AUSTRALIAN + NEW ZEALAND
BONE + MINERAL SOCIETY

28TH ANNUAL SCIENTIFIC MEETING

Meeting Handbook

anzbms.qteventmanagement.co.nz

ANZBMS 30TH ANNIVERSARY

2ND - 5TH SEPTEMBER 2018

RYDGES HOTEL, QUEENSTOWN, NZ

References: 1. Prolia Data Sheet available at medsafe.govt.nz. **2.** Cummings SR, et al. *N Engl J Med* 2009;361:756–65. **3.** Papapoulos S, et al. *Osteoporos Int* 2015;26:2773–83.
Prolia® is a registered trademark of Amgen.

For further information on Prolia or to report an adverse event or product complaint involving Prolia please call Amgen Medical Information on 0800 443 885.

PROLIA® (denosumab, 60 mg/mL) solution for injection is a Prescription Medicine. **INDICATIONS:** Treatment of osteoporosis in postmenopausal women to reduce risk of vertebral, non-vertebral and hip fractures. Treatment to increase bone mass in men with osteoporosis at increased risk of fracture. **CONTRAINDICATIONS:** Hypocalcaemia. Hypersensitivity to denosumab, CHO-derived proteins or any component. Pregnancy and in women trying to get pregnant. **PRECAUTIONS:** Correct hypocalcaemia prior to initiating therapy. Monitor calcium in patients predisposed to hypocalcaemia. Adequate intake of calcium and vitamin D is important. Severe renal impairment. Evaluate patients for risk factors for osteonecrosis of the jaw (ONJ); use with caution in these patients. Very rare reports of atypical femoral fractures. Multiple vertebral fractures may occur following discontinuation. **ADVERSE EFFECTS:** (see Data Sheet for complete list). Serious: Hypocalcaemia, skin infections (predominantly cellulitis) and pancreatitis. Common: back pain, arthralgia,hypertension, nasopharyngitis, pain in extremity, osteoarthritis. **DOSAGE AND ADMINISTRATION:** Single subcutaneous injection of 60 mg, once every 6 months. Ensure adequate intake of calcium and vitamin D. No dose adjustment required in the elderly or in renal impairment. **PRESENTATION:** Pre-filled syringe with automatic needle guard. Prolia is funded for some patients so please refer to the PHARMAC Special Authority criteria. Prolia is unfunded for all other patients - a prescription charge applies. Before prescribing, please review the Prolia Data Sheet available at www.medsafe.govt.nz/profs/Datasheet/p/proliainj.pdf Minimum PI based on Data Sheet 08.03.2018

Amgen (New Zealand) Limited, Auckland. Phone 0800 443 885. NZ-00036. Approved August 2018. TAPS Approval No: NA 10360

