita State University, USA, [2]Orthopaedic Research Institute, Via Christi Health, USA

Purpose: To examine variant types of orthopaedic biomaterials particles on differentiation and functions of bone MSC cell.

Methods: Bone marrow stromal cells (MSCs) were isolated from femurs of BALB/c mice by density centrifugation over Histopaque®-1083. Cells were cultured in regular culture medium or in complete-osteoblast-induction medium including 10mM β-glycerol phosphate, 100nM L-ascorbic acid, and 10nM dexamethasone. The cells were respectively co-cultured with micron-sized Ti, PMMA, UHMWPE, and Co-Cr particles at various doses. MTT assay was performed periodically for cell viability and proliferation, and immunocytochemical osteocalcin stain was also performed. Alkaline phosphatase (ALP) activities were assayed in the culture media and cell lysates; while real-time PCR performed to examine gene expression of RANKL, LRP, OSX, and Runx2.

Results: Challenge with low doses of Titanium, UHMWPE, or Co-Cr particles markedly promoted bone marrow cell proliferation while high dose of Co-Cr significantly inhibited cell growth ($p<0.05$). Interestingly, the MSCs co-cultured with PMMA particles appeared in slow growth pattern, even at low concentration (0.63mg/ml). However, co-culturing the cells with low dose of PMMA-particles (0.63 mg/ml) in complete induction medium revealed significantly stronger ALP activity whereas Ti, UHMWPE, and Co-Cr groups showed relatively minimal ALP activity, in comparison with non-particle controls ($p<0.05$). Further, immunocytochemistry stain exhibited considerably more osteocalcin-positive cells in PMMA-challenged cultures than other groups. Comparison of the gene expression profiles among the cells following Ti, HPE, or Co-Cr challenges did not find significant difference in LRP5, RANKL, Runx2, Osterix/Sp7; whereas all these gene expressions were elevated following PMMA challenge ($p<0.05$).

Discussion: Evidence suggests that adverse cellular responses to particulate wear debris are critical in the periprosthetic osteolysis and the pathogenesis of aseptic prosthetic loosening. While macrophage-mediated foreign body reaction and osteoclastogenesis are the major factors to resorb periprosthetic bone, wear debris-interacted bone marrow stromal cells may play an equally important role in regulation of differentiation and functions of osteoclasts and bone remodeling. The current study suggested that various types of wear debris particles behaved differently in the differentiation, maturation, and functions of osteoblasts. PMMA particles did not show marked influence on cell proliferation, unlike other types of particles which promoted cell proliferation at low concentration and resulted in accelerated cell death at high doses. Also, PMMA particles promoted the differentiation and maturation of bone MSCs with higher ALP activities, more osteocalcin+ cells and elevated expression of osteoblast markers.

***Disclosures: Yunpeng Jiang**, None.*

P36

Vitamin D_3 Supplementation Increases Intranuclear Vitamin D Receptor Expression in Human Skeletal Muscle.

Sathit Niramitmahapanya[1], Lisa Ceglia*[1], Susan Harris[1], Heike Bischoff-Ferrari[2], Roger Fielding[3], Bess Dawson-Hughes[1]. [1]Tufts University, USA, [2]University of Zurich, Switzerland, [3]Jean Mayer USDA HNRCA At Tufts University, USA

Background: The vitamin D receptor (VDR) has been identified in human skeletal muscle and is hypothesized to mediate effects of vitamin D on muscle cell differentiation and proliferation. Whether vitamin D supplementation alters VDR expression in skeletal muscle cells has not been reported.

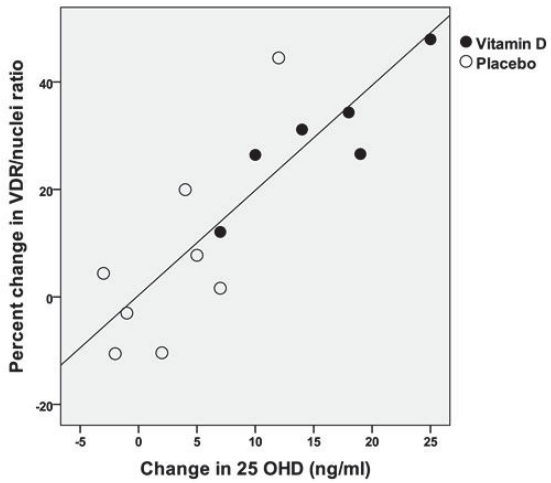
Purpose: To determine whether a daily vitamin D_3 supplement alters intranuclear VDR expression in skeletal muscle in older women with low vitamin D status. To assess if change in VDR expression is associated with change in 25-hydroxyvitamin D (25OHD) level.

Methods: In this randomized, double-blind, placebo-controlled pilot study, 14 healthy older women were given oral vitamin D_3 4000 IU/d or placebo for 4 mos. Serum 25OHD level and a muscle biopsy of the vastus lateralis were performed at baseline and 4 mos. Muscle cross-sections were probed for VDR using an immunofluorescent technique. Intranuclear VDR expression was calculated by counting the proportion of VDR-positive nuclei out of at least 300 nuclei per sample at baseline and 4 mos. We compared the percent (%) change (4 months-baseline) in intranuclear VDR expression in the 2 groups.

Results: In the whole sample, mean ($\pm$SD) age was 78 ± 3 y and mean baseline 25OHD was 18.3 ± 4.1 ng/ml; these characteristics did not differ significantly in the 2 groups. Mean final 25OHD differed in the two groups (placebo [n=8]: 22.4 ± 7.5 ng/ml; vitamin D [n=6]: 32.3 ± 5.7 ng/ml; p=0.006). Mean baseline % intranuclear VDR expression was similar in the 2 groups (placebo: $60.3 \pm 3.7\%$; vitamin D: $61.0 \pm 3.9\%$, p=0.72). Mean % change in intranuclear VDR expression was $6.8 \pm 18.2\%$ in the placebo group vs. $29.8 \pm 11.7\%$ in the vitamin D group (p=0.02). Change in 25OHD was positively associated with % change in intranuclear VDR expression independent of group (r=0.86, p=0.005; Figure).

Conclusion: Supplementation with vitamin D_3 for 4 mos significantly increased intranuclear VDR expression in skeletal muscle of older women with low vitamin D status. Analyses to determine whether the increase in VDR-positive nuclei is fiber-type specific are ongoing. The rise in VDR expression is strongly associated with the rise in 25OHD level. This small study lends support to the concept that vitamin D acts on skeletal muscle by activation of the VDR.

Figure



***Disclosures: Lisa Ceglia**, None.*

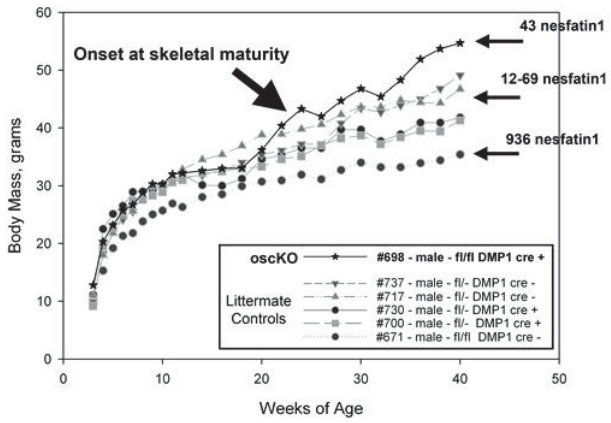
P37

Inactivation of SKI-1 in Osteocytes Leads to Obesity in Adult Mice and Suggests a New Bone to Brain Endocrine Pathway Regulating Body Mass.

Jeffrey Gorski*[1], Nichole T. Huffman[1], Anne C. Breggia[2], Clifford Rosen[2], Sridar Chittur[3], Amber Stern[1], Mark Dallas[1], Nabil G. Seidah[4], Lynda Bonewald[1]. [1]University of Missouri - Kansas City, USA, [2]Maine Medical Center, USA, [3]Center for Functional Genomics, University at Albany, [4]Institut de Recherches Cliniques de Montreal

Obesity is a major health problem and mechanisms controlling weight gain are incompletely understood. Osteocytes are endocrine cells embedded in bone secreting sclerostin, MEPE, and FGF-23 in response to load induced strain in bone. The hypothalamus regulates body mass and bone formation via similar leptin-serotonin pathways. Inactivation of the serine protease SKI-1 in osteocytes (oscKO) by breeding [SKI-1 (fl/-) DMP1 Cre (+/-)] males with [SKI-1 (fl/fl)] females leads to a rapid increase in body mass only upon reaching skeletal maturity (Fig. 1, representative of one of three litters). Similar findings were obtained in two separate breeding programs. SKI-1 is required for activation of transmembrane bound transcription factors including ATF-6, SREBP-1, SREBP-2 and CREB-like factors. Interestingly, maturity-dependent onset of obesity is not seen in leptin-deficient ob/ob mice. At 9-11 months of age, male oscKO are significantly heavier, 44.4 +/- 6.12 gm (n=8), than control littermates, 37.9 +/- 5.17 gm (n=12) ($p<0.02$, t-test). Female oscKO mice (n=2) trended in the same way but did not reach significance compared with same sex littermate controls. Although heavier, male oscKO mice exhibited the same or slightly smaller femoral cortical and trabecular bone volume by microCT, and ash weight mineral content as littermate controls. Similar to ob/ob, the oscKO mouse was hyperinsulinemic yet displayed elevated blood glucose. Nesfatin1, an appetite suppressant synthesized by osteocytes and released from nucleobindin-2, was almost 20-fold higher in serum from the leanest littermate (Fig. 1). Whole genome array studies on osteocyte mRNA showed 29 genes were reduced >2-fold in scKO compared with three control littermates, while 53 genes were increased by >2-fold in the oscKO compared to the controls. In summary, inactivation of floxed SKI-1 in osteocytes using DMP1 Cre leads to a dramatic increase in body mass only after reaching skeletal maturity, a phenotype distinct from leptin deficient ob/ob mice. We hypothesize that osteocytes, cellular endocrine sensors of load induced strain in bone, suppress appetite by release of a systemic anorexigenic factor like nucleobindin-2/nesfatin1 which is able to cross the blood brain barrier and act on the hypothalamic appetite control center. The known increase in bone stiffness occurring with skeletal maturity suggests that bone biomaterial properties also play a role in this proposed bone to brain signaling pathway.

Fig 1. DMP1 Cre SKI-1 cKO mice gain weight rapidly after skeletal maturity



***Disclosures: Jeffrey Gorski**, None.*

P38

Homogeneous Mutant Collagen in Osteogenesis Imperfecta Model Mice Leads to Improved Bone Phenotype through Multiple Pathways.

Adi Reich*[1], Wayne Cabral[2], Joan Marini[1]. [1]National Institute of Child Health & Human Development, USA, [2]Bone & Extracellular Matrix Branch, NICHD, NIH, USA

Classical osteogenesis imperfecta (OI) is caused by autosomal dominant mutations in type I collagen. The Brtl knock-in mouse, with a G349C substitution in one *col1a1* allele, models classical OI inheritance and bone phenotype. Brtl heterozygous (HET) pups have 30% prenatal lethality; survivors have a moderate bone dysplasia. Because dominant negative disorders are typically more severe in homozygous (HZ) form, it was surprising that HZ Brtl perinatal lethality was comparable to WT. Surviving HZ phenotype and bone mechanical properties were intermediate between HET and WT. We investigated two murine genotypes with homogenous mutant collagen, HZ (Brtl/Brtl) and Brtl/mov, a compound with the null mov13 *col1a1* allele. Brtl/mov also have normal perinatal survival and improved bone properties. We analyzed the differentiation of cultured calvarial osteoblasts (OB) from HZ and Brtl/mov mice during differention timecourse using Osteogenesis PCR Arrays and qPCR.

Expression of OB differentiation markers was variably altered in HZ and Brtl/Mov cells, rather than restored to WT levels. HZ OB displayed increased expression of *Cdh11*, *Intga2*, and the pre-osteocyte marker E11/Pdpn, compared to WT and HET OB. HZ OB had blunted expression of *Dmp1*, *Sost, Phex, Col1a1, Col1a2, Akp, Runx2* and *Bmp2.* In femoral tissue RNA, HZ mice had decreased expression of *Sost, Osx Bmp2* and *Vegfa.* Immunestaining of *Dmp1* showed lighter intensity in extracellular matrix of cortical bone, compared to HET and HZ mice. In contrast, Brtl/Mov OB do not display increased expression of *E11*, *cdh11* or *Intga2*, and have increased expression of *Dmp1, Sost, Col1a1, Col1a2*, & *Akp*, compared to WT, Mov13 and Brtl.

Cell-matrix exchange was performed with HZ OB to study the influence of matrix composition on OB differentiation. *Col1a1, Runx2* and *Sost* are modulated by matrix, and WT, HET and HZ cells have normal or intermediate levels on HZ matrix. In contrast, *Alp, BMP2* and *Itga2* are primarily determined by cellular genotype, and are hardly altered by the matrix exchange.

HZ and Brtl/mov mice achieve homogeneous mutant collagen by different mechanisms and apparently attain improved bone properties by distinct pathways. These expression patterns suggest that several compensation mechanism involving bone mineralization and remodeling, as well as cell-matrix attachment, occur in OB on homogenous mutant matrix. These pathways function to improve OI bone phenotype and could be the basis of novel therapeutic approaches

***Disclosures: Adi Reich**, None.*

P39

Comparative Analysis of Skeletal Muscle and Bone Gene Regulation by the Master Regulatory Factors Myod and Runx2.

Phillip W.L. Tai*[1], Hai Wu[1], Catherine L. Smith[3], Alexandra P. Lewis[3], Troy W. Whitfield[1,2], John C. Angello[3], Robert E. Welikson[3], Jonathan A.R. Gordon[1], André J. van Wijnen[1], Janet, L. Stein[1], Jane B. Lian[1], Stephen D. Hauschka[3], Gary S. Stein[1] [1]Department of Cell Biology, University of Massachusetts, [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Medical School, USA, [3]Department of Biochemistry, University of Washington, USA

The master regulatory factors Myod and Runx2 drive the processes of myogenesis and osteoblastogenesis, respectively. Although these factors are differentially expressed during development, recent reports show that Myod and Runx2 share many functional properties. These commonalities include the epigenetic control of genes during mitosis, and the regulation of protein synthesis through the control of ribosomal genes. There is also compelling evidence that myod and runx2 are directly or indirectly regulated by a common set of miRNAs. Another level of regulation by these "master genes" is being revealed with chromatin-immunoprecipitation followed by high-throughput sequencing (ChIP-seq) analyses. The genome-wide enrichment profiling of Myod by ChIP-seq has largely confirmed the presence of Myod at muscle-specific gene promoters. However, what remains puzzling is the observed Myod enrichment at far-distal regions and at non-promoter sequences. To address some of this complexity, we compared the publicly available Myod ChIP-seq enrichment tracks from primary myotubes with our own functional studies in muscle cultures and transgenic mice. We show that Myod binding at non-promoter sequences of the muscle creatine kinase (MCK) gene may concertedly control muscle-twitch type gene expression. We demonstrate that the MCK gene is controlled by a 5'-enhancer that drives fast-twitch muscle expression, and an intronic enhancer that drives expression in slow-twitch muscle. Oddly, these enhancers share common control-element motifs, and are equally enriched with Myod. These results prompt the question of how Myod can contribute to the differential expression of muscle genes in different muscle-types. We have also begun to analyze the enrichment profile of Runx2. Similar to Myod in muscle cultures, we observe widespread genomic binding of Runx2 in osteoblasts, as well as specific enrichment of Runx2 within intronic sequences. In fact, enrichment at intronic sequences outweighs the enrichment observed at promoter sequences - a feature also shared with Myod. These findings expand our understanding of the master regulator class of factors beyond that of simple transcriptional activators, to broaden function roles. The characterization of both Myod and Runx2 as widespread genomic interactions directly impacts future designs of gene therapy promoters that rely on the recruitment of these master regulatory factors to drive tissue-specific expression of therapeutic gene products.

***Disclosures: Phillip W.L. Tai**, None.*

Defective Mechanotransduction and Repair

P40

Protease Activated Receptor-1: Common Pathways in Bone and Muscle Repair?

Hyun Jin Yoo, Eleanor Mackie, Charles Pagel*. University of Melbourne, Australia

Prothrombin is expressed and activated by cholinergic stimulation in skeletal muscle tissue, suggesting that muscle cells are exposed to thrombin; moreover, bone cells are exposed to this coagulation protease during fracture. Protease-activated receptor-1 (PAR-1) is a G-protein-coupled receptor that is activated by proteolysis of its extracellular domain by thrombin. As osteoblasts and myoblasts express PAR-1 and stimulation of these cells with thrombin elicits similar functional responses, it seems likely that signaling via PAR-1 in response to thrombin is a common regulatory mechanism in bone and muscle tissue. As we have previously shown that PAR-1 plays an important role in the early stages of bone repair, the aim of this study was to identify and describe the effects of PAR-1 on skeletal muscle regeneration.

The right extensor digitorum longus muscle of littermate wildtype and PAR-1-null mice was subjected to whole muscle

autografting by dissecting it free from its muscle bed, before returning the muscle and re-suturing it in position. The contralateral muscle was used as a sham operated control. Mice were euthanazed and muscles were collected and processed for histological and morphometric analysis, 3, 5, 7, 10 and 14 days after surgery. Histological analysis indicated that grafted wildtype muscles were significantly larger 3 and 7 days post-grafting and contained a greater number of muscle fibers 3 and 14 days post grafting than grafted PAR-1 null muscles. The numbers of neutrophils and macrophages present in grafted wildtype muscles were significantly greater than in PAR-1 null muscles 3 and 5, and 5 days post-grafting, respectively. The area of fibrotic tissue, both within and surrounding the grafted muscles was also measured. Whilst no significant difference was found in intramuscular fibrosis between the two genotypes, significantly more fibrotic tissue was observed surrounding PAR-1 null than wildtype muscles.

The results presented here suggest that, as with bone repair, normal skeletal muscle regeneration involves signalling via PAR-1. A surprising finding of this study was the apparent ability of PAR-1 in suppressing fibrosis around injured muscles. Whilst the mechanism underlying this phenomenon remains unclear, it does highlight how thrombin signalling via PAR-1 may link repair of different tissues following trauma.

***Disclosures: Charles Pagel**, None.*

P41

Concomitant Volumetric Muscle Loss Impairs an Early Vascular Response in a Composite Bone-Muscle Defect Model.

Nick Willett*[1], Mon-Tsu Li[1], Brent Uhrig[1], Joel Boerckel[1], Gordon Warren[2], Robert Guldberg[3]. [1]Georgia Institute of Technology, USA, [2]Georgia State University, USA, [3]Parker H. Petit Institute for Bioengineering & Bioscience, USA

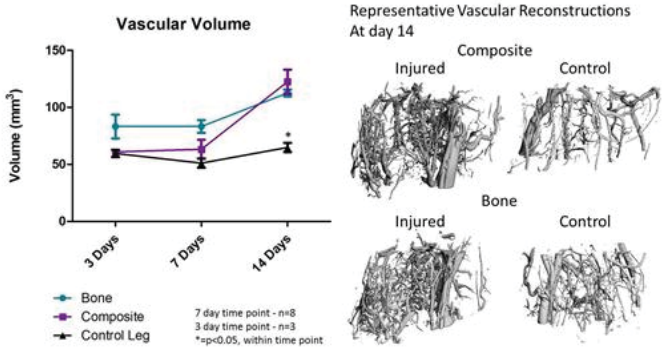
Purpose: Muscle has been implicated in bone regeneration as a critical source for vascularization, progenitor cells, osteogenic myokines, and biomechanical stimuli. In clinical cases involving severe muscle damage and a concomitant large bone defect, complications and delayed healing are common, often resulting in amputation of the limb. We have developed a pre-clinical rat model of composite bone-muscle injury that combines a critically-sized segmental bone defect with adjacent volumetric muscle loss. Our objective was to quantitatively assess the effect of muscle injury on limb vascular supply and subsequent vascular ingrowth and bone formation within the segmental defect.