AMGEN®

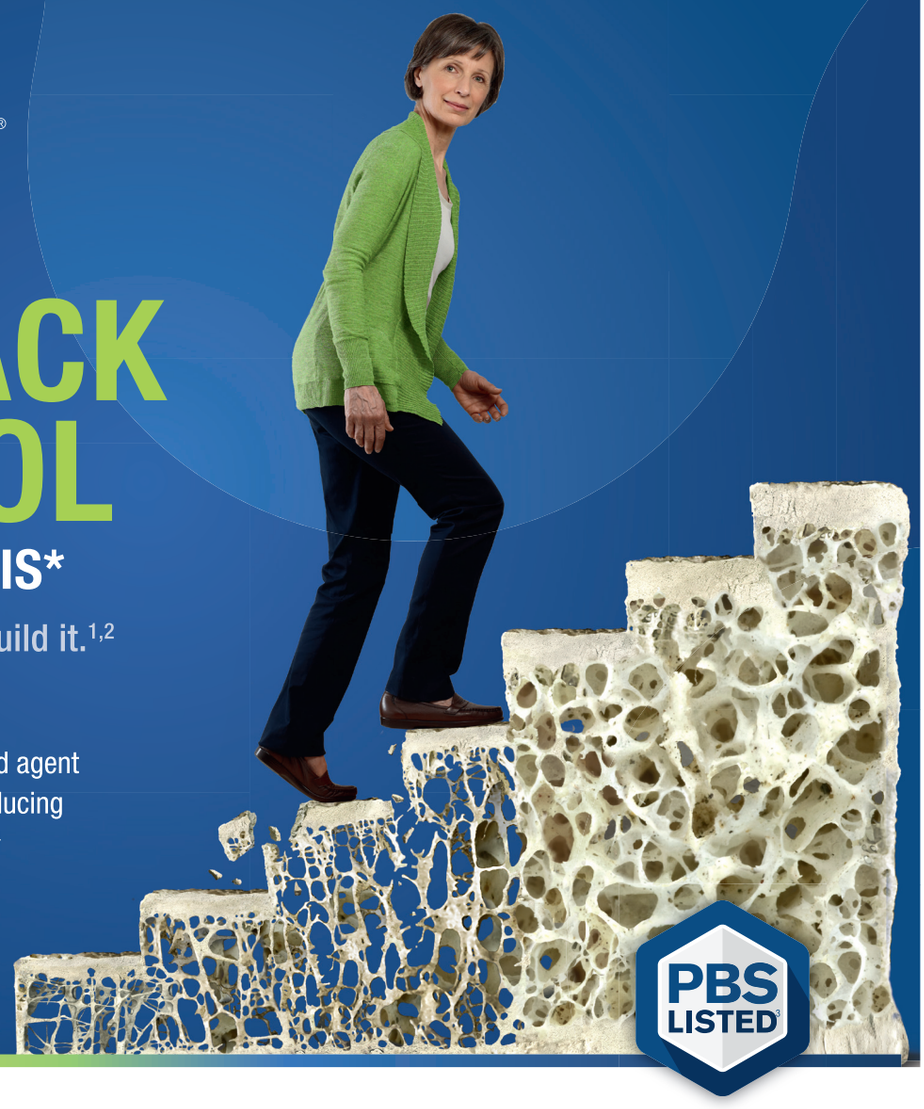
FORTÉO®
teriparatide (rbe)

TAKE BACK CONTROL

OF OSTEOPOROSIS*

*Don't just preserve bone. Build it.[1,2]

FORTEO® is the only TGA-approved agent that builds bone, significantly reducing the risk of future fracture[2–4]



WARNING: In male and female rats, teriparatide caused an increase in the incidence of osteosarcoma that was dependent on dose and treatment duration. The effect was observed at systemic exposures to teriparatide ranging from 3 to 60 times exposure in humans given a 20 mcg dose and occurred after treatment durations ranging from 6 to 24 months. Effects were dependent on dose and duration of treatment, but a no effect dose was not determined. The relevance of the rat osteosarcoma findings to humans has not yet been established. (See PRECAUTIONS, Carcinogenesis and ADVERSE REACTIONS – Spontaneous data.)

PBS Information: Authority Required. Initial treatment, as the sole PBS subsidised agent, by a specialist or consultant physician, for severe, established osteoporosis in a patient with a very high risk of fracture who: a) has a bone mineral density (BMD) T-score of -3.0 or less, and b) has had two or more fractures due to minimal trauma, and c) has experienced at least one symptomatic new fracture after at least 12 months continuous therapy with an anti-resorptive agent at adequate doses. Refer to the PBS schedule for full criteria.

Please review Product Information before prescribing. Product Information can be accessed at www.lilly.com.au/en/products/index.aspx

FORTEO® (teriparatide[rbe]) MINIMUM PRODUCT INFORMATION. Indication: Treatment of osteoporosis in postmenopausal women. Treatment of primary osteoporosis in men. Treatment of osteoporosis associated with sustained systemic glucocorticoid therapy in women and men at high risk for fracture. **Dosage:** 20 mcg sc once daily. Refer to User Manual for proper injection technique. **Contraindications:** Paget's disease. Hypersensitivity to product. **Precautions:** To minimise potential risk of osteosarcoma, maximum lifetime therapy duration is 18 months. Informed consent from patients required. Not recommended for patients with increased risk of osteosarcoma (unexplained elevations of alkaline phosphatase, open epiphyses, prior radiation therapy involving skeleton). Not recommended for bone disorders other than primary osteoporosis. Children, young adults. Potential for hypercalcaemia. Potential for interaction with digoxin. Hypotension. Urolithiasis. Pregnancy and lactation. Haematomas at injection sites with concomitant anticoagulants. **Adverse Reactions:** Leg cramps, muscle spasms, nausea, hyperuricaemia, local reaction and injection site. Allergic events soon after injection. Refer to PI for others. There has been a report of metastatic osteosarcoma with subsequent fatal outcome in a 72 year old woman with osteoporosis and low back pain who had received teriparatide for 14 months prior to presentation. Causality cannot be established on the basis of this single case and a surveillance program continues. Osteosarcoma occurs at a rate of approximately 4 in one million per year (1 in 250,000 per year) in the general population over 60 years old and at the same rate in women over the age of 70 years. At present it is not known if humans treated with FORTEO have an increased risk of osteosarcoma. Based on PI last amended: 2 November 2015.

References: 1. Arlot M *et al. J Bone Mineral Res* 2005;20:1244–1253. **2.** FORTEO (teriparatide) Product Information, 2 November 2015. **3.** Ebeling PR. Aust Prescr 2011;34:176–81. **4.** Neer RM et al. NEJM 2001;344:1434–1441.

PP-TE-AU-0033. Date of preparation: January 2017. FR7054/AOAB4/17.

Lilly

29th Australian and New Zealand Bone and Mineral Society Annual Scientific Meeting

SAVE THE DATE 27th – 30th October, 2019

Darwin Convention Centre

www.anzbmsconference.org

ANZBMS

SPONSOR ACKNOWLEDGEMENTS

The ANZBMS gratefully acknowledges the support of the following companies and organisations:

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EXHIBITORS

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ANZBMS President's Welcome

The 28th annual scientific meeting of the ANZBMS will be held in Queenstown from the 2nd to the 5th, of September 2018. Chaired by Rachel Davey and Rory Clifton Bligh, together with members David Findlay, Jill Cornish, Tim Cundy, Jackie Centre and Audrey Chan, the POC have put together a varied program of cutting-edge science that will appeal to both clinician and basic scientists in the musculoskeletal field.

Topics to be presented by our invited speakers from France, the USA, Australia and New Zealand include bone and vascular biology, osteoarthritis, fracture healing and 3D printing, exercise, nutrition and sarcopenia, designing the skeleton, bone cell biology, as well as an update on anabolic and anti-resorptive therapies. Our scientific program also includes basic and clinical abstract presentations, young investigator awards and an interactive poster tour session. The Early Career Investigator Committee will be hosting a 'Building your research profile" symposium and an evening networking event.

There will be ample time in the scientific program for networking and discussion including a free afternoon allowing delegates to make the most of our beautiful location, with the local organising committee organising a trip to one of Queenstown's nearby ski slopes. The depth and breadth of the scientific program, together with Queenstown's breath taking scenery, ensures for a vibrant and valuable meeting for all attendees.

Peter Croucher, President

Invited Overseas Speakers

Marie-Hélène Lafage-Proust, MD, PhD

Marie-Hélène Lafage-Proust, MD, PhD, was trained as a physician and rheumatologist at the Medical School of Bordeaux University. She graduated in Cell Biology at the University Jean Monnet, member of "Université de Lyon", Saint-Etienne, France. She joined the INSERM 1059 team in 1994, now directed by Dr *L Vico*, after a postdoctoral Research Fellowship at Merck Res Lab (West Point, PA, USA, 1991-1993) were she studied the effects of bisphosphonates on bone. Her lab focused on in vivo effects of mechanical strain on bone, developing unloading or physical exercise models in rodents, as well as on more fundamental aspects of mechanical stimulus signal transduction in bone cells. More recently, her main research aimed at further understanding of the role of bone microvascularisation in the bone response to anabolic signals such as mechanical load or PTH and the involvement of VEGF in the remodelling of vasculature and bone. In parallel, she participated to the research led by *Dr L Malaval*, in her group, on the role of the extracellular matrix Bone Sialo-Protein, a member of the SIBLINGs family.