Methods: Surgeries were performed on rats in three experimental groups: muscle injury (8 mm dia. full-thickness defect in the quadriceps), bone injury (8mm critically-sized defect in the femur), or composite injury combining the bone and muscle defects (n=3-8). Bone defects were treated with 2μg of BMP-2 delivered in a pre-gelled alginate injected into a cylindrical nanofiber mesh. Bone regeneration and angiogenesis were quantitatively assessed using mCT, and limb function was assessed using gait analysis and muscle strength measurements.

Results: At 3 and 7 days post-surgery vascular volume was elevated in the muscle surrounding the bone defect in bone injury animals compared to composite injury animals. No re-vascularization of the bone defect region was observed at these time points in either group. By 14 days, both groups showed a significant increase in vascular volume reaching comparable levels, both within the bone defect region and in the surrounding tissue. At 12 weeks post-surgery, bone regeneration, muscle function, and limb function were all impaired in composite injury animals compared to the single tissue injury animals.

Conclusions: Muscle damage impaired an immediate increase in vascular volume in the muscle surrounding the bone defect, which was potentially produced by a hyperemic response and vascular remodeling; however, there was no apparent effect of muscle injury on angiogenesis and revascularization of the bone defect region by 14 days. The differences in early vascular response after muscle injury may alter mechanisms such as inflammation, molecular signaling, or circulating stem cell recruitment, thereby affecting long-term bone formation and ultimately limb function.

Figure 1 – Vascular volume at 3, 7 and 14 days in the upper hindlimb.



Bone regeneration, muscle strength (12 weeks), and gait from bone, muscle and composite injuries

***Disclosures: Nick Willett**, None.*

P42

Unloading Stress Disturbs Muscle Regeneration through Perturbed Recruitment and Function of Macrophages.

Takeshi Nikawa*[1], Shohei Kohno[1], Yui Yamashita[1], Katsuya Hirasaka[1], Ayako Ohno[1], Shigetada Kondo[1], Yuushi Okumura[1], Ken-Ichi Miyamoto[2]. [1]The University of Tokushima Graduate School, Japan, [2]Tokushima University School of Medicine, Japan

Skeletal muscle is one of the most sensitive tissues to mechanical loading, and unloading inhibits the regeneration potential of skeletal muscle after injury. This study was designed to elucidate the specific effects of unloading stress on the function of immunocytes during muscle regeneration after injury. We examined immunocyte infiltration and muscle regeneration in cardiotoxin (CTX)-injected soleus muscles of tail suspended (TS) mice. In CTX-injected TS mice, the cross-sectional area of regenerating myofibers was smaller than that of weight bearing (WB) mice, indicating that unloading delays muscle regeneration following CTX-induced skeletal muscle damage. Delayed infiltration of macrophages into the injured skeletal muscle was observed in CTX-injected TS mice. Neutrophils and macrophages in CTX-injected TS muscle were presented over a longer period at the injury sites, compared with those in CTX-injected WB muscle. Disturbance of activation and differentiation of satellite cells was also observed in CTX-injected TS mice. Further analysis showed that the macrophages in soleus muscles were mainly Ly-6C-positive pro-inflammatory macrophages, with high expression of tumor necrosis factor-? and interleukin-1?, indicating that unloading causes preferential accumulation and persistence of pro-inflammatory macrophages in the injured muscle. The phagocytic

and myotube formation properties of macrophages from CTX-injected TS skeletal muscle were suppressed, compared with those from CTX-injected WB skeletal muscle. We concluded that the disturbed muscle regeneration under unloading is due to impaired macrophage function, inhibition of satellite cell activation and their cooperation.

***Disclosures: Takeshi Nikawa**, None.*

P43

Exercise during Recovery between Two Bouts of Disuse Mitigates Bone Loss on Second Exposure.

Yasaman Shirazi-Fard*, Estela Gonzalez, Joshua Davis, Ramon Boudreaux, Derrick Morgan, Kevin Shimkus, Susan Bloomfield, Harry Hogan. Texas A&M University, USA

The bone response to repeat exposures of disuse remains a concern for astronaut health and for clinical patients experiencing periods of bed rest or non-weightbearing. Previously, we reported that, regardless of age, bone loss during a subsequent period of disuse is milder than the initial exposure. This experiment assessed effects of resistance exercise during recovery between two bouts of disuse in distal femur metaphysis (DFM) and femur diaphysis (FD) bone. We hypothesized that exercise would not only enhance recovery but would also mitigate impact of the 2nd disuse.

Adult male Sprague-Dawley rats (6mo.) were assigned to age-matched cage controls (CC) and hindlimb unloaded (HU) groups by body weight and total (integral) vBMD. HU animals were exposed to 28d of hindlimb suspension (1HU), followed by 56d of recovery (1HU+R), and then a 2nd HU exposure (2HU). HU animals also performed squat jumping resistance exercise during recovery for 7 weeks (3d/wk) with exercise intensity increasing weekly. Groups (n=15) were euthanized at baseline, the end of the exercise protocol (1HU+EX, CC9), and after the 2nd HU (2HU+EX, CC10). Excised femora were scanned by pQCT at DFM and FD and tested to failure by 3-pt bending at FD and reduced platen compression at DFM.

DFM total BMC and vBMD for 1HU+EX recovered more completely (+23.1%, +14.3%) compared to 1HU+R (+3.6%, +3.9%). During the 2nd HU, tot BMC in 2HU+EX increased (+2.9%) while 2HU decreased (-1.9%). Tot vBMD decreased during the 2nd HU in both 2HU+EX (-2.0%) and 2HU (-6.9%). FD cortical BMC and vBMD recovered more in 1HU+EX (+10.7%, +2.4%) than 1HU+R (+7.4%, +0.16%). Cortical vBMD in 2HU+EX increased 3.8% vs. a decrease of 1.8% in 2HU. Exercise did not improve FD ultimate strength during recovery or during the 2nd HU. DFM ultimate stress from RPC testing increased +217% for 1HU+EX compared to 114% for 1HU+R and was significantly higher in 2HU+EX compared to 2HU by 342% or CC10 by 183%.

Exercise during recovery from HU proved beneficial to most densitometric and biomechanical properties of bone as measured at the end of recovery and after a subsequent period of disuse (2nd HU). Cancellous bone strength exhibits the most dramatic increases with exercise during recovery and is well preserved after the 2nd HU. Thus, incorporating resistance exercise during recovery from disuse not only provides benefits during reloading but also for subsequent unloading even with cessation of exercise.

***Disclosures: Yasaman Shirazi-Fard**, None.*

P44

Foxp1/2/4, new transcriptional regulators for the chondrocyte hypertrophy and osteoblast differentiation during skeletal ossification.

Xizhi Guo, Haixia Zhao*, Wenrong Zhou. Shanghai Jiao Tong University, Peoples Republic of China

Great progress has been achieved in the past years in understanding the transcriptional regulation of skeletal ossification, especially the activator for chondrocyte and osteoblast differentiation, such as Runx2, Osterix, etc. Yet the scenario of the transcriptional complexes for osteoblast differentiation is still not well-defined. Here we discovered that Foxp1/2/4, transcriptional factors of the forkhead gene family, contributed to the transcriptional control of osteochondrogenic differentiation. Through in situ hybridization and IHC, very specific and similar expression of Foxp1, Foxp2 and Foxp4 were detected in the perichondrium of skeleton during embryonic development. To understand the function of the Foxp1/2/4 proteins in bone development, genetic approaches were used including transgenic or gene knockout mice. Overexpression of Foxp1, Foxp2 or Foxp4 in the chondrocytes driven by Col2a1 promoter inhibited chondrocyte maturation in mice. Conversely, conditional inactivation of Foxp1 or Foxp2 promoted the hypertrophy and apoptosis in the chondrocytes. The double knockout of Foxp1 and Foxp2 exhibited additive defects in chondrocyte differentiation compared to the single knockout mice. These results are in line with the report that Foxp1/2/4 acts as homo- or heterodimer complex. Meanwhile, overexpression or inactivation of Foxp1/2 in osteochondrogenic progenitor cells both inhibited osteoblast differentiation. Molecularly, Foxp1/2/4 proteins repressed the transcriptional activity of Runx2 via directly binding to the Runt domain. Thus we concluded that Foxp1/2/4 proteins regulate the multiple steps of skeletal ossification, including chondrocyte hypertrophy, survival and osteoblast differentiation.

***Disclosures: Haixia Zhao**, None.*

P45

Site-specific Associations Between BMI and Bone Content and Density in Healthy Adults: A pQCT Study.

Amanda Lorbergs*[1], Stefan Jackowski[2], Andrew Frank[2], Ashlee McLardy[2], Lauren Sherar[3], Adam Baxter-Jones[2], Saija Kontulainen[2]. [1]McMaster University, Canada, [2]University of Saskatchewan, Canada, [3]Loughborough University, Canada

The effect of being overweight and/or obese on bone structure is controversial. This is in part because of the paucity of research concerning the site-specific effects of increased body mass index (BMI) on bone structure in males and females. The purpose of this cross-sectional study was to compare the radius and tibia content and density of normal, overweight, and obese adult males and females.

One hundred and fifty one healthy adults (79 males, 72 females; aged 24-53 years) from the Saskatchewan Growth and Development Study (SGDS) and the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) were separated into normal weight (NW), overweight (OW), or obese (OB) groups according to the World Health Organization criteria. Distal and shaft bone properties of the radius and tibia were measured using peripheral quantitative computed tomography (pQCT). Sex-specific MANCOVAs controlling for age, height, and limb-specific muscle cross-sectional area (MCSA) were used to assess BMI group differences in bone variables.

There were no significant differences in adjusted bone variables observed at the radius between BMI groups in either males or females ($p>0.05$). Male and female OW and OB groups had significantly greater adjusted total content (mg/mm; males: NW (mean $\pm$ SD) 401.4 ± 15.5, OW 429.3 ± 15.5, OB 448.9 ± 22.5; females: NW 293.2 ± 6.1, OW 313.2 ± 7.7, OB 317.7 ± 13.0) and adjusted total density (mg/cm^3; males: NW 319.4 ± 8.7, OW

341.1 ± 6.9, OB 352.5 ± 12.6; females: NW 283.7 ± 6.3, OW 305.1 ± 8.0, OB 310.1 ± 13.4) at the distal tibia ($p<0.05$). Respectively, OW and OB groups had greater adjusted cortical content (mg/mm; males: NW 440.5 ± 9.9, OW 461.3 ± 7.9, OB 466.2 ± 14.4; females: NW 354.4 ± 5.9, OW 361.2 ± 7.4, OB 366.6 ± 12.4) at the tibia shaft ($p<0.05$).

Being overweight and/or obese in adulthood may confer skeletal benefits to total bone density and cortical content at the weight-bearing tibia, but not at the non weight-bearing radius. These skeletal site-specific differences may be pertinent to fracture risk.

***Disclosures: Amanda Lorbergs**, None.*

P46

Viscoelastic Mapping of Transmenopausal Bone Biopsies.

Sara Campbell[1], Philip Yuya[2], Ben Polly[3], Donna Hurley[1], Joseph Turner[4], Joan Lappe[5], Robert Recker[5], Mohammed Akhter*[5]. [1]National Institute of Standard & Technology, [2]Clarkson University, [3]National Renewable Energy Laboratory, [4]University of Nebraska, USA, [5]Creighton University Osteoporosis Research Center, USA

Trabecular bone architecture has been shown to deteriorate in postmenopausal, osteoporotic women, yet the changes in the intrinsic material properties of bone during the transmenopausal period are uncertain. The general hypothesis for this work is that material properties of trabecular bone are affected by change in hormonal status at menopause soon after final menses. Here, we used atomic force microscopy (AFM) methods to measure viscoelastic properties (storage and loss modulus *E'* and *E''*, respectively) at the nanoscale. The nanoscale spatial resolution of AFM enables measurement of material properties without the contribution of voids and pore spaces. In a previously reported study (Recker *et al.*, 2004), we obtained transilial bone biopsies from women on entry (pre-menopausal and >age 46) and at 12 months past the last menstrual period. In this preliminary work, we applied contact resonance force microscopy (CR-FM), a dynamic contact mode of AFM, on a pair of specimens from the previous study. The specimens were prepared with a flat surface (~155 nm roughness) by grinding with 600, 800, and 1200 grit sand papers and then polishing with 1 mm and 0.25 mm diamond slurry. A total of 14 point maps were acquired, each consisting of a 10 mm x 10 mm region (32 x 32 = 1024 pixels, 0.32 mm/pixel width). Absolute values of *E' and E''* for the CR-FM data were obtained with use of dynamic nanoindentation values on the pre-menopausal sample as reference. Nanoindentation and CR-FM values for both *E'* and *E''* declined in the postmenopausal bone biopsy in this pair (Table), consistent with nanoindentation results in 15 biopsy pairs (Polly *et al.*, 2012). The difference in test frequency between the two techniques (~1 MHz for CR-FM and 200 Hz for nanoindentation) likely contributes to measurement variations. Increased remodeling rates have been reported in postmenopausal women (Recker *et al.*, 2004), suggesting greater heterogeneity with respect to gradation in mineralization. Although no fractures were reported in women from the original study, the greater variation in mineralization may be responsible for lower values of *E'* and *E''* in postmenopausal women. These results demonstrate how viscoelastic CR-FM mapping enables nanoscale characterization of material properties that may be affected by increased remodeling rates in postmenopausal women.

Table

sample	NI *E'* (GPa)	NI *E''* (GPa)	CR-FM *E'* (GPa)	CR-FM *E''* (GPa)
Pre	18.0 ± 1.83	0.219 ± 0.194	reference: 18.0	reference: 0.22
Post	16.2 ± 1.62	0.154 ± 0.108	15.0 ± 3.0	0.21 ± 0.04
Pre-premenopausal; Post-postmenopausal; NI-nanoindentation; *E'*-storage modulus; *E''*-loss modulus. Values represent the mean and one standard deviation of measurements.				

***Disclosures: Mohammed Akhter**, None.*

P47

Generation and Characterization of Osterix-Cherry Reporter Mice.

Sara Strecker*[1], Yu Fu[1], Peter Maye[2]. [1]University of Connecticut, USA, [2]University of Connecticut Health Center, USA

Objectives: Osterix is a zinc finger transcription factor which functions as a master regulator of osteoblast differentiation. We report here on the generation of Osterix-mCherry reporter mice.Methods: We subcloned a 40 kb region encompassing the Osterix gene from BAC clone RP24-362M3. An mCherry fluorescent reporter was then inserted into this subcloned genomic DNA region. Pronuclear injection was carried out with this construct and two founder lines were generated.

Results: Preliminary characterization of reporter gene expression at early postnatal ages indicates Osterix expression is largely restricted to cells of the osteoblast lineage. Robust mCherry expression can be detected in cells of the osteoblast lineage in the skull, long bones, and axial skeleton. Reporter expression can also be detected in the kidney. At adult ages, Osterix expression is not exclusive to the osteoblast lineage and is moderately expressed in hypertrophic chondrocytes and weakly expressed in cells present within the bone marrow. Osterix expressing cells in the bone marrow appear close to, but are not on the bone surface. These cells appear similar to reticular cells with regards to their cell morphology.

To validate the expression pattern of Osterix-mCherry reporter expression, we carried out immunostaining studies for endogenous Osterix protein on tissue sections. Also, qRT-PCR analysis for Osterix on FACS isolated cells from day 5 stromal cultures revealed that the mCherry positive cell fraction was approximately 35 fold higher in endogenous Osterix gene expression relative to the negative cell fraction.

Conclusions: These studies substantiate that our reporter accurately represents endogenous Osterix expression in the cortical bone and cells of the bone marrow. The identity and significance of these mCherry positive cell types is currently under investigation. Future studies will further validate and characterize the mCherry + reporter expression relative to endogenous Osterix gene expression.

***Disclosures: Sara Strecker**, None.*

P48

Trabecular Mineralization and Muscle Fiber Growth in Response to Dynamic Fluid Flow Stimulation.

Minyi Hu*, Robbin Yeh, Morgan Teeratananon, Yi-Xian Qin. State University of New York at Stony Brook, USA

Intramedullary pressure (ImP)-driven bone fluid flow acts as a mediator between an external load and bone cells, which then regulate bone remodeling. It is hypothesized that muscle compressions may increase vessel pressure gradient that can directly increase ImP. To establish the translational potential of ImP-induced fluid flow stimuli in bone, a dynamic hydraulic stimulation (DHS) approach has been developed to evaluate trabecular mineralization and muscle fiber growth in response to non-invasive, direct muscle compressions in a disuse osteopenia rat model. Sprague-Dawley virgin rats were randomly assigned to 5 groups: baseline control (n=15), age-matched control (n=12), hindlimb suspended (HLS, n=10), HLS+static pressure (n=10), HLS+DHS (n=14). Stimulations were given to right tibia by an inflatable cuff with 2Hz, 30mmHg static+30mmHg dynamic pressures, for 10min on-5 min off-10min on, 5d/wk for 4 wks. Two injections of calcein (10mg/kg) were given to the animals in two weeks apart during the study. The right tibiae, soleus and gastrocnemius were obtained at the animal sacrifice. The proximal tibiae were embedded with PMMA. Longitudinal sections were

made to 5μm. Histomorphometric measurements were done by tracing calcein labels in the trabecular bone in the metaphyseal region using the Osteomeasure software. Muscle samples were snap-frozen, and cross-sections were made to 8μm followed by H&E staining. Muscle fiber areas were determined by the Image J software. The histomorphometry data indicated that HLS significantly reduced BV/TV, MS/BS, MAR, BFR/BS and BFR/BV compared to age-matched controls (Table 1). HLS+static pressure alone did not show a significant difference compared to HLS, except for MS/BS. However, HLS+DHS rats showed significant increases in BV/TV and all other bone formation indices, except for MAR. HLS+DHS had increases compared to the HLS group by 34% in BV/TV, 121% in MS/BS, 190% in BFR/BS, and 146% in BFR/BV. On the other hand, stimulation-induced improvements on muscle fiber growth were not shown (Figure 1). DHS did not seem to rescue muscle atrophy due to HLS. While dynamic fluid flow stimulation by DHS significantly mitigate bone loss in disuse, the current effective DHS stimuli may be suitable for bone cell responses but not for muscle fiber alterations.

Table 1 and Figure 1

	BV/TV (Histo, %)	MS/BS (%)	MAR (mm/day)	BFR/BS (mm^3/mm^2/day)	BFR/BV (mm^3/mm^3/day)
Age-matched	46.61±7.32	10.75±2.24	0.73±0.15	0.08±0.03	0.23±0.07
HLS	33.22±7.99*	4.12±0.84**	0.53±0.16*	0.02±0.01**	0.07±0.03**
HLS + Static	40.99±14.84	6.81±1.18**#	0.59±0.19	0.04±0.01**	0.12±0.03**
HLS + DHS	44.65±8.99#	9.09±2.94##	0.71±0.18	0.06±0.02##	0.18±0.05##

*p < 0.05 vs. age-matched; #p < 0.05 vs. HLS; **p < 0.001 vs. age-matched; ##p < 0.001 vs. HLS; #p < 0.05 vs. HLS + static

Table 1. Static and dynamic histomorphometry analysis.