She is the current director of IFRESIS (Institut Fédératif de Recherche en Sciences et Ingénierie de la Santé) in Saint-Etienne. She has been responsible for the histomorphometry diagnosis of metabolic bone diseases at the University Hospitals of Bordeaux and now St-Etienne

As a clinician, she currently takes care of patients with metabolic bone diseases, especially osteoporosis and uremic bone disease in the Rheumatology Department of St-Etienne University Hospital. She is particularly interested in the patho-physiological mechanisms of bone fragility in uremic patients.

Roberto Civitelli

Roberto Civitelli, M.D,is the Sydney M. & Stella H. Schoenberg Professor of Medicine, Orthopaedic Surgery, and Cell Biology and Physiology; and Chief of the Division of Bone and Mineral Diseases. He also directs the Washington University Skeletal Disorders Training Program and is Co-Director of the Musculoskeletal Research Center. He is a physician-scientist, with active practice in bone and mineral diseases. His areas of clinical specialty include bone and mineral diseases, and osteoporosis in particular. His research focuses on cell-cell communication and signaling in the bone microenvironment, and the role of cell adhesion, communication and signaling on skeletal development and maintenance, in the pathobiology of various forms of osteoporosis and metabolic bone disorders, and in the relationship between bone cells and tumors. He is a recipient of the Fuller Albright Award (1994), and has served ASBMR in many capacities; member of the Publications Committee (1998-2001), Councilor (2009-2011), member of the Executive Committee (2012-2015) and President (2013-2014). He served as member of the Board of Directors of the Federation of American Societies of Experimental Biology (2015-2017). In 2018, he began a 5-year tenure as Editor-In-Chief of the *Journal of Bone and Mineral Research*.

Dr Buddy D. Ratner

Dr. Buddy D. Ratner is the Director of University of Washington Engineered Biomaterials (UWEB21) Engineering Research Center, co-director of the Center for Dialysis Innovation (CDI) and the Darland Endowed Chair in Technology Commercialization. He is Professor of Bioengineering and Chemical Engineering, University of Washington. Ratner received his Ph.D. (1972) in polymer chemistry from the Polytechnic Institute of Brooklyn. Ratner is a fellow of the American Institute of Medical and Biological Engineering (AIMBE), AVS, AAAS, ACS, POLY and the International College of Fellows Biomaterials Science and Engineering.

In 2002 Ratner was elected a member of the National Academy of Engineering, USA. He has been involved in the launch of seven companies and won numerous awards including the AVS Welch Award (2002), Society for Biomaterials Founders Award (2004), the BMES Pritzker Distinguished Lecturer Award (2008), the Acta Biomaterialia Gold Medal (2009), the Galletti Award (2011) the George Winter Award of the European Society for Biomaterials (2012) and the University of Washington School of Medicine Lifetime Innovator and Inventor Award (2014). His research interests include biomaterials, tissue engineering, regenerative medicine, polymers, biocompatibility, surface analysis and plasma thin film deposition.

Michael R. McClung

Michael R. McClung, MD is widely known as an educator, translating clinical research information into practical strategies of evaluation and treatment for other physicians. Dr. McClung is internationally recognized expert in the fields of osteoporosis and bone density testing. He is the founding director of the Oregon Osteoporosis Center. His Center has been involved in many of the important clinical studies that resulted in the availability of the medications now used to treat osteoporosis and Paget's disease of bone. He has published more than 200 papers and book chapters, is co-editor of a book for clinicians about disorders of bone and mineral metabolism and is a member of the editorial boards for several journals in his field.

He is an active member of multiple international societies focusing on bone diseases and their treatment. He serves as a member of the Council of Scientific Advisors for the International Osteoporosis Foundation, on the Scientific Advisory Board of the National Osteoporosis Foundation, and as a medical advisor for the Paget Foundation. He was a member of the World Health Organization Fracture Risk Task Force that led to the development of the FRAX® tool. He is a member of the global advisory boards for multiple companies and organizations. He has served on the Endocrinology and Metabolism Advisory Committee of the FDA and has participated in the development of evidence–based guidelines for the treatment of osteoporosis for several national and international societies.

Kenneth G. Saag

Kenneth G. Saag, MD, MSc earned his medical degree at Northwestern University in Chicago, Illinois and his Master of Science Degree with an emphasis in epidemiology, from the University of Iowa, Department of Preventive Medicine and Environmental Health (Iowa City, Iowa). Dr. Saag is a Professor of Medicine in the Division of Clinical Immunology and Rheumatology at the University of Alabama at Birmingham (UAB), as well as a rheumatologist and outcomes researcher at the same institution. His main areas of interest include rheumatoid arthritis, osteoporosis, and pharmacoepidemiology.

Dr. Saag is currently the Director for the Center for Education and Research on Therapeutics (CERTs) of Musculoskeletal Disorders at UAB. He is a Member of the Southern Society for Clinical Investigation, Chair of the Osteoporosis Abstract Selection Committee, American College of Rheumatology (ACR), and Associate Director of the UAB Center for Metabolic Bone Disease (CMBD) National Institutes of Health (NIH) T32 Institutional Training Grant entitled, "Comprehensive Training Grant in Bone Biology and Disease". Dr. Saag serves on numerous editorial boards including serving as Osteoporosis Section Editor with Current Rheumatology Reports and Outcomes Section Associate Editor with Arthritis, Research & Therapy. Dr. Saag has delivered hundreds of lectures and publications including, "Mend the Mind, but Mind the Bones! Balancing Benefits and Potential Skeletal Risks of Serotonin Reuptake Inhibitors" (Archives of Internal Medicine), and "Epidemiology, Risk Factors, and Lifestyle Modifications for Gout" (Arthritis Research & Therapy).

Dr. Saag has been a consultant/speaker and received honoraria from Amgen, Eli Lilly, Merck, Novartis, Roche, Savient and TAP.

ANZBMS OFFICE BEARERS 2018

President	Peter Croucher	Councillors	Rob Daly
President Elect	Natalie Sims		Allison Pettit
Secretary	Paul Anderson		Mark Forwood
Treasurer	Nathan Pavlos		Jillian Cornish
Past President	Emma Duncan		Mark Cooper

Past ANZBMS OFFICE BEARERS *1988 – 2017*

Year	President	President Elect	Secretary	Treasurer	Councillors
1990 (Steering Group)	T J Martin		M Hooper		A Need, R Prince, J Eisman, I Reid, K Ibbertson, D Fraser, P Sambrook, E Seeman
1991-93 (Inaugural Council)	T J Martin		M Hooper	M Hooper	J Eisman, A Goulding, D Perry-Keen, J Wark, A Need, N Kent
1993-95	J Eisman	I Reid	N Kent	J Wark	P Sambrook, A Need, R Prince, D Perry-Keene, E Seeman
1995-97	I Reid	N Kent	J Moseley	P Ebeling	P Sambrook, A Need, R Prince, D Perry-Keene
1997-99	N Kent	P Ebeling	J Moseley	P Ebeling	R Prince, I Reid, M Hooper, H Morris, M Forwood
1999-01	P Ebeling	M Hooper	J Cornish	M Forwood	J Moseley, H Morris E Mackie, M Zheng
2001-03	M Hooper	E Seeman	J Cornish	M Forwood	R Mason, R Price, G Nicholson, D Findlay
2003-05	E Seeman	J Cornish	D Findlay	M Forwood	R Mason, R Price, G Nicholson, P Sambrook
2005-07	J Cornish	P Sambrook	D Findlay	R Price	G Nicholson, R Mason, M Gillespie, P Nash
2007-09	P Sambrook	R Mason	M Gillespie	R Price	P Nash, T Cundy, N Fazzalari, M Kotowicz
2009-11	R Mason	M Gillespie	N Fazzalari	R Price	P Sambrook, N Sims, M Seibel, G Thomas, N Gilchrist
2011-13	M Gillespie	M Seibel	G Aktins	G Thomas	N Pocock, M Bolland, C Inderjeeth, N Sims, R Mason
2013 - 15	M Seibel	E Duncan	G Atkins	G Thomas	N Sims, M Gillespie, N Pocock, N Pavlos, E Dennison
2015 - 17	E Duncan	P Croucher	P Anderson	N Sims	N Pocock, R Buchbinder, E Dennison, R Daly, A Pettit, M Seibel