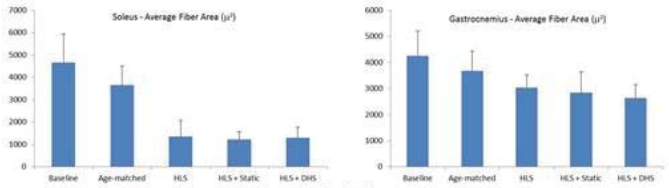


Figure 1. Muscle fiber areas.

***Disclosures: Minyi Hu**, None.*

Emerging Areas

P49

Adult Patients With Cerebral Palsy Show Negative Bone Balance Specifically More In Spastic Type.

Won Jin Kim*[1], Sung-Rae Cho[2], Yumie Rhee[1]. [1]Department of Internal Medicine, College of Medicine, Yonsei University, South Korea, [2]Department of Rehabilitation Medicine, Yonsei University, College of Medicine, South Korea

Patients with cerebral palsy (CP) patients are known to have low bone mass with increased risk of fragility fracture. CP is classified into four major types: spastic, ataxic, dyskinetic and mixed. Spastic CP is the most common type which is characterized by hypertonicity and impaired neuromuscular mobility. In comparison, dyskinetic CP shows mixed muscle tone with involuntary motions. The aim of this study is to discover the relationship between bone mineral density (BMD) by dual X-ray absorptiometry according to the type of CP. Fifty four patients with CP (age 19 to 60 years, mean age 36.4 y.o., 29 males and 25 females) were included for this cross-sectional analysis. Lumbar spine (LS) and femoral BMD Z-score were measured. Bone markers, including C-telopeptide (CTx) and osteocalcin (OCN), were also analyzed. Among these patients, there were 22 spastic and 32 dyskinetic type of CP. Z-score of LS BMD was not different between two types of CP. Meanwhile, Z-score of total hip and trochanteric BMD were significantly lower in spastic type than dyskinetic type (-1.62±0.25 vs. -1.06±0.19, -1.64±0.23 vs. -0.95±0.20, respectively, $p<0.05$). As their ambulatory ability varied, all patients were sub-classified into 3 groups: totally dependent non-ambulatory group, wheelchair-bound group, and independently ambulatory group. Totally dependent ambulatory patients showed significantly lower BMD in hip including trochanteric and total region in spastic CP patients ($p<0.05$), but not in patients with dyskinetic type. More than half of the patients with either type of CP showed abnormally elevated CTx but about 90% of patients showed normal OCN level compared to the normal control range. These results reveal that reduced weight bearing and immobilization of CP patients cause negative bone balance due to increased bone resorption leading to lower bone mass. Hypertonicity with clonus and muscle spasms of affected limbs in spastic CP patients resulted in more deteriorated bone mass compared with dyskinetic CP type. In conclusion, different type and degree of activity or ambulation in CP patients should be considered as important factors affecting bone metabolism.

***Disclosures: Won Jin Kim**, None.*

P50

Sarcopenia, Exercise and Fall Prevention in the Elderly.

Eduardo Abreu*[1], Leticia Brotto[1], Keyna Chertoff[1], Patricia Kelly[1], Marco Brotto[2]. [1]School of Nursing, Muscle Biology Research Group, University of Missouri-Kansas City, USA, [2]University of Missouri - Kansas City, USA

Sarcopenia is defined as the age-related loss of skeletal muscle mass, strength, and function, a condition that begins in the 4th decade of life. A huge disconnection between atrophy, force and power (muscles atrophy by 20-30%, force drops by 40-50%, power drops by 50-60%) suggests other factors may be involved. Overall, the loss of muscle mass and function can be both a fundamental cause and a contributor to disability and disease progression, especially when combined, as quite frequently happen, with osteoporosis. For example, weaker individuals are more susceptible to falls, which can aggravate the deleterious consequences of osteoporosis. Despite its importance, sarcopenia remains underdiagnosed. The identification of biomarkers for sarcopenia are needed and will not only help this conditions to be better diagnosed, but also to improve our understanding of its relationship with osteoporosis and the efficacy of preventive measures (e.g., resistance training).

Sixty-three elderly individuals (ages between 64 and 94, 17 males and 56 females) were evaluated for BMI, blood pressure, functional activities, grip strength, and serum determination of lactate dehydrogenase (LDH), muscle creatine kinase (CKM) and muscle Troponin T (TnT) before and after a 10-week exercise program designed to improve balance, strength/resistance, flexibility and endurance. Exercise resulted in a discrete but significant increase in grip strength (right hand, from 21.4 to 22.3 kg, $P<0.05$) and an overall improvement in the performance of functional activities. In terms of muscle biomarkers, LDH and CKM increased (from 77.1 to 83.1mU/mL, $p<0.005$, and from 106.9 to 114.0μg/mL, $p<0.05$, respectively), with TnT decreasing from 68.5 to 52.3pg/mL, $p<0.05$. The slight increases in LDH and CKM, two key muscle enzymes, indicate higher utilization of skeletal muscles, while the decrease in serum levels of TnT suggests a strengthening of skeletal muscles, making them less likely to be damaged.

In conclusion, a 10-week exercise program was able to help elderly individuals improve their muscle strength, which was attested by improvement in functional activities and positive

changes in muscle biomarkers. This is the first study to combine grip strength with blood markers to evaluate an elderly population before and after exercise training. It is also the first time that TnT is proposed as a specific marker for skeletal muscle status.

***Disclosures: Eduardo Abreu**, None.*
This study received funding from: State of Missouri Clay County Senior Services

P51

Cellular Mechanisms of Tendon-muscle Crosstalk.

Janalee Isaacson[1], Sandra Romero-Suarez[2], Leticia Brotto[1], Chenglin Mo[2], Marco Brotto[2], Eduardo Abreu*[1]. [1]School of Nursing, Muscle Biology Research Group, University of Missouri-Kansas City, USA, [2]University of Missouri, Kansas City, USA

Tendons are mechanosensitve tissues responsible for the transmission of force between bones and muscles, as evidenced by tendon injuries that occur from overuse and by the careful progression of exercises designed to promote tendon healing in the rehabilitative phase. In addition to the mechanical load, practitioners recognize many other factors that may affect the patient's recovery course, including their age, nutrition, level of activity, genetic, and epigenetic factors. Intriguingly, a more elementary aspect of tissue biology (i.e., tissue to tissue crosstalk) has not been considered as a major factor for the healing process. Due to the spatial and functional relationship between muscles, bones and tendons, it is reasonable to expect interaction between these tissues. Although transmission of mechanical load is the most obvious relationship between muscles, tendons and bones, new evidence has shown that these tissues might also communicate through biochemical factors. In fact, previous results from our lab suggest the existence of crosstalk from bone to muscle cells and vice-versa. To investigate a possible crosstalk between tendons and muscles, we tested the hypothesis that conditioned media from tenocytes (CM-T) would exert noticeable effects on myogenic differentiation. Myoblasts (C2C12) were treated either with the differentiation media (DIFF, DMEM + 2.5% horse serum + 1% antibiotic), control group, or with DIFF plus 10% CM-T media, treatment group, for up to 7 days. Tenocytes were obtained from explants of C57BI6 WT mouse flexor digitorum brevus (FDB) tendon. CM-T was obtained from 90% confluent tenocytes cultures. Results revealed CM-T had a profound effect to promote myogenic differentiation. There were significant increases in mean myotube area and fusion index, but not in number of myotubes. Furthermore, CM-T caused the upregulation of myogenic regulatory factors (MRF), including MyoD1, and of genes related to the Calcineuring-NFAT pathway.

These findings provide further evidence that tenocytes are able to biochemically signal to muscle cells. The potential clinical implications for patients suffering from musculoskeletal disorders compel our group to continue work understanding the biochemical communication between muscles and tendons. Uncovering these signaling mechanisms that underlie tendon to muscle crosstalk might lead to a better understanding of the bone-muscle-tendon unit, which is a major focus of our current studies.

***Disclosures: Eduardo Abreu**, None.*
This study received funding from: This work was supported by a Missouri Life Sciences Research Board Grant to MB and a NIAMS 1RC2AR058962-01 GO Grant to MB

P52

The Role of *Drosophila* Trim-32 in Myofbril Stabilzation.

Erika Geisbrecht*. University of Missouri-Kansas City, USA

Myopathic diseases are a substantial economic burden for long-term care; while symptoms themselves may be treated there are currently no cures available. In order to better approach this elusive long-term medical goal, more information is needed to bridge the current gaps in our knowledge of how muscle tissue is formed and maintained. Studies have revealed that the Tripartite-motif (Trim)-containing proteins are important for proper development and maintenance of both cardiac and skeletal muscle. Specifically, mutation in a gene called *Trim32* is associated with Limb-girdle muscular dystrophy (LGMD2H), a disease characterized by progressive muscle wasting of the shoulder and pelvic muscles. It is known that *Trim-32* encodes for an E3-ubiquitin ligase and is widely implicated in the ubiquitination of actin and other cytoskeletal elements. To date, the mechanism that links defective Trim-32 function and progressive muscle wasting is unknown. Understanding of the function of Trim proteins in normal and disease states is necessary for identifying crucial points of interaction between other elements of the cell.

Proteins required for proper muscle structure and function are highly conserved between humans and model organisms such as *Drosophila.* We have identified the *Drosophila* homolog of Trim-32, which is encoded by the *another b-box affiliate (abba)* locus. Abba is expressed at high levels in *Drosophila* skeletal muscles and is localized to the Z-line in larval body-wall muscles. Knockdown of Abba function, either by RNAi or by the creation of *abba* null alleles, results in pupal lethality. Analysis of larval mature muscles deficient for Abba protein show a loss of sarcomeric striations and muscle size, consistent with degeneration of the muscle fibers. Immunolocalization studies demonstrate that the normal function of Abba is to maintain the proper localization of protein components within the muscle fiber. These data indicate that *abba* plays an important role in maintaining healthy muscle, possibly thorough regulating the subcellular distribution of key proteins.

***Disclosures: Erika Geisbrecht**, None.*

P53

Expression Profiling of Smooth Muscle Alpha Actin-Expressing Periosteal Cells During Fracture Callus Formation.

Brya Matthews*[1], Danka Grcevic[2], Liping Wang[1], Douglas Adams[1], Ivo Kalajzic[1]. [1]University of Connecticut Health Center, USA, [2]University of Zagreb, Croatia

Fracture healing is a complex process that involves many cell lineages. Studies of gene expression in whole fracture callus do not distinguish contributions from different cell lineages. Our previous work has shown that smooth muscle alpha actin (αSMA) is a marker of mesenchymal progenitor cells. We aimed to define changes in gene expression in a mesenchymal progenitor population during commitment to callus formation. To identify and trace these cells we used αSMA promoter-driven inducible Cre expression (αSMA-CreERT2) combined with a Cre-activated tomato reporter (SMA9 mice) to label and trace mesenchymal progenitors in periosteum and bone marrow (BM). Tibias were fractured in 3-4 month old SMA9 mice pretreated with tamoxifen. Periosteum/soft callus was collected 2 days after treatment (unfractured), and 2 and 6 days after fracture. Histology indicated that SMA9+ periosteal cells form chondrocytes and osteoblasts in the callus. FACS analysis showed that SMA9+ cells comprised 0.8% of cells in unfractured periosteum, and 1.2% and 3.5% 2 and 6 days after fracture, respectively. Gene expression in these sorted cell fractions was evaluated using Illumina microarrays.

At fracture day 2 many upregulated genes were associated with mitosis or immune response (for example chemokines *Cxcl2* and *Ccl9*). By day 6, upregulated genes were associated with bone and cartilage, with >50-fold increase in *Acan* and *Col2a1*, and elevated *Ibsp* and osterix. Numerous downregulated genes were associated with vascular and muscle development, including the expected decrease in αSMA. Notch signaling components were downregulated, including Notch1, 3, 4, Hes1 and Hey1. These changes were confirmed by real time PCR.

Further analysis indicated that SMA9+ cells in the periosteum have a different phenotype to SMA9+ from the BM, although they are both capable of multilineage differentiation. FACS analysis of cell surface markers has also shown differences between the populations, and while the BM resident cells show perivascular localization, periosteal cells are more abundant and show fibroblastic morphology.

In summary, this is the first study to characterize gene expression in a defined subset of cells involved in fracture healing. After fracture, proliferation is stimulated in SMA9+ periosteal progenitor cells followed by differentiation into chondrocytes and osteoblasts. Downregulation of muscle genes and Notch signaling may be important in this process.

***Disclosures: Brya Matthews**, None.*

P54

Ryanodine Receptor 1 Remodeling in Cancer Associated Muscle Dysfunction.

David Waning*[1], Khalid Mohammad[2], Daniel Andersson[3], Sutha John[4], Patricia Juarez-Camacho[4], Steven Reiken[3], Andrew Marks[5], Theresa Guise[2]. [1]Indiana University School of Medicine, USA, [2]Indiana University, USA, [3]Department of Physiology & Cellular Biophysics, College of Physicians & Surgeons of Columbia University, USA, [4]Indiana University Simon Cancer Center & Indiana University School of Medicine, USA

Muscle weakness is common in advanced cancers and is a cause of significant cancer-related morbidity and mortality. The mechanisms of cancer-associated muscle dysfunction are unknown and no effective treatment exists. Ryanodine receptor 1 (RyR1) is the skeletal muscle sarcoplasmic reticulum calcium release channel required for excitation-contraction coupling. The RyR1 channel is a macromolecular complex that is comprised of 4 RyR1 monomers and regulatory proteins that modulate channel function. RyR1 undergoes remodeling in disease states, such as muscular dystrophy and ageing, characterized by oxidation and nitrosylation of the channel and loss of the stabilizing subunit, calstabin1 resulting in leaky channels. We hypothesized that muscle weakness in cancer could be due to remodeling of RyR1 resulting in intracellular calcium leak that causes impaired muscle function.

We used a mouse model of breast cancer metastases to bone during which mice develop significant cachexia. Five-week-old female nude mice were inoculated with MDA-MB-231 breast cancer cells via intra-cardiac inoculation and compared to non-tumor bearing controls. Mice developed osteolytic lesions 12 days after inoculation. Tumor bearing mice lost significant weight by 4 weeks compared to controls (20.5 ± 0.6 vs. 23.2 ± 0.4; $p<0.0002$). This was associated with a significant reduction in total body tissue, lean mass and fat in tumor bearing mice, as assessed by DXA ($P<0.01$), with no difference in total body %lean mass or %fat. Muscle specific force production of the extensor digitorum longus (EDL) muscle was significantly decreased in tumor bearing mice compared to controls ($p<0.001$). The reduction in muscle force correlated with larger osteolytic lesions ($p<0.05$). Immunoprecipitation and immunoblotting of RyR1 from EDL of tumor bearing mice showed that RyR1 were oxidized,nitrosylated and depleted of calstabin1, consistent with leaky channels. Transmission electron microscopy showed dysmorphic mitochondria in tumor bearing mice.

Our data show that MDA-MB-231 bone metastases are accompanied by loss of muscle function and remodeling of RyR1 channel complex resulting in leaky channels. Similar remodeling of RyR1 has been shown to cause muscle weakness in muscular dystrophies and sarcopenia. Targeted therapy against leaky RyR1 channels improves muscle function and exercise capacity in murine models of muscular dystrophy and sarcopenia and may be an effective therapy for cancer-associated muscle weakness.

***Disclosures: David Waning**, None.*

P55

Ultrastructural Detection and Histological Characterization of Extracellular Membrane Vesicles and Myofibroblasts in Osteosarcoma-Potential Implications in Tumor-Stromal Intercommunication.

Rama Garimella*, Priyanka Bhamidi, H. Clarke Anderson, Ossama Tawfik, Peter Rowe. University of Kansas Medical Center, USA

Osteosarcoma (OS) is the most common malignancy of bone, mainly affecting children, adolescents and young adults. It is a highly aggressive tumor of bone and typically metastasizes to lungs. Bone is a unique and favorable microenvironment which supports the growth and colonization of cancer cells. In recent years the role of exosomes or nano-sized extra-cellular membrane vesicles (EMVs) as mediators of intercellular communication is emerging as a topic of great interest in cancer and/or bone biology. It is our hypothesis that EMVs derived from cancer cells mediate transdifferentiation of normal stromal fibroblasts or mesenchymal stem (MSCs) cells to myofibroblasts. Myofibroblasts are specialized stromal fibroblasts expressing α-smooth muscle actin and play an important role in tumor progression and metastasis via secretion of Transforming growth factor-β and proteolytic enzymes like metalloproteinases. The long term goal of the study is to investigate the role of exosomes or EMVs in OS pathobiology. In this study, we determined and characterized myofibroblasts of OS by immunohistochemistry and electron microscopy (EM). Myofibroblasts were identified by their spindle shape in histological sections and α-smooth muscle actin (α-SMA; generously provided by Dr. Smirnova, University of Kansas Medical Center) and desmin immunoreactivity. Since there are other cells such as smooth muscle cells, pericytes, endothelial cells that express α-SMA, EM plays an important role in identifying myofibroblasts based on their ultrastructural features (Eyden BP, 2009). Tumor-bearing tibial sections excised from mouse *in vivo* OS experimental model were fixed in 2% glutaraldehyde and processed for electron microscopy. Myofibroblasts were identified by the presence of prominent rough endoplasmic reticulum (RER), peripheral myofilaments, fibronexus junction, Golgi apparatus and collagen. Electron microscopy revealed the presence of nano-sized extracellular membrane vesicles in the ultra-thin sections of the tumor tissue. Future studies are needed to investigate the role of EMVs derived from OS cells in facilitating differentiation of myofibroblasts from MSCs and elucidate the underlying molecular mechanisms of exosome or EMV mediated differentiation. In conclusion, our study reports ultrastructural detection and histological characterization of EMVs and myofibroblasts in OS and suggests EMVs as potential mediators in tumor-stromal intercommunication.

***Disclosures: Rama Garimella**, None.*

This study received funding from: Study was supported from start-up research funds to Dr. Garimella from the School of Health Professions, The University of Kansas Medical Center

P56

MiR-133a in Human Circulating Monocytes: A Potential Biomarker Associated with Postmenopausal Osteoporosis.

Yang Wang*[1], Ling Li[1], Benjamin Moore[1], Xianhao Peng[1], Xiang Fang[2], Joan Lappe[1], Robert Recker[1], Peng Xiao[1]. [1]Creighton University Osteoporosis Research Center, USA, [2]Biostatistical Core, Office of Research & Compliance, Creighton University, USA

MicroRNAs (miRNAs) are a class of highly conserved, non-coding RNAs (~ 22 nt) involved in posttranscriptional gene regulation, which regulate gene expression by targeting mRNAs. Circulating monocytes play important roles in osteoclastogenesis by acting as osteoclast precursors and secreting osteoclastogenic factors, such as IL-1, IL-6 and TNF-α. This study aimed to find significant miRNA biomarkers in human circulating monocytes underlying postmenopausal osteoporosis. We used ABI TaqMan® miRNA array followed by qRT-PCR validation in circulating monocytes to identify miRNA biomarkers in 10 high and 10 low BMD postmenopausal Caucasian women. MiR-133a was up-regulated ($P = 0.007$) in the low compared with the high BMD groups in the array analyses, which was also validated by qRT-PCR ($P = 0.044$). We performed bioinformatic target gene analysis and found three potential osteoclast-related target genes, CXCL11, CXCR3 and SLC39A1. In addition, we performed Pearson correlation analyses between the expression levels of miR-133a and the three potential target genes in the 20 postmenopausal women. We did find negative correlations between miR-133a and all the three genes though not significant. Many studies demonstrated that miR-133a is important in the development of muscle, such as skeletal and cardiovascular muscle. In bone, particularly, miR-133a has been found to regulate osteoblastogenesis by targeting and regulating Runx2 expression. A recent study also demonstrated that miR-133a was up-regulated in osteoblast-like periodontal ligament stem cells treated with ibandronate, a nitrogen-containing bisphosphonate that inhibits bone resorption and is widely used to treat osteoporosis. However, our study for the first time suggests that miR-133a in circulating monocytes is a potential miRNA biomarker and regulatory element for post-menopausal osteoporosis.