PROGRAM ORGANISING COMMITTEE

Co-Chair: Rachel Davey
Co-Chair: Rory Clifton-Bligh

Jill Cornish
David Findlay
Jackie Centre
Tim Cundy
Audrey Chan

LOCAL ORGANISING COMMITTEE

Jill Cornish
Ian Reid
Peter Croucher
Nathan Pavlos
Ashika Chhana
Ivone Johnson
Doreen Presnall

ANZBMS Secretariat

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ANZBMS Meeting Manager

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QT Event Management
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ROGER MELICK YOUNG INVESTIGATOR AWARD

This award is presented to commemorate the contribution of Dr Roger Aziz Melick to endocrinology and student education. Roger Melick died in November 1986 after a long battle with cancer. He trained in endocrinology with Fuller Albright, in Boston, and joined The Royal Melbourne Hospital as the third member of the foundation Department of Medicine. He was appointed Dean of the Clinical School in April 1979 and he was forced to retired because of his illness during 1986. Roger Melick was particularly known for his kindness, consideration and empathy for both patients and students. The prize is awarded annually to young members of the Society working towards a higher degree (including FRACP).

1996	Vicky Kartsogiannis
1997	Linda Crofts
1998	Janelle Barry
1999	Liza-Jane Raggatt
2000	Sandra Iuliano-Burns
	Nathan Pavlos
2001	David Good
2002	Kun Zhu
2003	Agatha Labrinidis
	Xiaofang Wang
2004	Susan Allison
	Kirk Ho Man Yip
2005	James Doecke
2006	Yosuke Kawasaki
2007	Stella Foley
	Garry Williams
2008	Jonathan Gooi
2009	Nicola Lee
2010	Irene Zinonos
2011	Chiaming Fan
2012	Farzin Takyar
	Audrey Chan
2013	Asiri Wijenayaka
2014	Hua Ying
	Irina Kulina
2015	Niloufar Ansari
2016	Christina Vrahnas
2017	Mahmoud Bakr

CHRISTOPHER AND MARGIE NORDIN YOUNG INVESTIGATOR POSTER AWARD

This Award is named in honour of the outstanding and major clinical investigations into disorders of bone and mineral metabolism made by Professor Christopher Nordin and his contributions to the ANZBMS. Professor B.E Christopher Nordin was a senior specialist at the Institute of Medical and Veterinary Science in Adelaide, and the man credited with drawing the medical community's attention back to the link between calcium deficiency and osteoporosis (brittle bones).

1997	Anne Nelson
	Hidenori Murata
1998	Marianne Holzherr
1999	Tanya Uebergang
2000	Josef Kaplan
2001	Rebecca Jackson
2002	Nathan Pavlos
2003	Nicole Walsh
	Rouha Granfar
2004	Laura Gregory
	Mark Bolland
2005	Mark Bolland
	Catherine Wang
2006	Andrew Hattam
	Estabelle Ang
2007	Taksum Cheng
2008	Hasnawati Saleh
2009	Ee-Cheng Khor
2010	Kylie Alexander
2011	Shek Man Chim
2012	Alvin Ng
	Marie-Luise Wille
2013	Yu Wen Su
2014	Masato Koike
2015	Dzenita Muratovic – Clinical
	Scott Youlten – Basic
2016	Audrey Chan
2017	Scott Youlten

CHRISTINE AND T JACK MARTIN RESEARCH TRAVEL GRANT

This grant is offered by the ANZBMS in memory of Christine Martin and to honour the outstanding and major scientific contributions of Professor T Jack Martin to bone and mineral research and his contributions to associates and trainees in teaching, research, and administration.