***Disclosures: Yang Wang**, None.*

This study received funding from: The State of Nebraska (LB692 and LB595) and NIH (R01AR04054496-02S1)

P57

Development of Carbon Foam Materials for Implantable Orthopedic Devices.

Steve Miller*, Joel White, Tala Sandid, Nora Zacharias, Andi Meyer, Kim Reuter, Stephen Miller Shang-you Yang, Paul Wooley. CIBOR, Inc., USA

Purpose: Pyrolyzed carbon foam materials share many physical properties with cancellous bone including porosity, pore size and compressive strength. Accordingly, developmental projects aimed at assessing the suitability of carbon foam materials for orthopedic applications have been initiated.

Methods: In vitro analyses of cell-material interactions included enzyme assays, real-time PCR, histochemistry and SEM. Evaluative animal models included ectopic bone formation, and repair of femoral and calvarial bone defects.

Results: Preliminary studies with rat bone marrow stromal cells demonstrated that an exemplary material, ERG DuoCell 80ppi carbon foam, presented no cytotoxicity in the L929 assay (not shown), was permissive for cell adhesion by S EM (not shown) and supported cell replication (Fig. 1A) and differentiation (Fig. 1B). The data in Fig. 1 (MTT metabolism and alkaline phosphatase induction) were compiled for cells cultured for 6 days under non-inducing (BMSC) or inducing (BMP-2 or osteo-supplements) conditions.

Other studies of cell differentiation established that BMP-2 binds very tightly to carbon foam and further, that the bound cytokine fraction retains biologic activity. Results for 3 exemplary carbon foams representing vitreous (DuoCell and Ultramet) and graphitic (K-Foam) materials are presented in Fig. 2. Immersion or the materials in BMP-2 solution followed by BMP-2 ELISA of the depleted soak solution demonstrated % BMP-2 binding values that ranged from 25-65% of input (Fig. 2A). A bioassay employing C2C12 mouse myoblasts to assess residual biologic activity in the depleted and foam fractions demonstrated clear AP inductive activity for both fractions for all 3 carbon foam materials. DuoCell was superior to the 2 other materials based upon both the amount of active BMP-2 retained and the residual activity of the cytokine contacting the foam (Fig. 2B).

Conclusions: The physical similarities between cancellous bone and porous carbon foams are recapitulated in osteopromotive potential as assessed by in vitro cell differentiation (above) and animal studies (not shown). Material properties contributing to these outcomes likely include inter-connective porosity, pore diameter, stiffness and surface chemistry that promotes cytokine binding. Studies ongoing are directed at developing a clearer mechanistic and clinically-relevant picture of these outcomes.

Fig. 1. Effects of carbon foam binding and inducers on MTT metabolism and AlkPhos induction by BMSCs

Fig. 2. BMP-2 binding and assessment of biological activity by C2C12 cells exposed to carbon foams.

Figure 1A

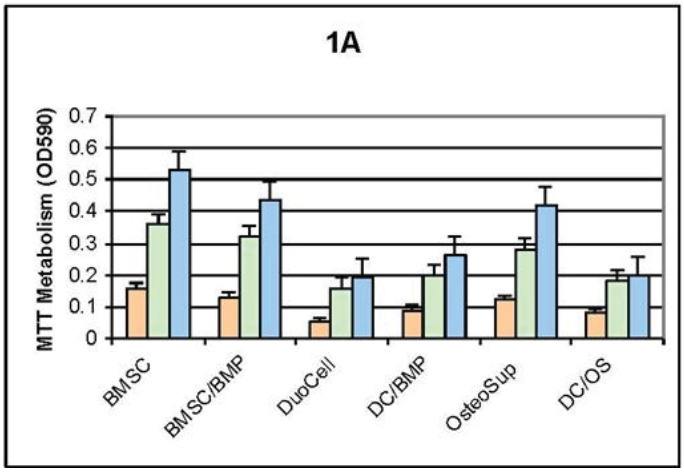


Figure 1B

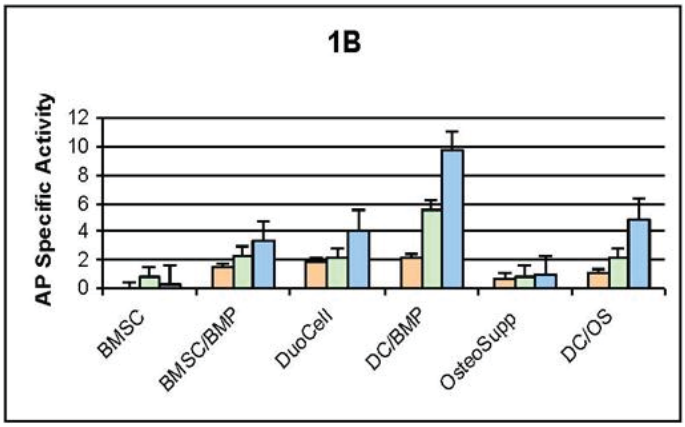


Figure 2A

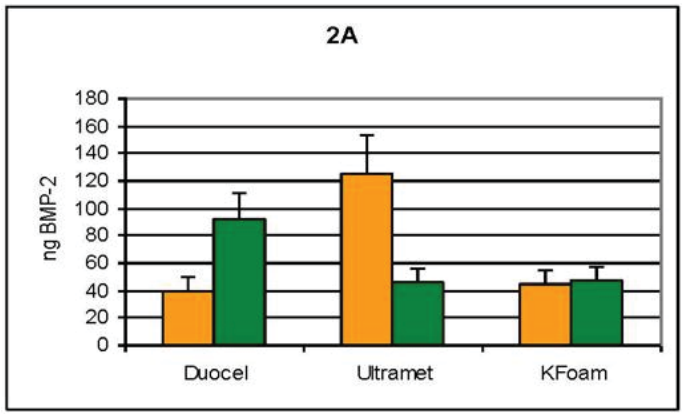
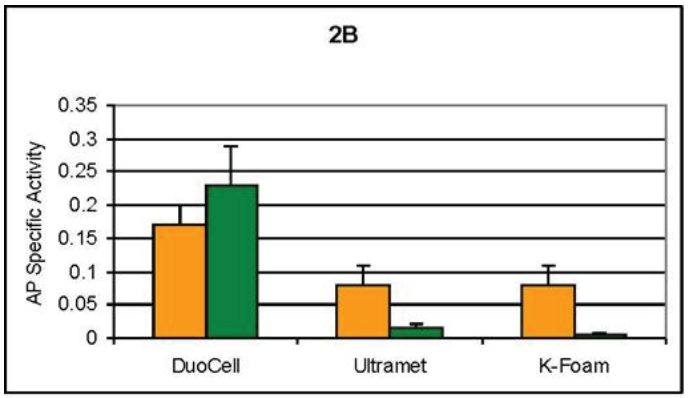


Figure 2B



***Disclosures: Steve Miller**, CIBOR, Inc, 3*
This study received funding from: CIBOR, Inc

P58

Mice Mimicking Hypophosphatasia Phenotype Present a Mineralization Deficit in the Musculoskeletal Enthesis.

Alexandre Caron*[1], Isabelle Lemire[1], Pierre Leonard[2], Philippe Crine[1], Deb Ramsdell[3], Hal Landy[3]. [1]Enobia Pharma, Inc., Canada, [2]Alexion, Canada, [3]Alexion, USA

Hypophosphatasia (HPP) is a rare, inherited metabolic disorder characterized by impaired skeletal mineralization and multisystemic effects including seizures, nephrocalcinosis, muscle weakness and pain due to deficient activity of the tissue nonspecific alkaline phosphatase (TNALP). TNALP-null mice (Akp2-/-) phenocopy human infantile hypophosphatasia, and are well characterized for their skeletal and dental hypomineralization. However, no published studies have described their musculoskeletal phenotype.

The present study aims to evaluate the presence of phenotypic alterations in 15-day old Akp2-/- mice compared to wild-type littermates. Histological assessment was performed using standard staining procedures, and included bones (undecalcified tibia and calcaneus), skeletal muscles (masticatory, glossal, and hindlimb muscles), and insertion sites (Achilles tendon and patellar ligament entheses).

Akp2-/- mice presented a marked to severe mineralization deficit in the calcified fibrocartilaginous zone of both the Achilles tendon and patellar ligament entheses. The mineralization defect was also observed in the adjacent bone/cartilage of the secondary ossification centers, with absence of cellular anomalies. Phenotypic alterations were however more evident in the tibial bone vs. the calcaneus in Akp2-/- mice, with increased endosteal osteoid accumulation. Although gastrocnemius muscle weight / body weight was significantly reduced in Akp2-/- mice, preliminary assessment of skeletal muscle histology revealed no signs of degeneration, necrosis, atrophy or major inflammation. The periodontal ligament also appeared normal, even though hypomineralization of the tooth acellular cementum, previously reported, is thought to impair the anchoring to the periodontal ligament.

In conclusion, histological analysis allowed identification of a clear lack of mineralization in the musculoskeletal enthesis of Akp2-/- mice. This finding could explain the changes in muscle function described in HPP patients since the amount of calcified tissue at the tendon enthesis has been shown to correlate with the force that the tendon transmits. In vivo studies are currently being performed to gain new insights on the efficacy of asfotase alfa, a recombinant targeted alkaline phosphatase enzyme-replacement therapy, in restoring normal mineralization in the enthesis of the Akp2-/- mouse model.

***Disclosures: Alexandre Caron**, Alexion, 3*
This study received funding from: Alexion

P59

Bone and Muscle Interactions during the Progression of Nfat1 Deficiency-Mediated Osteoarthritis.

Qinghua Lu, Brent Furomoto, H. Clarke Anderson, Jinxi Wang*. University of Kansas Medical Center, USA

Purpose: Osteoarthritis (OA) is the most common form of joint disease in middle-aged and older individuals. No pharmacologic therapy is currently available to cure the disease, largely because the pathogenetic mechanisms for initiation and progression of OA remain unclear. OA involves multiple joint tissues (e.g., articular cartilage, subchondral bone, and synovium) and possible interactions between these tissues have been reported. However, it is not clear whether interactions between a joint tissue and peri-articular skeletal muscles are involved in the pathogenesis of OA. This study aimed to investigate whether metabolic interactions between the subchondral bone and peri-articular skeletal muscles play a role in the pathogenesis of OA.

Methods: Transcription factor Nfat1-deficient (Nfat1-/-) mice were used in this study. Age-matched wild-type (WT) mice were used as controls. All animal procedures were approved by the institutional animal care and use committee. Joint tissues with skeletal muscles around the joint were harvested from hips, knees, and shoulders of Nfat1-/- and WT mice at 1, 2, 3, 4, 6, and 12 months of age for histopathological, immunohistochemical, and gene expression analyses.

Results: At 3-6 months of age, focal loss of proteoglycan staining with chondrocyte clustering was seen in the articular cartilage of Nfat1-/- joints. Chondrocyte hypertrophy occurred in the deep-calcified zones of Nfat1-/- articular cartilage. Some of the subchondral bone marrow cells differentiated into chondrocytes, which subsequently underwent endochondral ossification, leading to thickening of the subchondral bone. These bony changes were accompanied by abnormal chondrocyte differentiation and endochondral ossification in the periosteum and deep layer of skeletal muscles, forming chondro-osteophytes near joint margins in the later stage. The expression of bone morphogenetic protein (BMP)-2 and (BMP)-4 was significantly increased in Nfat1-/- subchondral bone than WT subchondral bone before and during the abnormal chondrocyte differentiation in peri-articular muscles.

Conclusions: During the process of Nfat1 deficiency-mediated OA, overexpression of BMP in the subchondral bone may stimulate pathological chondrocyte differentiation and endochondral ossification in periosteum and deep layer of muscles near the

joint margins. This in turn compromises the function of affected joints and speeds the progression of OA.

***Disclosures: Jinxi Wang**, None.*

P60

Instant and Clinically Accessible Method to Measure Total Body Protein with DXA.

Joseph Wilson*[1], Boyd Strauss[2], Bo Fan[3], Frederick Duewer[3], John Shepherd[3]. [1]University of California Berkeley-UCSF Graduate Program in Bioengineering, USA, [2]Monash Medical Centre, Australia, [3]University of California, San Francisco, USA

Purpose: Dual energy X-ray absorptiometry (DXA) reports bone mineral, fat, and lean body mass. Unfortunately, lean body mass is primarily water found in both adipose and parenchymal tissue. Functional protein mass is a small fraction compared to water mass. We will present a clinically accessible method to measure total body protein using whole-body DXA and bioimpedance analysis, where results can be reported immediately after the acquisition of the scans. We compare our method, as well as two additional protein estimates from the literature, to a criterion protein measure, neutron activation analysis (NAA).

Methods: Our study design was a retrospective analysis of a sample of convenience recruited at the Body Composition Laboratory of the Monash Medical Centre. A total of 207 participants (130 Female) were included with a mean age of 37.64 ± 15.45 years and mean BMI of 24.46 ± 7.80 kg/m^2. Each participant received the following body composition examinations: total body protein by neutron activation analysis, whole body DXA (GE Lunar Prodigy), air displacement plethysmography (BodPod), water by bioimpedance analysis, scale weight, and height. The study population was split into calibration and validation datasets using simple random sampling by sex, BMI category, and age decade. Using our calibration dataset, we generated a ''directly-calibrated'' total body protein measure (*DC-TBPro*) using linear regression of DXA-reported mass components and water from bioimpedance to the criterion protein measure (*NAA-TBPro*). Using this calibration equation, *DC-TBPro* measures were calculated for the validation dataset. Additionally, protein was estimated from DXA bone mass, body volume (BodPod), and total body water (bioimpedance) using the Lohman 4-compartment model (*4CL-TBPro*) and from DXA fat-free mass, age, and sex using the Wang model (*W-TBPro*).

Results: From our validation dataset, *DC-TBPro* was highly correlated to *NAA-TBPro* (R^2=0.89, RMSE=0.83kg) with no significant Bland-Altman slopes or intercepts. Both *4CL-TBPro* and *W-TBPro* had lower correlations to *NAA-TBPro* (R^2=0.69 and 0.81, respectively) and higher RSME values (1.39kg and 1.09kg, respectively) with significant Bland-Altman slopes and offsets.

Conclusions: The combination of whole body DXA and bioimpedance results can be used to generate an accurate measure of total body protein. With our novel method, protein mass could be available instantly after a 15-minute exam.

***Disclosures: Joseph Wilson**, None.*

P61

Muscle Derived Factor(s) Enhance the Activation of the PI3K/Akt Pathway in the Osteocyte in Response to Fluid Flow.

Nuria Lara*[1], Leticia Brotto[2], Marco Brotto[1], Lynda Bonewald[1], Mark Johnson[3]. [1]University of Missouri-Kansas City, USA, [2]University of Missouri-Kansas City School of Nursing, USA, [3]University of Missouri-Kansas City Dental School, USA

Skeletal muscle is known to apply load to the skeleton. We sought to determine if muscle also secreted factors that might condition the response of the skeleton to loading. Osteocytes are the primary mechanosensory cells in bone and so we studied the effects of conditioned media (CM) from various muscle cell/fiber types on the response of the MLO-Y4 osteocyte-like cells to fluid flow shear stress (FFSS), as an *in vitro* model system for mechanical loading. C2C12 cells were induced to undergo myogenesis and CM was collected at various stages of myogenic differentiation. Also, adult mouse Soleus (Sol), an oxidative, slow twitch muscle type, and Extensor Digitorum Longus (EDL) muscle, a glycolytic, fast twitch muscle type, were isolated and stimulated *ex vivo* to produce CM for these studies. We observed that under resting conditions the addition of 10% C2C12 CM induced a rapid, but transient activation of the Akt signaling pathway, peaking at 15 minutes and returning to baseline by 2 hours in a concentration and cell type dependent manner. This activation was observed as early as Day 3 of C2C12 differentiation when myoblast fusion had started, but maximal activity was observed at day 5 coinciding with the formation of fully mature myotubes. FFSS applied to MLO-Y4 cells for 2 hours in the presence of 10% myotube CM resulted in a 6 fold increase in pAkt compared to a 3 fold increase under non-treated conditions or when 10% CM from myoblasts or blank C2C12 differentiation media was present. A similar enhanced increase in pAkt in response to FFSS was observed in the presence of EDL CM, but not in the presence of Sol CM suggesting muscle specific effects. Downstream of increases in pAkt, we observed increased β–catenin nuclear translocation in the presence of CM from myotubes in MLO-Y4 cells. We also observed activation of β–catenin only when EDL CM was present. These data provide evidence that specific muscle cell types produce soluble factors that alter the sensitivity of MLO-Y4 osteocyte like cells to fluid flow shear stress. These data support our hypothesis that beyond a mechanical interaction there is a very specific molecular coupling of muscle and bone. These data could provide a molecular basis for the high concordance between the diseases of osteoporosis and sarcopenia that exists within the aging human population.

***Disclosures: Nuria Lara**, None.*

P62

Acute Exposure to Fibroblast Growth Factor 23 Increases Cardiac Contractility.

Chad Touchberry*[1], Troy Green[2], Vladimir Tchikrizov[3], Lori Wetmore[2], Lynda Bonewald[3], Michael Wacker[1]. [1]University of Missouri-Kansas City School of Medicine, USA, [2]William Jewell College, USA, [3]University of Missouri-Kansas City, USA

Fibroblast growth factor 23 (FGF23) is a hormone secreted by bone that regulates vitamin D and phosphate metabolism. Recent clinical data shows a correlation between chronically elevated levels of serum FGF23 in patients with chronic kidney disease (CKD) and a decline in cardiac function. Our laboratory has been studying the effects of chronic FGF23 exposure in the development of cardiac hypertrophy. However, it is currently unclear if acute exposure to FGF23 contributes to changes in cardiac function. The purpose of this study was to elucidate the acute effects of FGF23

on cardiac contractility. We exposed electrically paced left ventricular muscle strips from wild-type mice (C57BL6; n=6-9) to pathophysiological concentrations of FGF23 (90, 900, 9000 pg/ml). Muscle contractile data were analyzed using LabChart software. Treatment with FGF23 increased the magnitude of isometric force in a concentration dependent manner when compared to vehicle treatment ($p<0.01$). Isometric force nearly doubled within 20 minutes of exposure to 9000 pg/ml with corresponding increases in the rate of force development (slope) and the area (the integral) ($p<0.01$) associated with the contractile waveforms. However, the rate of relaxation (tau) of the left ventricular muscle strips was not affected ($p>0.05$). Increased cardiac contractility is tightly coupled to increases in calcium-induced calcium release (CICR). Our data showing increased isometric tension and rate of force development following acute FGF23 exposure suggest that FGF23 may alter CICR mechanisms in cardiac muscle. Since calcium is involved in the induction of pathological hypertrophy, disruption of calcium homeostasis may be a common mechanism by which FGF23 increases contractility acutely and pathological hypertrophy over time. Further studies are needed to confirm this hypothesis and elucidate the specific receptor mediated mechanism for these changes in cardiac performance.

***Disclosures: Chad Touchberry**, None.*

P63

Appendicular DXA BMC and Lean Mass Strongly Associated to Hip Fracture Risk.

John Shepherd*[1], Serghei Malkov[1], Kathleen Peters[2], Jane Cauley[3], Steven Cummings[4], Tamara Harris[5], Rachel Murphy[6], Suzanne Satterfield[7], Marjolien Visser[8], Joseph Wilson[9], Joseph Zmuda[3], Peggy Cawthon[10]. [1]University of California, San Francisco, USA, [2]California Pacific Medical Center, USA, [3]University of Pittsburgh Graduate School of Public Health, USA, [4]San Francisco Coordinating Center, USA, [5]Intramural Research Program, National Institute on Aging, USA, [6]NIA/NIH, USA, [7]University of Tennessee Health Sciences Center, USA, [8]University of Amsterdam, Netherlands, [9]University of California Berkeley-UCSF Graduate Program in Bioengineering, USA, [10]California Pacific Medical Center Research Institute, USA

Progression of osteoporosis, especially in the early stages of disease, is elusive to detect. There is growing evidence that a better model is needed to better select individuals for high fracture risk and for fracture prevention strategies. Cross-sectional muscle area (CSA) and muscle X-ray attenuation of the thigh, measured using computed tomography (CT), have been shown to be independent predictors of hip fracture risk. Nevertheless, adding CT scans to a clinical osteoporosis workup is problematic because of the increased cost, increased radiation exposure, and limited availability.

Purpose: We aim to determine the utility of quantifying DXA-derived surrogates of CT measures including thigh cross-sectional muscle area (CSA) and muscle attenuation.

Methods: We used a retrospective case-cohort study design using data from the Health Aging and Body Composition (HealthABC) study. Cases were 111 participants that had a hip fracture during the 8.7-year study period. A cohort of 212 non-fracture participants was randomly selected from the entire study population. Each participant received a DXA whole body scan and a single-slice mid-thigh CT scan. Thigh muscle CSA and attenuation were estimated from DXA using in-house algorithms and geometric modeling to CT measures. Strength testing and an extensive questionnaire were also available from each visit.

Results: In a fully-adjusted model (age, race, site, gender, chronic disease, physical activity, self-rated health, MMSE, drinking, smoking, education, hip BMD), we found BMC and lean mass variables were associated ($p<0.05$) to fracture risk [Relative Hazard/SD, 95% confidence interval, p-value] including appendicular BMC [4.1, 1.72-9.69, 0.001], and arm lean mass [2.5, 1.12-5.49, 0.03]. DXA and CT thigh muscle attenuation values were similar and not significant in the model: [0.75, 0.5 – 1.11, 0.15] and [0.79, 0.55-1.12, 0.18] respectively.

Conclusions: Strong associations for DXA BMC and lean mass variables, but not thigh muscle attenuation, to hip fracture risk were found in a fully adjusted model.

***Disclosures: John Shepherd**, None.*

P64

Evaluation of αSMA Expressing Cell Contribution To Muscle Heterotopic Ossification.

Elena Torreggiani*[1], Danka Grcevic[2], Brya Matthews[1], Ivo Kalajzic[1]. [1]University of Connecticut Health Center, USA, [2]University of Zagreb, Croatia

Heterotopic ossification is characterized by the formation of bone in atypical locations including muscle and subcutaneous tissues. Enormous progress has been made in understanding the identity of the cells that participate in the lesion formation, and molecular mechanisms underlaying the induction of the osteogenic commitment. Several studies addressed to identify the role of BMP signaling in the heterotopic ossification have been performed. However, the identity of progenitor cells that contribute to BMP-induced heterotopic ossification is still unknown.

The aim of our current work is to evaluate whether the cells that reside in the perivascular niche can actively contribute to the bone formation. We have observed the expression of a smooth muscle a-actin promoter directed green fluorescent promoter (αSMA-GFP) in the perivascular location in both muscle and subcutaneous tissue. The critical evidence of the transition of the cellular phenotype can be obtained by combinatorial approach of using visual markers for transgene expression and Cre/loxP recombination system that will allow for the lineage tracing. Therefore we have bred αSMACreERT2 mice with reporter mice in which RFP Tomato expression is controlled by ubiquitious promoter and dependent on Cre activation by tamoxifen. To assess the transition of αSMA expressing cells to osteoblast lineage we have used a bone specific Col2.3 promoter driving GFP. These mice were intercrossed to generate a triple transgenic model αSMACreERT2/Ai9/Col2.3GFP.

Heterotopic ossification was induced by intramuscular injection of 2.5μg of BMP2/Matrigel. The formation of bone ossicles was evaluated by x-ray analysis and mice were sacrificed at different time points. The contribution of αSMACreERT2/Ai9 labeled cells to bone was evaluated using fluorescence microscopy. Our results suggest that aSMA expressing cells can give rise to a population of mature osteoblasts present within heterotopic lesions. Currently, we directed our efforts to better define the cellular phenotype of the αSMACreERT2/Ai9 labeled progenitor cells. We plan to evaluate the αSMACreERT2/Ai9 expression to the presence of endothelial (CD31), hematopoietic (CD45), satellite cells (SM/C2.6) and mesenchymal stem cells (PDGFRα, PDGFRβ and Sca1) markers by flow cytometry. This study provides new insight into the cellular identity of cells that participate in the formation of the heterotopic ossification.

***Disclosures: Elena Torreggiani**, None.*

P65

Comparison of 3D UTE (Ultrashort Time-to-Echo) MRI Versus Micro-CT For Quantitative Evaluation of the Temporomandibular Joint (TMJ) Condylar Morphology.

Won Bae[1], Sheronda Statum*[1], Daniel Geiger[2], Koichi Masuda[1], Jiang Du[1], Christine Chung[1]. [1]University of California, San Diego, USA, [2]Sapienza University of Rome, Italy

Purpose: Temporomandibular dysfunction involves osteoarthritis of the temporomandibular joint, including degeneration of the mandibular condyle. Morphologic changes of the bone and fibrocartilage occurs, and it would be useful to evaluate them using non-invasive means. The purpose of this study was to determine the accuracy of novel 3D UTE MRI versus micro CT (mCT) for quantitative evaluation of mandibular condyle morphology.

Material and Methods: Three TMJ condyles specimens were harvested from cadavers. Novel 3D UTE MRI (TR=50 ms, TE=0.03 ms, voxel size=104 μm) was performed on 3-Tesla General Electric HDx, and mCT was performed using Skyscan 1076 (18 μm isotropic voxel size). MR datasets were spatially-registered with mCT dataset using FSL-FLIRT software. Bony contour of the condyles were segmented (ImageJ Segmentation Editor plugin) using global threshold (Figure 1A; mCT) or manual approach (Figure 1B; MRI, performed by two observers). Manual segmentation took less than 20 min per sample. Using Matlab, bone surface coordinates (Figure 1CD), volume of segmented regions, and Gaussian curvature (Figure 1EF) were determined for quantitative evaluation of joint morphology. Agreement between techniques (MRI vs. mCT) and observers (MRI vs. MRI) were determined by the means of intraclass correlation coefficient (ICC) analysis.

Results: Between MRI and mCT, the average deviation of surface coordinates (Figure 1G) was 116 um, approximately the resolution of MRI. Average deviation (Figure 1H) of the Gaussian curvature and the volume, from MRI to mCT, was 2.2 and 4.4%, respectively. ICC coefficient for the three measures (surface, volume, curvature) was each greater than 0.992. Between observers, the ICC coefficients were all greater than 0.997.

Conclusions: 3D UTE MR evaluation of TMJ condyle morphology including shape, volume and curvature shows high accuracy against mCT and between observers. The technique maybe useful for clinical evaluation of temporomandibular joint dysfunction. Future work includes cartilage evaluation, as well as comparison of normal and degenerated mandibular condyles.

Figure 1

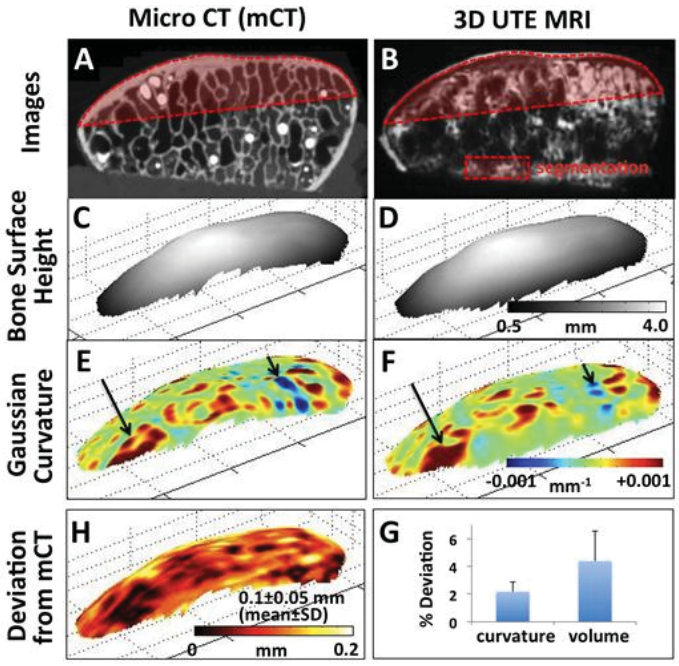


Figure 1. (A) Micro CT and (B) 3D UTE MR datasets were segmented to determine (C,D) bone surface coordinates and (E,F) Gaussian curvature in 3D. Gaussian curvature shows regions with bowl-shape (positive curvature; long arrows) and saddle-shape (negative curvature; short arrows). (G) The deviation of bone surface determined from MRI was in close agreement with that from micro CT, with a mean deviation of approximately 100 um. (H) Deviation of the curvature and volume were small as well, showing a high accuracy of MR technique to evaluate condyle morphology.

***Disclosures: Sheronda Statum**, None.*

P66

The Identification and Characterization of New Proteins Essential for *Drosophila* Myotendinous Junction Formation.

Zong-Heng Wang*, Ze (Cindy) Liu, Erika Geisbrecht. University of Missouri-Kansas City, USA

The myotendinous junction (MTJ) is the primary site for force transmission from the interior of the muscle cell, across its membrane, and to the extracellular matrix (ECM). In healthy muscle tissue, the MTJ provides resistance against the mechanical stress generated during muscle contraction, and it is now known that any decrease in MTJ formation and/or stability leads to muscle detachment in diverse organisms. Most significantly, it is this detachment phenotype that typifies a series of congenital, progressive myopathies in humans. While it is know that integrins are required to form cohesive attachments with the ECM that lies between the muscle and tendons, the molecular mechanisms that govern formation and maintenance of MTJs in any model system are poorly understood. Because the functional conservation of proteins required for muscle development across diverse species is well-established, we have chosen to use the genetically tractable organism *Drosophila melanogaster* to better understand MTJ formation and how defects in MTJ stability may lead to the onset and progression of myopathies.

As an entry point to uncover proteins that contribute to MTJ development and maintenance, we utilized an *in vivo* proteomics approach. We have identified three new proteins that are required

for the stable attachments of muscles to their target tendon cells. One of these evolutionarily conserved candidates, named Clueless (Clu), has been chosen for further study. A series of *clu* mutant alleles were generated using P-element excision techniques. Immunostaining using an antibody against Myosin heavy chain reveals that the muscles in *clu* mutant embryos migrate to their target tendon cells. Moreover, the expression and localization of molecular markers that function in muscle migration, such as dGit1 and activated focal attachment kinase, are not affected in the *clu* mutant embryos. However, upon muscle contraction, the muscles detach from their corresponding tendon cells and round up. The expression of integrin components at the MTJ are reduced in *clu* mutant embryos, suggesting a failure to form strong interactions between the muscle and the tendon cell. Other data will be presented to determine in what cell type Clu functions in muscle-tendon attachment; which domains of Clu are important for function; and how Clu functions at the molecular level to mediate proper MTJ formation and/or maintenance.

***Disclosures: Zong-Heng Wang**, None.*

P67

Wnt/ Ca^{+2} Signaling Pathway Takes Shape in Muscle-bone Cross-talk.

Sandra Romero-Suarez*, Cheng Lin Mo, Mark L Johnson, Lynda Bonewald, Marco Brotto. University of Missouri-Kansas City, USA

Integrative biological approaches have led to the discovery of new physiological roles for bone and skeletal muscle as autocrine, paracrine, and endocrine organs. We tested the effects of conditioned media from MLO-Y4 osteocyte-like cells (MLO-Y4-CM) and primary osteocytes (PO- CM) derived from 4 months wild type mouse, and found that MLO-Y4 CM and PO- CM enhanced myogenic differentiation of C2C12 myoblasts into myotubes as detected by histomorphometric measurements. Wnt signaling is involved in various aspects of bone and skeletal muscle development and regeneration. In osteocytes, Wnts are over expressed upon fluid flow shear stress; also they are released by osteocytes. To test our hypothesis that osteocytes can biochemically signal to muscles via the Wnt pathway, we treated C2C12 muscle cells with low, physiological concentrations of Wnt3a. Wnt3a accelerated myogenic differentiation and enhanced nuclear translocation of β-catenin, which is required for transcription of the key muscle transcription factor, MyoD. This pathway showed to play a crucial role in the enhancement of myogenic differentiation produced by 10ng Wnt3a, 10% and 1% MLO-Y4 CM and PO-CM respectively. The potent effects of Wnt3a on stimulating the ß-catenin signaling pathway in C2C12 muscle cells, strongly correlated with an increased sensitivity to calcium released from the sarcoplasmic reticulum by caffeine, suggesting adaptive changes of the excitation-contraction coupling machinery. These data were consistent with the upregulation of some of the essential genes related to intracellular calcium homeostasis (i.e., IP3R types 1 and 3; Stim-2; Camk2d; and SERCA-1 and 2). These findings Suggest that MLO-Y4 CM and PO- CM through activation of ß-catenin signaling pathway may alter intracellular Ca^{+2} release and activate an alternative Wnt/Ca^{+2} pathway that promotes or assists with myogenic differentiation. The molecular mechanisms leading to these effects of bone CM in osteocytes are currently under investigation.

***Disclosures: Sandra Romero-Suarez**, None.*
This study received funding from: Acknowledgments: This work was supported by a Missouri Life Sciences Research Board Grant to MB and a NIAMS 1RC2AR058962-01 GO Grant to MJ, LB, MB.

P68

Molecular Characatarization of GDF5/ActRIIB Complex.

Abdulhafez Selim*[1], Osama Haji Ahmed[2]. [1]Center for Chronic Disorders of Aging, Philadelphia College of Osteopathic Medicine, USA, [2]Faculty of Medicine Ain Shams University

Introduction: The growth/differentiation factors (GDFs) are a subfamily of the highly conserved group of bone morphogenetic protein (BMP) signaling molecules known to play a diverse set of roles in the skeletal system. GDFs 5, 6, and 7 in particular have been grouped together on the basis of the high degree of amino acid sequence homology in the C-terminal signaling region of these proteins. On the basis of the available evidence to date, GDF-5 may hold promise as a possible therapeutic agent for various clinical applications

Members of the TGF-β superfamily transduce their signals through the formation of heteromeric complexes of two different types of serine/threonine kinase receptors, *i.e.* type I receptors and type II receptors. Previous studies (Nishitoh et al, 1996) demonstrated the biological effect of GDF-5 on osteoprogenitor-like cell lines and identified type I and type II receptors for GDF-5; BMPR-IB and BMPR-II, but not BMPR-IA, bound GDF-5 in ROB-C26 cells and other cell types. Moreover, the studies showed that GDF-5 transduces its signal through heteromeric complexes of BMPR-IB and various type II receptors. Kotzsch et al (2009) studies characterized the GDF5/BMRIB complex.

There are no studies available that characterize the GDF5/ActRIIB complex. Previous attempts to achieve this task had failed due to technical challenges (Kotzsch et al, 2009). Our study is the first study that describes this complex. In this work, we modeled the GDF5/ActIIRB complex and identified the regions of GDF5 interacting with ActRIIB.

Methods: 1-GDF5 PDB model was obtained from PDB.org (3EVS; chains B).

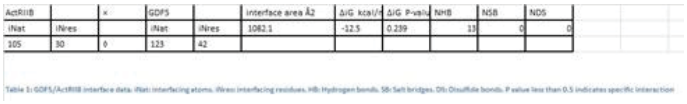
2-ActRIIB PDB model was obtained from PDB.org (2GOO; chains C and F).

3-GDF5/ActRIIB complex was modeled using Vakser Lab server.

4-GDF5/ActRIIB complex interface was analyzed using PDB ePISA server.

5-Covalent bond quality was evaluated using PROSSES server. The GDF5/ActRIIB complex passed the quality test.

Results and Conclusions: Our data demonstrated that GDF5 interacts with ActRIIB at lower affinity compared to the GDF5 / BMPRI complex. GDF5 regions interacting with ActRIIB are not localized in one region. There are three GDF5 regions that interact with ActRIIB. The interaction is formed by thirteen hydrogen bonds. No salt bridges or disulfide bonds are involved in the interaction (GDF5 /BMPRI complex is formed by six hydrogen bonds and one salt bridge). These data could be used to develop agonists and antagonists to modulate the functions of GDF5.



ActRIIB		x	GDF5		Interface area Å2	ΔG kcal/M	ΔG P-value	NHB	NSB	NDS
iNat	iNres		iNat	iNres	1082.1	-12.5	0.239	13	0	0
105	30	0	123	42						

Table 1: GDF5/ActRIIB interface data. iNat: interfacing atoms. iNres: interfacing residues. HB: Hydrogen bonds. SB: Salt bridges. DS: Disulfide bonds. P value less than 0.5 indicates specific interaction

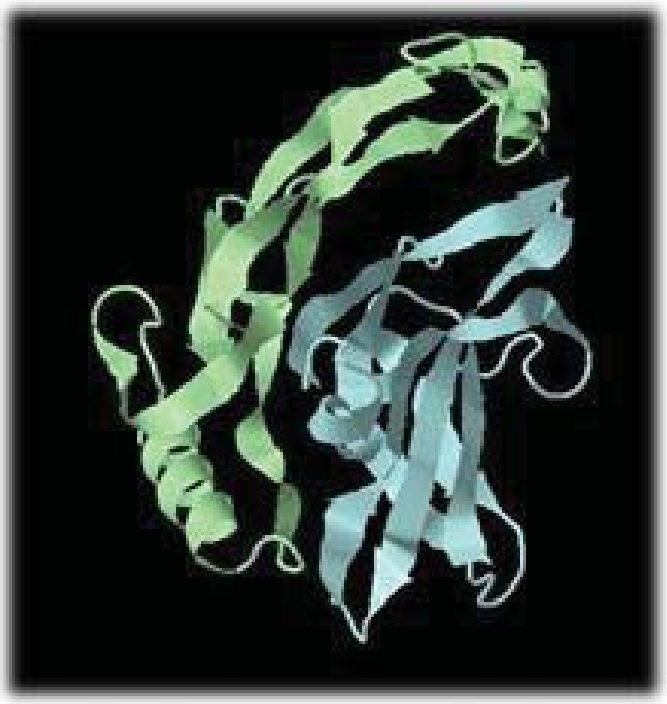


Figure 1: GDF5/ActRIIB complex. GDF5 is green. ActRIIB is blue. Image was generated using Jmol.

***Disclosures: Abdulhafez Selim**, None.*

P69

PDGFBB Promotes PDGFR Alpha-positive Cell Migration into Artificial Bone in vivo.