2002	Catherine Middleton-Hardie
2003	Vicky Kartsogiannis
2004	Kerrie Sanders
2005	Susan Allison
2006	Mark Forwood
2007	Brya Matthews
2008	Roger Zebaze
2009	Bich Tran
2010	Garry Williams
2011	Julie Quach
2012	Ashika Chhana
2013	Yohann Bala
2014	Michelle McDonald
2015	Christina Vrahnas
2016	Audrey Chan
2017	Alexander Rodriguez

AMGEN / ANZBMS OUTSTANDING ABSTRACT AWARD

The Council of ANZBMS wishes to recognise the high standard of bone and mineral research presented at the Annual Scientific Meeting. The Program Organising Committee will award a prize to the basic and clinical abstracts receiving the highest scores.

2003	Rob Will, Amanda Devine
2004	Roger Zebaze
2004	Christine Rodda
2005	Markus Seibel, Julian Quinn
2006	Yosuke Kawasaki, Julie Kuliwaba, Stella Foley, Dana Bliuc, Jonathan Gooi
2007	Colin Dunstan, Richard Prince, Maria Chiu, Natalie Sims, Paul Baldock, Ian Parkinson, Hong Zhou
2008	Robert Kalak, Andrew Grey
2009	Vicky Kartsiogiannis, Nguyen Nguyen
2010	Markus Seibel, Emma Walker, Iris Wong Sarah Brennan, Jasreen Kular, Markus Seibel, Hugh Zhang
2011	Ian Reid, Asiri Wijenayaka
2012	Ego Seeman, Rachelle Johnson
2013	Peter Ebeling, Rossana Nogueira, Simon Junankar, Narelle McGregor, Jinwen Tu
2014	Allison Pettit Paul Baldock, Jinwen Tu, Roger Zebaze, Kirtan Ganda
2015	Michelle McDonald, Allison Pettit, Audrey Chan, Le Phong Thao Ho, Ayano Nakayama
2016	Nicola Lee Steven Watson Emma Walker Natalie Hyde Michelle McDonald
2017	Victoria D Leitch Emma M Wade Michelle M McDonald Ego Seeman

MSD / ANZBMS CLINICAL RESEACH EXCELLENCE AWARD

MSD, through the Merck Research Labs have a long history in bone research and continue to achieve clinical innovations in this field. While the scientists at Merck are dedicated to exploring new ways to address health problems, they also recognize that the best scientific discoveries often emerge from collaboration with clinical researchers outside Merck laboratories. This award is offered to recognise and support clinicians in or within 10 years of postgraduate training who are contributing to clinical research in the field of bone-related disorder.

2012	Belal Khan
2013	Syndia Lazarus
2014	Masakazu Kogawa
2015	Hanh Nguyen
2016	Thach Tran
2017	Weiwen Chen

KAYE IBBERTSON AWARD ON METABOLIC BONE DISEASE

This Award is named in honour of the outstanding career and major investigations into skeletal disorders made by Professor Kaye Ibbertson, and his contributions to the ANZBMS.

2005	Roger Zebaze
2006	Julie Pasco
2007	Tania Winzenberg
2008	Paul Baldock
2009	Mark Bolland
2010	Kun Zhu
2011	Susannah O'Sullivan
2012	Emma Duncan
2013	Tara Brennan-Speranza
2014	Nicole Yu
2015	Joshua Lewis Rachelle Johnson
2016	Michelle McDonald
2017	Feitong Wu

SOL POSEN RESEARCH AWARD

This Award is named in honour of Professor Sol Posen who was one of the pioneers in the field of bone and mineral endocrinology in this country. Sol Posen's contributions span the range from basic biochemistry – his citation classic in Clinical Chemistry described the first means of distinguishing alkaline phosphatase of bone origin – to clinical studies in metabolic bone disease, including Paget's disease, osteoporosis, hyperparathyroidism and tumour-induced osteomalacia. He attended meetings and journal clubs, where his presence was marked, as ever, by his propensity to ask incisive questions.

2006	Nathan Pavlos
2007	Aaron McDonald
2008	Haotian Feng
2009	Ming-Kang Chang
2010	Tak Sum Cheng
2011	Kylie Alexander
2012	Julie Quach
2013	Farzin M Takyar
2014	Heath McGowan
2015	Dana Bliuc
2016	Paul Lee
2017	Thao P. Ho-Le

PHILIP SAMBROOK AWARD

The Professor Philip Sambrook Award is presented annually to an outstanding early career researcher to honour the major clinical and scientific contributions by Professor Philip Sambrook to the field of rheumatology and osteoporosis. Successful applicants must be passionate about bone research, results driven and committed to giving back to the community.