Shigeyuki Yoshida*[1], Ryotaro Iwasaki[2], Hiromasa Kawana[2], Taneaki Nakagawa[2], Takeshi Miyamoto[3]. [1], Japan, [2]Dentistry & Oral Surgery, [3]Keio University School of Medicine, Japan

Purpose: Bone defects caused by traumatic bone loss or tumor dissection are now treated with auto- or allo-bone graft, or sometime artificial bone transplantation. Large bone defects often cause difficulties in providing auto- or allo-graft bones and thus artificial bones are used to fulfill the bone defects. Implantation of artificial bones often results in bone affinity failure likely because the artificial bones contain no mesenchymal stem cells/pre-osteoblastic cells. Growth factors may induce cell migration into artificial bones, however, several growth factors are known to inhibit osteoblastogenesis. Thus we searched a growth factor, which induce cell migration but not inhibit osteoblast differentiation.

Methods: Mouse osteoblastic cell line, MC3T3-E1, cells were seeded in 96-well tissue culture plates at a density of 1×10^4 cells/well. On achieving con?uence, cells were cultured in α-MEM containing 10% FBS in the presence or absence of bone morphogenetic protein-2 (BMP-2) (300 ng/ml) with or without platelet derived growth factor BB (PDGFBB) (10 ng/ml), hepatocyte growth factor (HGF) (10ng/ml), fibroblast growth factor (FGF2) (10 ng/ml) and Transforming growth factor-beta1 (TGF-b1) (10ng/ml) for 72 hours. Thereafter, cells were subjected to realtime PCR. β-TCP was impregnated with or without 2 ug of PDGFBB, followed by implantation into hamstring muscles of mouse hind paws. At 3 and 7 days post-implantation, mice were euthanized by cervical dislocation, and β-TCP were dissected and subjected to histological analyses.

Results: We found that PDGFBB did not inhibit or even stimulated osteoblast differentiation shown by Alkaline Phosphatase (ALP), osteocalcin (OCN), Runt-related transcription factor 2 (RUNX2), and Osterix (OSX) expression compared with TGFβ, HGF, and FGF. PDGFBB also promotes significant increase in migration of PDGF receptor alpha (PDGFRa)-positive mesenchymal stem cells/pre-osteoblastic cells into artificial bones *in vivo*.

Conclusion: These results suggest that combination of artificial bones and PDGFBB is of benefit to promote host cell migration into artificial bones without inhibiting osteoblastogeneis.

***Disclosures: Shigeyuki Yoshida**, None.*

P70

Discordant Bone and Muscle Adaptation to Multiple Microgravity Exposure with Interposed Resistance Exercise.

Yasaman Shirazi-Fard*, Kevin Shimkus, Jacqueline Perticone, Derrick Morgan, Joshua Davis, James Fluckey, Susan Bloomfield, Harry Hogan. Texas A&M University, USA

Bone and muscle responses to repeated exposures to microgravity remain a concern for astronaut health. Previously, we showed that incorporating resistance exercise during recovery from disuse not only provides benefits to bone during reloading but also for subsequent exposure to disuse even with cessation of exercise. This study focuses on primary muscle outcomes as well as the bone-muscle relationship.

Adult male Sprague-Dawley rats (6mo.) were assigned to age-matched cage controls (CC) and hindlimb unloaded (HU) groups by body weight and total (integral) vBMD. HU animals were exposed to 28d of hindlimb suspension, followed by 56d of recovery, and then a 2nd HU exposure (2HU). HU animals also performed squat jumping resistance exercise protocol during recovery (2HU+EX). Animals were operantly conditioned, then trained over 6 weeks (3d/wk) with exercise intensity increasing weekly. At baseline and then every 28 days, longitudinal *in vivo* pQCT scans were taken at proximal tibia metaphysis (PTM) and a group (n=15) was euthanized. The soleus, plantaris, and gastrocnemius muscles were excised, weighed, and snap-frozen in liquid nitrogen. Rates of muscle protein synthesis were assessed by deuterium oxide incorporation and measured via gas chromatography-mass spectrometry.

Exercise led to a significant increase in relative muscle mass (i.e., normalized by body weight) in the soleus (91%) and gastrocnemius (24%), but it did not protect against muscle loss during the 2nd HU exposure. Rates of protein synthesis were depressed due to unloading in soleus (-10%, n.s.) and gastrocnemius (-18%). Resistance exercise did not alter rates of protein synthesis after the 2nd HU as 2HU+EX was not different from 2HU or age-matched control. However, 2HU was significantly lower than CC, which suggests beneficial effects of exercise. Considering the ratio of PTM total BMC to total muscle mass (MM), bone and muscle recovers with a constant upward trend for 2HU+EX ratio during exercise, which is significantly higher than the declining trend for 2HU ratio. During the 2nd HU, BMC/MM for 2HU+EX increases significantly and at a higher rate than 2HU. These data suggest that there is a mismatch between bone and muscle adaptation to microgravity or recovery therefrom; interestingly, moderate resistance exercise prior to a subsequent bout of unloading is more beneficial to bone than to muscle.

***Disclosures: Yasaman Shirazi-Fard**, None.*

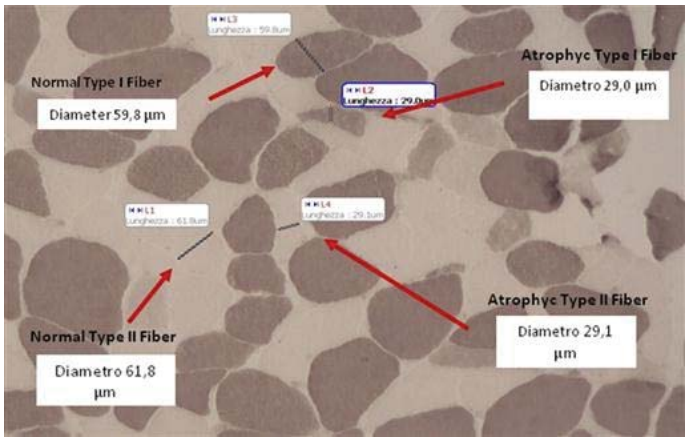
P71

Hip Fracture and Sarcopenia: A Model of Osteoporosis-Related Muscle Atrophy.

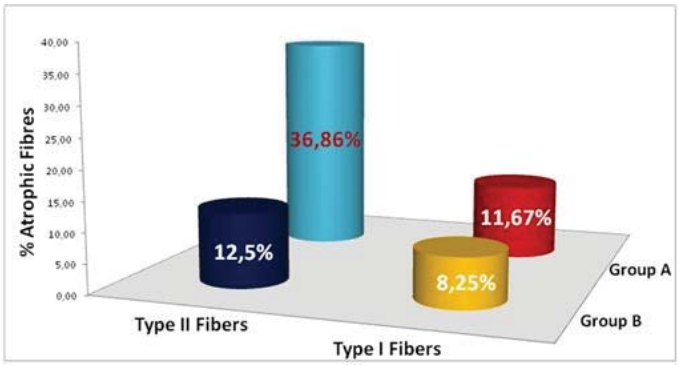
Umberto Tarantino*[1], Monica Celi[2], Jacopo Baldi[3], Fabio Luigi Perrone[3], Federico Maria Liuni[3], Elena Gasbarra[3]. [1]Azienda Ospedaliera PTV, Italy, [2]University of Rome Tor Vergata, Italy, [3]Orthopaedic Department University of Rome Tor Vergata

Osteoporosis and sarcopenia are considered two of the hallmarks of the aging process; the reduction of muscle mass and power may affect bone strength and Bone Mineral Density (BMD) by modifying loads to the skeleton. This could lead to an increased risk of fragility fracture, functional limitations and motor dependency. Aim of the study was to evaluate the degree of muscular atrophy in patients with osteoporosis and osteoarthrosis and to determine the role of IGF-1/PI(3)/Akt signaling pathway in the genesis of a specific type II osteoporosis-related muscle atrophy. For this study we performed vastus lateralis biopsy in 60 women (mean age 71,53 $\pm$ 9,74) underwent orthopaedic surgery; 30 women with osteoporosis undergoing primary Total Hip Arthroplasty (THA) for hip fracture (Group A); 30 women underwent surgery for hip osteoarthritis with no significant functional limitations (Group B). Muscle fibers were measured and classified by ATPase reaction. To evaluate whether Akt and pAkt are involved in osteoporosis-related muscle atrophy, muscle homogenates of twelve patients from Group A presenting higher percentage of type II fiber atrophy and twelve age-matched control biopsies from Group B were immunoblotted. In Group A atrophic type-II fibers were 3-fold more frequent than atrophic type I fibers (36,86%; $p<0.01$); in Group B the same fibers atrophy were 1.5-fold more frequent than type I atrophy (12,50%; $p<0.001$). Muscular atrophy significantly correlates with osteoporosis degree ($p<0.05$) in Group A; in Group B it significantly correlates disease duration, degree of pain and functional impairment of hip joint. Furthermore patients with higher percentage of type II fiber atrophy from Group A shown values of intramuscular Akt two and a half times (60%; $p<0,01$) lower than the same values from Group B. Immunoblotting didn't verify the activation status of intracellular pAkt probably due to its low expression in atrophic fibers. Osteoporotic patients shown a preferential and diffuse type II fiber atrophy, that seems to be related to disease severity. On the opposite, in osteoarthritic patients, atrophy involves seems to be caused by disuse and pain. The reduction of Akt observed in the muscle of individuals from the osteoporotic group, may be one of the possible moments of compromise in intracellular IGF-1/PI(3)/Akt signaling pathway that is likely to stimulate protein synthesis and cell survival as a result of activation of the complex mTOR/p70S6K22.

Muscle histomorphometry



Rate of atrophy of Type I and Type II muscle fibers in Group A and Group B



***Disclosures: Umberto Tarantino**, None.*

P72

Cyclooxygenase-1 plays an important role in C2C12 myogenic differentiation.

Chenglin Mo*[1], Orisa Igwe[2], Marco Brotto[1]. [1]University of Missouri-Kansas City, USA, [2]University of Missouri

Our previous studies have shown that prostaglandin E_2 (PGE_2) signaling through EP1 receptor is critical for myogenic differentiation in C2C12 myoblasts. Treatment with 50nM PGE_2 considerably increased myotube cell area and number of fully matured myotubes, whereas treatment with SC51322, a specific EP1 inhibitor, significantly attenuated myotube development. Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are the rate-limited enzymes in PGE_2 synthesis. To determine which COX is responsible for PGE_2 production in myogenesis, we first investigated the expression of COX-1 and COX-2 by Real-Time (RT) PCR and Western Blot (WB) analyses in C2C12 myoblasts. Both PCR and WB results indicated that COX-1 had higher expression than COX-2. Next, we did a time-course study of mRNA expression of COX-1, COX-2, and the three known isoforms of PGE_2 synthase (mPGES-1, mPGES-2 and cPGES) during myogenic differentiation. After the onset of differentiation, COX-1 expression was upregulated by 2-fold at 6 and 12h, and returned to baseline levels (time 0) at 24h, followed by a 2-fold downregulation at 48h. On the other hand, COX-2 expression was downregulated over this period, reaching a 5-fold downregulation at 48h. The WB results showed similar trends to those observed with RT-PCR; only COX-1 was upregulated at 12h, and returned to baseline levels at 48h. For PGES, the gene expression of mPGES-1 and cPGES did not show significant changes, but mPGES-2's profile was similar to that of COX-1, showing upregulation by 4- and 2-fold at 6 and 12h, respectively. Furthermore, the effects of the specific COX-1 inhibitor (FR122047), specific COX-2 inhibitor (NS-398), and non-specific COX-1/2 inhibitor (Indomethacin) on myogenic differentiation were tested. FR122047 (7.5 µM) and indomethacin (100 µM), but not NS-398(50 µM), significantly decreased myotube area after 96h of differentiation (myotube area was ~45% and ~53% of control, respectively). The co-treatment with EP1 agonist 17-phenyl trinor and PGE_2 successfully rescued myotube cell area reduction that had been induced by treatments with FR122047 and Indomethacin. Our results provide strong evidence that EP1 signaling is essential for myogenesis, and indicate that COX-1 may play a more prominent role in myogenic differentiation than COX-2.

Acknowledgements: This work was supported by NIDCR DE021888 (OJI), and by Missouri Life Sciences Research Board and an NIH RC2 AR058962 (MB).

***Disclosures: Chenglin Mo**, None.*

Preventing and Treating Muscle and Bone Loss

P73

Muscle-myopathy & X-linked Hypophosphatemic rickets (HYP).

Lesya Zelenchuk, Anne-Marie Hedge, Peter Rowe*. University of Kansas Medical Center, USA

Hypophosphatemia, rickets, myopathy, muscle-pain, increased bone-cell cathepsin-D and PHEX-related proteases (Neprilysin, ECEL1/DINE) occur in PHEX-defective mice (HYP). The increased proteases accelerate the release of protease-resistant ASARM-peptides from up regulated bone-SIBLING proteins (MEPE for example). Here we use several murine models and osmotic pump-infusion experiments to unravel the role of cathepsin D, Neprilysin-like activity and cystatin-C (a serine protease inhibitor) in the pathology of HYP.

HYP mice and "MEPE-null/ASARM-peptide" transgenic mice (MnAt) were used as ASARM-peptide over expressing groups. Wild type (WT) and MEPE null mice (Mn) were used as normal and reduced ASARM-peptide groups respectively. WT and HYP mice were infused with vehicle (VE), ASARM-peptide or a synthetic peptide (SPR4-peptide) that binds and neutralizes ASARM activity (WT-VE, WT-ASARM, HYP-VE and HYP-SPR4 respectively). Serum-urine chemistries were measured and protein/mRNA expressions calculated from femurs and kidneys. Bone marrow stem cell cultures were used to calculate cathepsin D and B enzyme activities. Femurs and kidneys were scanned by micro computed tomography (μCT).

HYP mice, MnAt mice, and WT-ASARM mice had hypophosphatemia with increased fractional excretion of phosphate (FEP), serum FGF23 and mineralization defects. Bone cultures from HYP mice and WT-ASARM mice displayed increased cathepsin D & B activities. Increased expression of sclerostin occurred with HYP, MnAt and WT-ASARM mice compared with WT mice. Increased cystatin-c expression occurred in HYP mice and MnAt mice compared to WT mice. SPR4 peptide infusion prevented the increased cystatin c expression and suppressed sclerostin expression.

In conclusion, loss of PHEX in HYP-mice results in increased expression of PHEX-related proteases (ECEL1/DINE) and cathepsin D activity. Cathepsin D then activates cathepsin B and inactivates cystatin-C, a serine-protease inhibitor. The resulting increased protease activity degrades matrix SIBLINGs including MEPE and releases protease-resistant ASARM-peptides. The increased acidic ASARM-peptide levels and protease activity likely contributes to the myopathy and bone defects. Also, cystatin-C is a TGFb responsive protein that influences matrix-metalloproteinase 2 (MMP2) and SPARC regulation of myogenesis. Further studies of HYP muscle pathology will increase our understanding of musculoskeletal physiology in disease and health.

***Disclosures: Peter Rowe**, None.*

P74

A Potential "FRAX-like" Approach to Sarcopenia Diagnosis.

Bjoern Buehring, Ellen Fidler, Jessie Libber, Bryan Heiderscheit, Jennifer Sanfilippo, Diane Krueger, Neil Binkley*. University of Wisconsin, Madison, USA

Background: Sarcopenia (SP) has adverse consequences including falls and fractures. Various SP definitions, including those of 2 recent consensus conferences exist. These are based on muscle mass, appendicular lean mass/height^2 (ALM/ht^2) ratio or a combination of muscle mass + function, (low ALM/ht^2 + slow gait speed and/or low grip strength). Current definitions are imperfect as they may not identify the same individuals as sarcopenic and do not consider fat mass. We hypothesized that a "FRAX-like" approach to SP diagnosis by combining risk factors might better identify those at risk for falls and fractures. To begin evaluating this concept, our study aim was to compare SP prevalence using current definitions with that obtained using a scoring system based on muscle, fat and bone mass, muscle function, falls history and age.

Methods: Community dwelling adults age 70+ underwent DXA body composition measurement and performed a battery of muscle function tests. DXA results were used to calculate ALM/ht^2 and a potential measure of muscle fat, the leg fat/lean mass ratio. This ratio is an attempt to include effects of obesity on function (i.e. sarcopenic obesity). A fat/lean ratio $\geqslant$2.5 SD above the mean of 329 young athletes (178M/151F) was defined as high. SP prevalence was determined using low ALM/ht^2, the European consensus, (low gait speed or grip strength + low ALM/ht^2) and the International consensus (low gait speed + low ALM/ht^2). SP prevalence using an alternative ("FRAX-like") 0 - 7 scoring system with 1 point each for low ALM/ht^2, grip strength, and gait speed, high leg fat/lean ratio, low BMD, history of falls in the last year and age $\geqslant$65 was explored.

Results: 97 older adults (49 F/48M; mean age 81 years) were studied. SP prevalence was 24%, 20% and 10% based on ALM/ht^2, the European and International approach respectively. SP prevalence was 40% using a "FRAX-like" score of $\geqslant$4. All differences were significant by chi-square test (p-values $\leqslant$ 0.001).

Conclusion: Current criteria do not identify the same proportion of older adults as sarcopenic. A risk score combining several measures important for adverse outcomes related to SP identifies a larger proportion as potentially being at risk. As ~50% of adults over age 50 fall annually, it is possible that this "FRAX-like" approach may be a more sensitive predictor of adverse outcomes. Future research is necessary to validate proposed SP definitions.

***Disclosures: Neil Binkley**, None.*

P75

Risk Factors, Frequency and Treatment of Bone Mineral Loss in Survivors of Childhood Allogeneic Bone Marrow Transplantation: A Single Institution Review.

Carla McCrave*, Celia Gonzales, Nancy Shreve, Rukhsana Rahmetulla. Children's Mercy Hospital, USA

Recent data from the International Bone Marrow Transplant Registry reported that 14,309 persons less than 21 years of age are alive after undergoing allogeneic bone marrow transplantation (allo BMT) between 1968 and 2002. As a result of the growing success of pediatric allo BMT, an increasing number of long-term survivors are at risk for disease-and-therapy-induced adverse effects, including diminished bone mineral density and osteonecrosis. Little is known about what factors contribute to bone loss in the pediatric allo BMT patient and limited guidelines are available to direct management of these patients.

Purpose: Evaluate risk factors, frequency, severity and management of bone mineral loss in survivors of pediatric allo BMT.

Methods: We will conduct a retrospective review of bone mineral loss in survivors of pediatric allo BMT at Children's Mercy Hospital from 1997 to 2011 to evaluate the following variables: demographic information, bone mineral density using dual photon x-ray absorptionmetry (DEXA), parameters significant to bone metabolism (calcium, phosphorus and vitamin D), contributing factors associated with bone loss, and management strategies of bone mineral loss.

Summary: Investigation of decreased bone mineral density in children who have undergone an allogeneic BMT is needed to determine risk factors, long-term implications, appropriate preventive measures and potential interventions.

***Disclosures: Carla McCrave**, None.*

P76

Genetic Variant on *PLIN4* is Associated with Obesity Phenotypes and BMC in Females.

Mai Abdel-Ghani*[1], Laura Tosi[2], Joseph Devaney[3], Todd Spock[4], Karin Kuhn[4], Eric Rupe[4], Clare Griffis[5], Heather Gordish-Dressman[3], Eric Hoffman[3], Priscilla Clarkson[6]. [1]George Mason University, USA, [2]Children's National Medical Center, USA, [3]Research Center for Genetic Medicine Children's National Medical Center, USA, [4]George Washington University School of Medicine, USA, [5]F. Edward Hébert School of Medicine Uniformed Services University of the Health Sciences, USA, [6]University of Massachusetts Amherst, USA

The purpose of this study was to examine a single nucleotide polymorphism (SNP) rs8887 in Perilipin 4 (PLIN4) due to its expression in adipose tissue and its functional role in obesity and possible impact on bone mineral density. Whole blood samples were collected from 133 healthy subjects of whom 67 are males and 69 are females average age of 22.05 $\pm$ 4.82 yrs for females and 23.31 $\pm$ 5.61 yrs for males. A Dual energy xray absorptiometry (DXA) measured bone mineral density (BMD) lean mass, fat mass. Genomic DNA was extracted from blood for genotyping. Genotypes for rs8887 in PLIN4 were obtained using a TaqMan allelic discrimination assays. All PCR reactions were analyzed using an ABI 7900 Quantitative Real Time PCR system. Mean quantitative adiposity and bone measurements were compared in relation to SNP genotypes using analysis of covariance (ANCOVA) methods. Significant associations were found in both males and females. In males, significant associations with PLIN4 were seen with total fat mass (p=0.0136), total lean mass (p=0.0268) and lean mass of the arms (p=0.0097). Males homozygous for the T allele showed increased total fat mass to males with a single copy of the C allele; TT (N=13; 21727 $\pm$ 1269 g), CC (N=25; 17819 $\pm$ 909 g),CT(N=29; 17187 $\pm$ 845g).Males who were homozygous for the minor T allele had reduced total lean mass than males with a single copy of the C allele; TT (N=13; 55370 $\pm$ 1249g), CC (N=25; 58880 $\pm$ 894g), CT(N=29; 59450 $\pm$ 831g). Males homozygous for the T allele showed decreased arms lean mass in contrast to males with a single copy of major C allele; TT (N=13; 6524 $\pm$ 207g), CC (N=25; 6866 $\pm$ 148g),and CT (N=29; 7275 $\pm$ 137g). In females, significant associations were found with total fat mass (p=0.0157) and bone mineral content (p=0.0216). Females who were homozygous for the C allele showed a greater mean total fat mass in comparison to both heterozygotes and those homozygous for the T allele;TT (N=17; 20097 $\pm$ 757g),CC (N=19; 22882 $\pm$ 706g),CT (N=31; 20553 $\pm$ 551g). Females homozygous for the T allele exhibited increased mean BMC in comparison to those homozygous for the T allele;TT (N=17; 2608 $\pm$ 70), CT (N=31; 2430 $\pm$ 52), CC (N=19; 2359 $\pm$ 65). There was a suggestion of an association between total BMD and PLIN4, although it did not reach statistical significance (p=0.0523). This study demonstrates an association between a variant in PLIN4 and adiposity phenotypes and BMC in females.

***Disclosures: Mai Abdel-Ghani**, None.*

P77

Animal Models of Sarcopenia: Orchidectomized Rat and Monkey Models.

Aurore Varela*[1], Elisabeth Lesage[1], Nancy Doyle[1], Solomon Haile[1], Joseph Arezzo[2], Susan Y. Smith[1]. [1]Charles River Laboratories, Canada, [2]Albert Einstein College of Medicine, USA

Sarcopenia with aging is devastating for quality of life, and related healthcare expenditures are enormous. Androgens may indirectly affect musculoskeletal homeostasis through interaction with body composition and the GH-IGF-I axis. In aging humans, abnormalities in GH/IGF1 signaling may contribute to reduced functional efficiency in the muscle–bone system. This study evaluated the effects of orchidectomy (ORX) and testosterone depletion on muscle in aged Sprague-Dawley rats and cynomolgus monkeys (NHP).

Animals (10 six month-old male rats or 20 male NHP aged $\geqslant$ 9 years old randomized by body weight (BW) or BMC) were ORX or Sham operated. In rats, fat/lean analysis by pQCT was measured at 0, 6 and 13 weeks at the proximal tibia metaphysis and diaphysis. Muscle weights were recorded at necropsy after 13 weeks. Conduction velocity was recorded in the mixed caudal nerve, the sensory digital nerve, and the distal motor branches of the tibial nerve innervating plantar muscles of the foot at 0, 6 and 12 weeks. In NHP, muscle mass was assessed by DXA, pQCT at 4, 8, 12 and 16 months post-ORX. Another subgroup of NHP (n=7) was used for muscle weight at necropsy 10 weeks post-ORX: tibialis cranialis, gastrocnemius, EDL (extensor digitorum longus), biceps brachii.

In rats, muscle area by pQCT was decreased 6% and 13% at Weeks 6 and 13, respectively, with no significant changes in muscle density or fat area. Paired muscle weights showed consistent trends for lower gastrocnemius and EDL weights by 4%. The induced changes in muscle were not associated with slowing of either sensory or motor nerve conduction velocity. In NHPs, BW decreased by 20% during the first 4 months after ORX and then stabilized. Muscle area decreased 18% compared to pre-ORX. There was no effect on % body fat. After 10 weeks, paired muscle weights showed lower biceps, gastrocnemius and EDL weights by 23, 25 and 21%, respectively, for ORX NHPs compared to shams.

The ORX rat and NHP are established models of osteoporosis with bone loss associated with increased bone turnover; the present study indicates that these models can also be valuable to evaluate androgenic compounds and agents that may modify androgen deprivation-induced sarcopenia. Associated with muscle biomarkers and muscle histomorphometry, these tests provide a comprehensive evaluation of muscle mass and function for preventing and treating muscle loss.

***Disclosures: Aurore Varela**, None.*

P78

The Relationship between Changes in Lean Body Mass and Bone Mineral Density in Active, Adult Males with Osteopenia after a 12-Month Exercise Intervention.

Melissa Carter*, Timothy Sinak, Jiang Juan, Peggy Nigh, Pamela Hinton. University of Missouri, USA

Weight-bearing exercise may positively affect bone via muscle contractile and impact forces; however, the importance of these forces to the osteogenic effect of exercise remains controversial. Thus, we examined the effects of resistance training (RT, a high-muscle-force activity) and plyometrics (PLY, a high-impact activity) on bone mineral density (BMD) in active ($\geqslant$4hr/wk), osteopenic, but otherwise healthy men between the ages of 25 and 60y. In addition, we examined the relationships between changes in lean body mass (LBM) and BMD of the whole body, weight-bearing (leg, hip) and non-weight-bearing (arm) sites. Participants were randomized to 12 mo of supervised RT (2x/wk, N=10) or PLY (3x/wk, N=10). Each participant received supplemental calcium (1200 mg/d) and vitamin D (10 μg/d) for the duration of the study. LBM and BMD of the whole body, weight-bearing (legs and hips) and non-weight-bearing (arms) sites were measured at baseline and after the intervention using dual-energy X-ray absorptiometry. Statistically significant changes in whole body and regional (i.e., upper/lower body) LBM and BMD were evaluated using a 2x2 ANOVA (time, group). Relationships between percent changes in LBM and BMD were assessed using

Pearson's product moment correlations. Whole body (time, p=0.036) and leg (time, p=0.020) BMD significantly increased after the intervention with no differences between RT and PLY. By contrast, hip BMD increased in the RT group, but remained unchanged in PLY (group x time, p=0.035). Whole body and leg LBM did not change significantly after 12 mo of either RT or PLY. Arm LBM significantly decreased in the PLY group and remained unchanged in the RT group (group x time, p=0.011). Whole body LBM was significantly correlated with Hip BMD (r=0.570, p=0.043) in the PLY, but not the RT, group. In conclusion, the results of the present study suggest that weight-bearing exercise that elicits either high- muscle-contraction forces or high-impact forces may positively affect whole body and leg BMD, while hip BMD increased following high-muscle-contraction force exercise in physically active men with osteopenia.

***Disclosures: Melissa Carter**, None.*

P79

Improved Osteochondral Allograft Preservation using Serum-free Chemically-defined Media.

Joseph Garrity*, James Cook, Aaron Stoker. University of Missouri, USA

Purpose: Osteochondral allografts (OCAs) are currently preserved at 4℃ and used within 28 days of donor harvest. The window of opportunity for implantation is limited to 14 days due to a two week disease testing protocol. This study was performed to assess the effects of storage up to 56 days in a serum-free chemically defined media at 37℃.

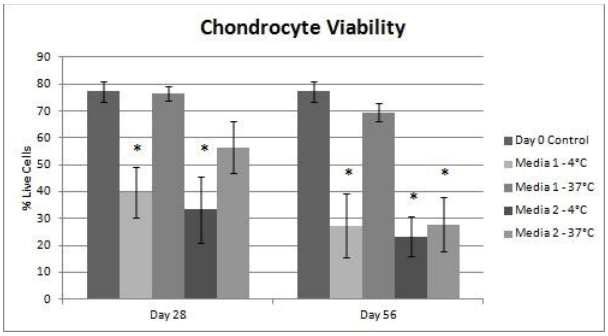
Methods: OCAs from 15 adult canine cadavers were aseptically harvested within four hours of death. Medial and lateral femoral condyles were stored in Media 1, a basic media similar to the current standard, or Media 2, an anti-inflammatory and chondrogenic media containing dexamethasone and TGFb3, at 4℃ or 37℃ for up to 56 days. Chondrocyte viability, proteoglycan (GAG) and collagen (HP) content, biomechanical properties, and collagen II and aggrecan content were assessed at Days 28 and 56. Five femoral condyles were stored overnight and assessed the next day to serve as controls.

Results: Storage in Media 1 at 37℃ maintained chondrocyte viability at significantly higher levels than in any other media temperature combination and at levels not significantly different from controls. OCAs stored in either media at 4℃ showed a significant decrease in chondrocyte viability throughout storage. Glycosaminoglycan (GAG) and hydroxyproline (HP) were maintained through 56 days of storage in OCAs in Media 1 at 37℃. There were no significant differences in elastic or dynamic moduli among groups at Day 56. Qualitative immunohistochemistry demonstrated the presence of collagen II and aggrecan throughout all layers of cartilage.

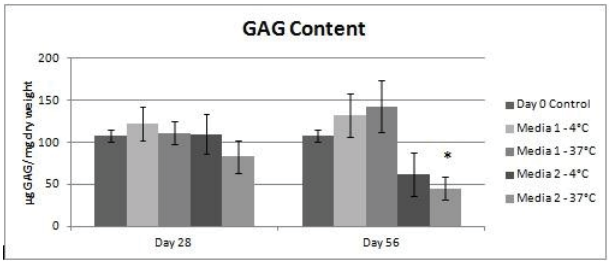
Conclusions: OCA viability, matrix content and composition, and biomechanical properties were maintained at ''fresh'' levels through 56 days of storage in media 1 at 37℃. OCAs stored at 4℃ were unable to maintain viability or matrix integrity through 28 days of storage. These findings suggest that storage of OCAs in a defined media at 37℃ is superior to current protocols (4℃) for tissue preservation prior to transplantation. Storage of OCAs in a serum-free chemically-defined media at 37℃ can ''increase the window of opportunity'' for implantation of optimal tissue from 14 days to 42 days after disease testing clearance.

The use of tissues from dogs that have been humanely euthanized for unrelated reasons with documented consent for disposal at the university is allowed by the general rules and regulations of the IACUC.

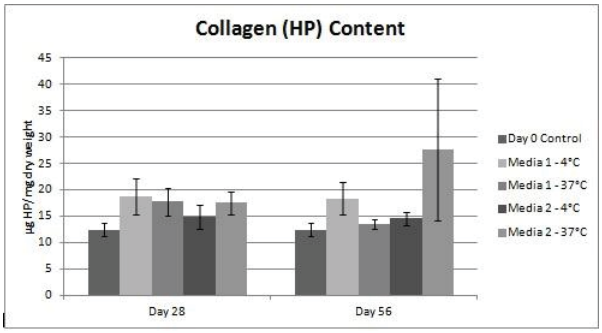
Chondrocyte viability



GAG Content



HP Content



***Disclosures: Joseph Garrity**, None.*

P80

Estrogen and Estrogen Receptors are both Involved in the Recovery of Atrophic Skeletal Muscle in Mice.

Johan Ferreira*, Andrew Dunn, Madoka Spence, James Langworthy, Eric Mayfield, Marybeth Brown. University of Missouri, USA

Prior studies from our laboratory revealed that estrogen (E2) plays a role in the recovery of atrophic muscle in rats. E2 effects reportedly are mediated through the estrogen receptors (ER) alpha and beta although a third receptor (GPER) has been postulated to be involved in E2 signaling. In addition, growth factors other than E2 have been reported to activate ER.

Purpose: To further delineate the roles of E2 and ER in the recovery of atrophic muscle we studied mice that were intact, ovariectomized (OVX), or treated with the ER blocker ICI 182780.

Methods: Intact and OVX 5-6 mos C57BL6 female mice were each allocated to 1 of 4 groups: control, hindlimb unweighted (HLU) for 28 days, 3 days of recovery following HLU, or after 3 days of recovery from HLU + ICI. The two ICI groups were given 0.25mg daily for the final 2 weeks of HLU and through recovery.

Body weight, muscle mass and contractile force (Po) of soleus, gastrocnemius, tibialis anterior, plantaris were determined at terminal experiment. Subsequently, muscles were sectioned at 10μ in a cryostat and stained with H&E for determination of cross-sectional area (CSA).

Results: Body weight did not change with HLU. OVX independently resulted in a decline inPoin all muscles studied even though muscle mass increased. HLU resulted in a significant decline in muscle mass in all 4 muscles studied in OVX and intact mice (p

***Disclosures: Johan Ferreira**, None.*

This study received funding from: MU Research Board Grant, HDO5834

P81

Interactions of Hindlimb Unloading, Ovariectomy, and Recovery on Hindlimb Muscle Function and Femoral Architecture and Strength in Female Mice.

Charlotte Phillips*[1], Johan Ferreira[1], Stephanie Carleton[2], Andrew Dunn[1], Mark Johnson[3], Marybeth Brown[1]. [1]University of Missouri-Columbia, USA, [2]Unversity of Missouri, USA, [3]University of Missouri, Kansas City Dental School, USA

Low sex hormone levels and bed rest are known to contribute to bone loss and muscle atrophy.

Purpose: To evaluate the interactions of simulated bed rest [hindlimb unloading (HU)] and low estrogen (E2) levels [ovariectomy (OVX)], and recovery on lower extremity muscle mass and function and bone geometry and strength in female mice.

Hypothesis: The combination of HU and low E2 levels will result in additive losses in muscle and bone, and attenuated recovery following HU.

Methods: 5-6 mo C57/BL6 mice were randomized into 6 groups: 1) CRTL (intact), 2) HU (sham OVX, HU-28 days), 3) HU+R3 (sham OVX, HU-28 days, 3 days re-ambulation), 4) OVX, 5) OVX-HU (OVX, HU-28 days), and 6) OVX-HU+R3 (OVX, HU-28 days, 3 days ambulation). Following HU or recovery contractile function studies were performed on the soleus (S), plantaris (P), gastrocnemius (G), and tibialis anterior (TA). Tibias and femurs were removed for μCT analyses of bone geometry/architecture and torsional loading to failure to evaluate whole bone biomechanical strength and bone material properties.

Results: HU induced atrophy and loss of muscle force in all muscles studied in sham and OVX mice. G, TA, P and S failed to recover muscle mass and contractile tension after 3 days re-ambulation in OVX-HU+R3, in contrast to HU+R3 mice. By μCT, we demonstrated that femoral marrow cavity diameter increased and cortical bone width decreased in OVX-HU and OVX-HU+R3 mice as compared to CTRL mice (normal cage ambulation). Femurs from OVX-HU and OVX-HU+R3, exhibited an 18% and 15% decrease in the whole bone parameter, ultimate breaking strength, T_{max}, respectively, which reflects not only changes in geometry, but also decreases in tensile strength (Su) of the bone material. OVX-HU femoral Su appeared able to respond to 3 days re-ambulation (OVX-HU+R3), though T_{max} and energy to failure had not. OVX-HU+R3 femoral energy to failure was reduced by 19% compared to CRTL mice. Tibias examined by μCT demonstrated that the trabecular bone volume/total volume (BV/TV) of the OVX-HU proximal tibias had decreased 70% compared to CRTL tibias. However, 3 days of re-ambulation resulted in an increase in the tibial BV/TV such that the BV/TV was only decreased by 48% of CRTL in OVX-HU+R3. These data suggest rapid recruitment or activation of osteoblasts upon re-ambulation or loading.

Conclusions: E2 is critical for the complete recovery of muscle and bone mass and strength following loss due to simulated bed rest in female mice.

***Disclosures: Charlotte Phillips**, None.*

P82

A Muscle Specific Factor Increases Survival of Dexamethasone-Stressed Osteocytes.

Katharina Jähn*[1], Leticia Brotto[1], Nuria Lara[1], Chenglin Mo[1], William Gutheil[1], Mark Johnson[2], Marco Brotto[1], Lynda Bonewald[1]. [1]University of Missouri-Kansas City, USA, [2]University of Missouri, Kansas City Dental School, USA

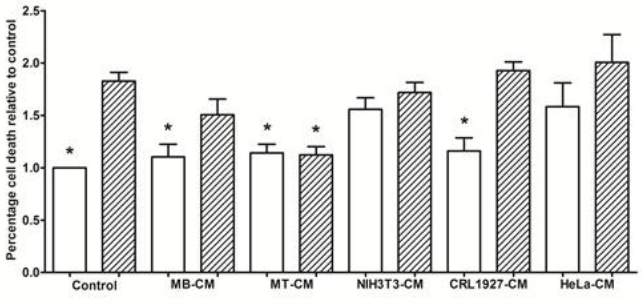
Exercise is beneficial for maintaining health, potentially through loading of both muscle and bone. We showed previously that mechanical loading induces the production of prostaglandin E2 (PGE_2) by osteocytes, which protected these cells from glucocorticoid-induced apoptosis. Muscles have been shown to produce factors termed 'myokines' that are released into the muscle interstitial space and the global circulation. The hypothesis for the present studies is that muscle may also produce factors that would promote osteocyte viability. Osteocyte viability has been shown to be important for skeletal maintenance and function. In this study, MLO-Y4 osteocyte-like cells and primary osteocytes were exposed to conditioned media (CM) from C2C12 myotubes (MT), myoblasts (MB) or *ex vivo* working EDL and Soleus muscle that were electrically stimulated to produce either a twitch (1Hz) or tetanic force (80Hz). Cell death was quantified using Trypan Blue exclusion assay and cell apoptosis was determined using the nuclear fragmentation assay. MT-CM but not MB-CM protected against dexamethasone-induced MLO-Y4 apoptosis. EDL CM from muscle stimulated at 80Hz was more potent than soleus CM stimulated at the same frequency and several magnitudes greater than CM from muscles stimulated at 1Hz.

Next we determined if the effects of the muscle CM were tissue specific. CM from MTs was compared to CM from HeLa epithelial cells, NIH3T3 fibroblasts, and CRL-1927 kidney mesangial cells for their ability to maintain osteocyte survival. Cell number/media volume was controlled for each cell type. No significant protective activity was found using non-muscle CM.

PGE_2 was not responsible for the protective effects of the muscle CM as muscle produces very low amounts that are insufficient to block apoptosis in this assay. In order to identify the active factor in MT- CM, we conducted experiments that indicate the active factor(s) is smaller than 10kDa, soluble in ethyl acetate and volatile. High pressure liquid chromatography (preparative C18 column, acetonitrile gradient) of ethyl acetate extracted CM showed active fractions could be collected. Mass spectrometric analysis of these fractions revealed peaks of 344, 353, 355 and 357 m/z that were only apparent in the MT not in the MB-CM.

These results support the concept that healthy, mature skeletal muscle promotes osteocyte survival upon exposure to dexamethasone through the secretion of muscle-specific factor(s).

MT-CM but not MB, NIH3T3, CRL or HeLa-CM protects against dexamethasone-induced cell death in MLO-Y4



***Disclosures: Katharina Jähn**, None.*

P83

Improving Outpatient Follow-up for Osteoporosis Management After a Hip Fracture.

Anika Alarakhia*, Robert Quinet. Ochsner Medical Center, USA

Purpose: Patients who are hospitalized for hip fractures are asked to follow-up with Rheumatology in 6-8 weeks after their hospitalization to assess their risk of osteoporosis and their need for a bisphosphonate and/or other approved treatments. Outpatient follow-up in our clinic after being hospitalized for a hip fracture has notoriously been poor. The purpose of this study was to determine the rate of outpatient follow-up of patients after a hip fracture and then to implement an intervention to increase the follow-up rate.

Methods: We conducted a chart review involving 50 hospitalized hip fracture patients prior to intervention, and 50 hospitalized hip fracture patients after intervention. The intervention included an informational handout which explained the risk of osteoporosis and risks that can lead to further fractures, the importance of receiving a bone density scan, and different treatments to help prevent further fractures that can be implemented as an outpatient. This informational handout was given to the patient and/or family prior to hospital discharge. The results were documented comparing clinic follow-up rates prior to intervention and follow-up rates after intervention.

Results: After retrospectively reviewing fifty charts from July 2006 to March 2011 of hospitalized hip fracture patients, it was noted that only 3/50 (6%) of patients followed up to the Rheumatology clinic after their hospitalization. A prospective review is currently being done on charts from May 2011, and is still ongoing. Out of the patients who received intervention, 15/41 (36.5%) of patients have followed up to the Rheumatology clinic after their hospitalization. This shows a significant increase in follow-up rates after the intervention was initiated.

Conclusion: We believe that outpatient follow-up for a patient after being hospitalized for a hip fracture is extremely important to prevent further fractures which can increase a patient's morbidity and mortality. After receiving an informational handout while in the hospital regarding osteoporosis and treatment options that can be provided as an outpatient, we found that the follow-up rate increased dramatically. It is concluded that once patients and their families fully understood the importance of seeing a Rheumatologist to assist in preventing further fractures, they were more willing to make a follow-up appointment with a Rheumatologist in the outpatient setting.

***Disclosures: Anika Alarakhia**, None.*

P84

Change of Muscle Strength, Muscle Mass, Muscle Related Markers in Rheumatoid Arthritis Patients Treated with Tocilizumab.

Akihide Nampei*[1], Makoto Hirao[2], Hideki Tsuboi[3], Shosuke Akita[3], Kosuke Ebina[2], Kenrin Shi[2], Hideki Yoshikawa[2], Jun Hashimoto[3]. [1]Osaka Rosai Hospital, Japan, [2]Osaka University, Graduate School of Medicine, Japan, [3]National Hospital Organazation, Osaka Minami Medecal Center, Japan

Background: Rheumatoid arthritis (RA) patients appear decreased muscle mass and muscle strength. The reason was considered as reduced physical activity from joint pain and stiffness, and increased resting energy expenditure from excess produced inflammatory cytokines. Previous reports showed that muscle related markers, such as serum creatine kinase (CK) and creatinine (Cr) were low level in RA patients.

Hypothesis: Does strong medication, such as potent biologic cytokine antagonists, improve the decreased muscle mass and the low level of muscle related markers in RA patients ?

Objectives: To investigate the muscle strength, muscle mass and muscle related markers sequentially against RA patients treated with tocilizumab (IL-6 receptor antibody).

Methods: Eleven RA patients treated with tocilizumab were enrolled. Disease activity score (DAS28CRP), C-reactive protein (CRP), matrix metalloproteinase-3 (MMP-3), modified health assessment questionnaire (MHAQ), grip strength, pinch strength, lean body mass, CK, Cr, Cystatin-C were measured before and 1, 3, 6 months after tocilizumab treatment.

Results: Mean age was 51.1 years. Disease duration was 7.7 years. Baseline CRP was 2.3 ± 2.2 mg/dl, MMP-3 was 321 ± 202 ng/ml, DAS28CRP was 4.3 ± 0.5. DAS, MMP-3 decreased immediately at 1 month and MHAQ at 3months. Grip and pinch strength were increased significantly at 3, 6 months (p=0.003, 0.001) and 6 months (p=0.019) respectively. Lean body mass tended to increase at 6 months, but not significantly (p=0.055). CK was not changed during 6 months, Cr was elevated significantly at 6 months (p=0.012), and Cystatin C was not changed during 6 months. The ratio of Cr/CystatinC was increased at 1 month significantly (p=0.045) and persisted until 6 months.

Discussion: Previous reports showed that grip and pinch strength were improved in RA patients treated with TNF antagonists, but lean body mass was not significantly changed at 12 weeks. Our results were consistent with these anti TNF reports. The reason of unchanged muscle mass might be that observation period was too short. Our results also showed CK was not changed, but Cr was elevated significantly. Since Cystatin C was not increased, the meaning of Cr elevation might not be the deterioration of renal function, but the early response of muscle mass increase

Conclusion: It might be possible that tocilizumab not only suppress the inflammation, but also improve the muscle metabolism.

***Disclosures: Akihide Nampei**, None.*

P85

Histomorphometric Analysis of Marrow Adipocytes after Treatment with Cinacalcet or Parathyroidectomy for Renal Hyperparathyroidism.

Aiji Yajima*[1], Yasuo Imanishi[2], Masaki Inaba[2], Yoshihiro Tominaga[3], Shigeru Satoh[4], Akemi Ito[5], Sharon Martin Moe[6]. [1]Akita University, School of Medicine, USA, [2]Osaka City University Graduate School of Medicine, Japan, [3]Nagoya 2nd Red Cross Hospital, [4]Division of Renal Replacement Therapeutic Science, Department of Urology Akita University, [5]Ito Bone Histomorphometry Institute, Japan, [6]Indiana University, USA

Purpose: Marrow cellular differentiation may be altered by changes in PTH. We hypothesized that PTH lowering by either parathyroidectomy or cinacalcet would alter marrow adipocytes.

Methods: In the 45 hemodialysis (HD) patients with secondary hyperparathyroidism, marrow adipocytes were evaluated by histomorphometric analysis of transiliac bone biopsy before and after treatment with cinacalcet (Cin; n=12 with baseline and 1 year, Age=59 $\pm$ 6, HD duration=10 $\pm$ 5 years) or total parathyroidectomy with reimplantation (PTX, n= 16 at 4 weeks, n = 4 at one year; Age=57 $\pm$ 9, HD duration=16 $\pm$ 7 years). Serum intact PTH was measured on the day of bone biopsy. Adipocyte volume (Ad.V/Ma.V; %), adipocyte number (N.Ad/Ma.V; N/mm^2) and mean adipocyte volume (Ad.V/N.Ad; $\times(10^{-3})$/mm^2) were calculated as the secondary parameters.

Results: The PTH dropped in the PTX group at 4 weeks from 1070 to 11.5 pg/ml. At 4 weeks in these patients, there was improvement in the parameters of osteitis fibrosa, and an increase in the marrow adipocyte volume (Adv/MaV; 20 $\pm$ 12 vs. 31 + 15%,

$p < 0.001$) and the number of adipocytes/marrow volume (N.Ad/Ma.V; 143 ± 86 to 189 + 76, p = 0.001). At one year, both treatments improved PTH (PTH decreased by 1162 ± 338 in PTX vs. 649 ± 326 pg/ml in the Cin groups), with a final PTH was 20 ± 15 in the PTX group vs. 100 ± 50 pg/ml in the Cin group, p = 0.006). The histologic parameters of osteitis fibrosis improved with marked decrease in osteoclast surface, osteoblast surface, and fibrosis in both treatments. However, after one year, the patients who underwent a PTX had no osteoclast surface Oc.S/Bs of 0.03 ± 0.05 % vs. those treated with cinacalcet (1.4 ± 1.9 %; p = 0.023) and no osteoblast surface Ob.S/BS (0 ± 0 vs. 12.3 ± 0.1%, p 0.004). After one year, there was a difference in the proportion of marrow filled with adipocytes in the PTX group (28.9 ± 14.4%) compared to the Cin group (44.4 ± 12.3%, p = 0.054). The number of adipocytes normalized by marrow volume in the PTX group was greater than the Cin group (91 ±104 vs. -12 ± 62, p = 0.03).

Conclusion: An acute reduction of iPTH increased marrow adipocytes 4 wks after parathyroidectomy. However at one year, this effect was lessened and no different than a more gradual reduction of iPTH with cinacalcet.

***Disclosures: Aiji Yajima**, None.*

P86

Bioactive PLGA/TCP/Icaritin Composite Scaffolds Reduce Collapse Incidence of Femoral Head in a Bipedal Emu Model of Steroid-Associated Osteonecrosis.

Lizhen Zheng*[1], Zhong Liu[2], Ming Lei[3], Le Huang[2], Yixin HE[2], Ge Zhang[4], Ling Qin[2]. [1]Hong Kong, [2]Chinese University of Hong Kong, Hong Kong, [3]Shenzhen Second People's Hospital, [4]Price of Wales Hospital, Hong Kong

Icaritin is a novel bioactive and osteogenic phytomolecule found in serum as a metabolite of phytoestrogenic herbal epimedium. Bioactive porous PLGA/TCP/Icaritin was fabricated using our established rapid prototyping technique. We have established a bipedal emu model of steroid-associated osteonecrosis (SAON), induced by a combination of methylprednisolone and Lipopolysaccharide, with hip joint collapse similar to humans that is desirable for preclinical research of osteonecrosis and subsequent joint collapse. This study is to evaluate treatment efficacy of PLGA/TCP/Icaritin scaffolds implanted for repairing steroid-associated osteonecrosis in emu femoral head after core decompression.

SAON was induced on 15 adult male emus (30 hips). 12 weeks after SAON induction, core decompression (bone tunnel) of a 6mm in diamter was created at proximal femur. A custom-made PLGA/TCP/Icaritin cylinder composite was implanted into the drill bone tunnel (P/T/I group, n=10), respectively. PLGA/TCP was used as vehicle control (P/T group, n=10) and no scaffold implanted group was used as empty control (Control group, n=10). 12 weeks after implantation, femora were collected and microCT was used to evaluate the collapse and local osteogenesis within volume of interests (the entire tunnel of 6mm diameter and the centre tunnel of 4mm diameter).

No animal died after SAON induction. Femoral head collapse incidence was 70% in the Control group, 30% in the P/T group and 10% in the P/T/I group. (Fig.2-A). Within the bone tunnel, newly formed bone was found in P/T/I group and P/T group while almost no new bone formed in Control group in micro-CT images (Fig.1). Micro-CT quantitative analysis showed that the BMD and BV/TV of newly formed bone in tunnel in the P/T/I group were both significantly higher than those in the P/T group (Fig.2-BC), indicating that PLGA/TCP/Icaritin composite scaffolds reduced incidence of collapse in femoral head by enhancing the repair of SAON lesions in femoral head in bipedal emus. No pathological changes were observed macroscopically.

This was the first efficacy study using bipedal emu model for prevention of joint collapse after core-decompression. Clinical trial registration is on the way before applying for SFDA approval for clinical research and applications. The innovative and bioactive PLGA/TCP/Icaritin porous scaffolds were able to reduce incidence of joint collapse in steroid-associated osteonecrotic femoral head of bipedal emus.

Figures

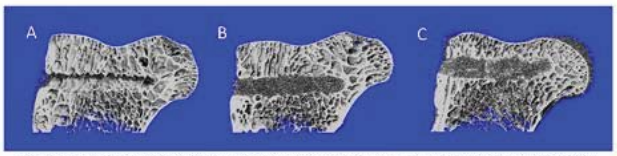


Figure 1: Representative 3-D micro-CT images of Control group (A), P/T group (B) and P/T/I group (C).

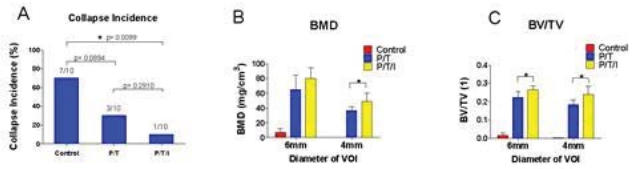


Figure 2: collapse incidence in each group (A), BMD (B) and BV/TV (C) in each group. (*P<0.05)

***Disclosures: Lizhen Zheng**, None.*

P87

KLF10 is a Critical Mediator of Wnt Signaling in Valve Interstitial Cells.

Muzaffer Cicek[1], Malayannan Subramaniam[1], John Hawse[2], Thomas Spelsberg[1], Nalini Rajamannan*[3]. [1]Mayo Clinic, USA, [2]Mayo Clinic College of Medicine, USA, [3]Northwestern University Medical School, USA

We have previously demonstrated that β-catenin plays important roles in valve calcification with a specific osteogenic phenotype defined by increased bone mineral content and overall valve thickening. Recent studies indicate that KLF10 may be involved in mediating the Wnt signaling pathway in bone, which is known to play critical roles in osteoblast differentiation and bone mineralization. Therefore, we sought to test the role of KLF10 in mediating Wnt signaling, as well as differentiation and mineralization, in valve interstitial cells (VICs). Therefore, we analyzed the Wnt pathway genes in valve interstitial cells (VICs) isolated from porcine valves. Exposure of VICs during the course of differentiation, led to increased the expression of Runx2, Sox9 and osteocalcin consistent with endochrondral gene activation. Differentiated cells also stained positive with Von Kossa while undifferentiated cells stained negative confirming the induction of an osteogenic phenotype were increased. As expected, expression of both Lef1 and Co-expression of both β -catenin and LEF1 led to co-activation of the top-flash reporter when transfected into VICs. However, when KLF10 was co-expressed with LEF or β -catenin, there was a significant decrease in reporter activity was observed. These data suggested that KLF10 regulates, LEF and β-catenin to form a transcriptionally active protein complex leading to enhanced Wnt signaling in VICs. This possibility was further confirmed by the observation that KLF10 and β-catenin co-localize with one another in the nucleus of VICs following stimulation with LiCl and/or TGF- β treatment. Taken together, these data implicate an important role for KLF10 in mediating Wnt signaling and LEF transcriptional activity in VICs, and implicate in an important role for canonical the Wnt signaling pathway in the observed osteogenic bone phenotype of cardiac aortic valves.

***Disclosures: Nalini Rajamannan**, None.*

P88

Anabolic small molecule cellular in vitro bone mineralization screen using MC3T3-E1.

JP Rey[1], F. Scott Kimball[1], Robert Young[2], James MacDonald[3], Debra L., Ellies[1] OsteoGeneX Inc, Kansas City, USA, [2]Simon Fraser University, Canada, [3]Chrysalis Pharma, USA.

OsteoGeneX is developing small molecule inhibitors of the bone formation target Sclerostin (SOST) for the treatment of osteoporosis and fracture repair. Through genomic approaches sclerostin was identified as a master regulator of bone mass affecting both men and women (Brunkow et al, 2001). Using proteomic approaches Dr.'s Ellies and Krumlauf discovered and patented Sclerostin's mechanism of action (Ellies, JBMR 2006; Krumlauf, US patent 2002). Dr. Ellies' team, at OsteoGeneX, developed a high throughput cellular screen for identifying small molecules able to block Sclerostin function, hence building bone. In so doing, OsteoGeneX has identified lead candidates capable of building bone both in vitro and in vivo. The development of new anabolic therapeutics relies upon good cellular screens for bone mineralization. We have found that the osteoblast cell line MC3T3-E1 clone varies greatly in its ability to differentiate and mineralize. Herein we compare the parent E1 clone, E1 clone 14, and the E1-BF clone's ability to mineralize. We can induce each of the E1 clones to mineralize, however they mineralize at varying days post differentiation (dpd; varying from 5 to 15dpd). In summary, the E1 clone is a valuable in vitro tool to help discover new compounds able to induce direct bone mineralization in an anabolic fashion, and understanding the limitations of these various E1 subclones is extremely valuable.

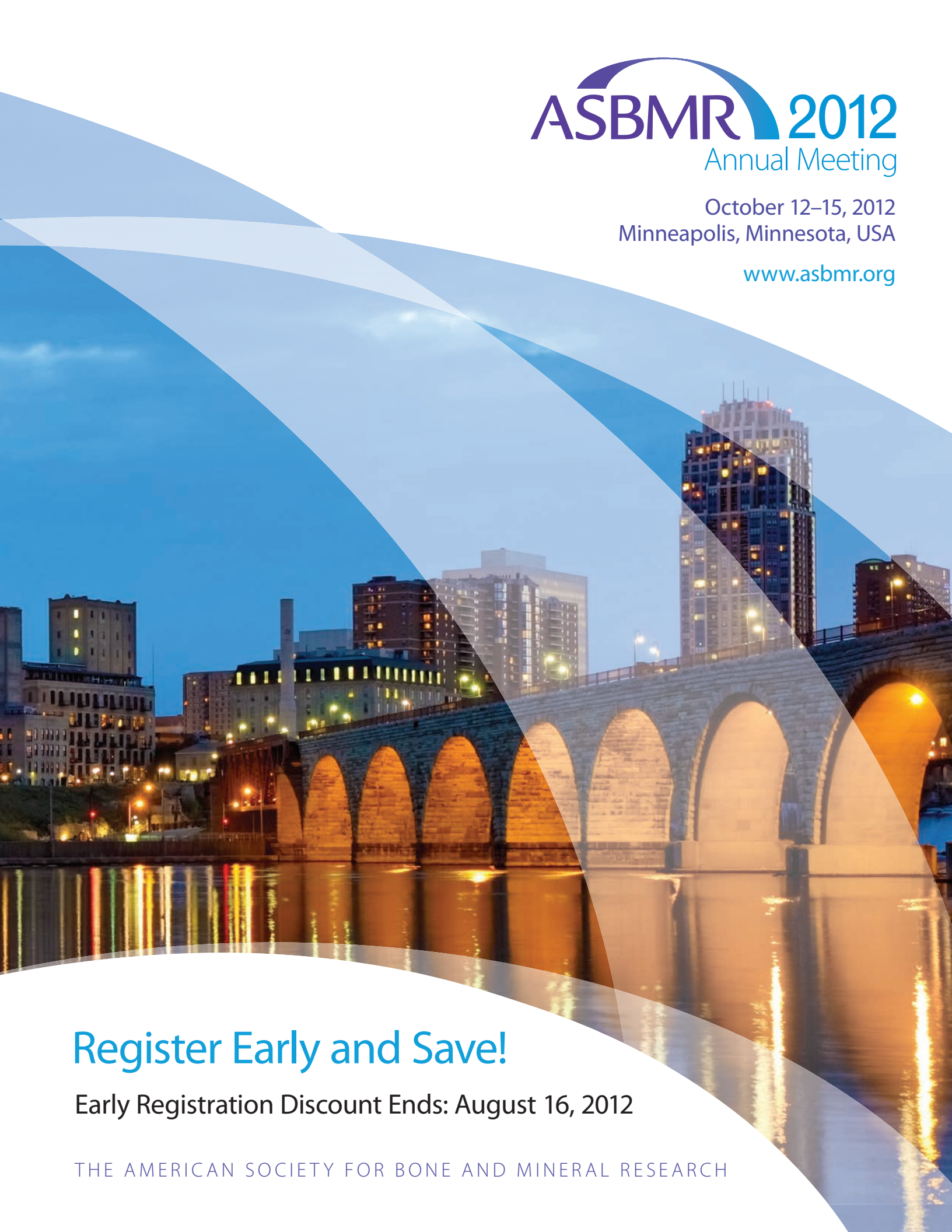
Disclosures: JP Rey, *OsteoGeneX 1,3,4*

Author Index

ASBMR 2012
Annual Meeting

October 12–15, 2012
Minneapolis, Minnesota, USA

www.asbmr.org



Register Early and Save!

Early Registration Discount Ends: August 16, 2012

THE AMERICAN SOCIETY FOR BONE AND MINERAL RESEARCH