2012	Gustavo Duque	2015	Sharon Brennan-Olsen
2013	Emma Duncan	2016	Jinwen Tu
2014	Kirtan Ganda	2017	Jasna Aleksova

ANZBMS INTERNATIONAL TRAVEL AWARD

This award is offered by the ANZBMS to support travel to attend the IBMS Herbert Fleisch Workshop.
The objective of this workshop is to provide a Gordon-conference style forum for students, post-docs and early stage principal investigators to present work in progress, discuss thoroughly and network with peers, and get constructive feedback from experienced senior scientists.

2014	Tara Brennan-Speranza	2015	Campbell Macgregor
	Ashika Channa		Megan Thomas
	Christina Vrahnas		Scott Youlten

ANZBMS CAREER ACHIEVEMENT AWARD

This esteem award recognises outstanding and major scientific or clinical contributions, and excellence in teaching and service to and within the bone and mineral field. The award will be widely publicised and presented annually during the society's Annual Scientific Meeting. The awardee will receive free registration to the Annual Scientific Meeting and invitations to the annual and the president's dinner the year the award is received.

2014	Jillian Cornish	2015	Howard Morris
	Ego Seeman	2016	Jack Martin

ANZBMS Mid-Career Fellowship

In recent years, funding for mid-career researchers has become increasingly difficult to obtain, jeopardising the future career of many of our colleagues in bone and mineral research. The ANZBMS has therefore created a new Fellowship Award with the intention to bridge shortfalls in salary funding for outstanding mid-career scientists.

2016	Allison Pettit
2017	Nicki Lee
	Rachel Davey

ANZBMS LIFE MEMBERS

Professor Henry Kaye Ibbertson (deceased)
Associate Professor Jane Moseley
Professor B.E.C Nordin (deceased)
Dr Solomon Posen (deceased)
Professor Thomas J. Martin
Dr Donald Gutteridge
Dr Ailsa Goulding
Associate Professor Michael Hooper
Professor John Eisman
Professor Ego Seema

PROGRAM INFORMATION

Speaker Presentations

It is important that **all** speakers giving oral presentations check in with the technicians in the conference room as early as possible during the conference so that their presentations can be loaded and checked. Please report to the technicians even if you are not using a PowerPoint presentation so that this can be noted. Microsoft Office PowerPoint will be used during the sessions.

If you have any questions, or if these arrangements pose a problem for you, please contact the registration desk.

Posters

Poster sessions times are below:

Plenary Posters
Sunday 2nd September 17.30 – 19.00

Poster Session 1 (Even Poster Numbers)
Tuesday 4th September 10.10 – 10.55am

Poster Session 2 (Odd Poster Numbers)
Tuesday 4th September 3.15 – 4.00pm

All posters must be removed at the end of afternoon tea on Tuesday 4th of September.

Photography

The use of photo equipment, cameras, audio-taping devices, and video-taping equipment are strictly prohibited in all scientific session venues without the express written permission of the ANZBMS. Unauthorized use of such equipment may result in the confiscation of the equipment or the individual may be asked to leave a scientific oral or poster session or be prohibited from viewing the poster displays.

GENERAL INFORMATION

Venue

Plenary sessions from Sunday to Wednesday will be located in the Coronet Room at the Rydges Hotel

Registration Desk

The registration desk will be open at the following times:

Sunday 2 September	08:00 – 19:00
Monday 3 September	07:00 – 20:00
Tuesday 4 September	07:30 – 17:00
Wednesday 10 September	08:00 - 12:30

Name Badges

Each conference delegate will receive a name badge on registration. The badge will be your official pass and must be worn to gain entry to all sessions, lunch and refreshment breaks. If a namebadge for a partner attending a social function is required, please ask at the registration desk.

ANZBMS 2018 EXHIBITION

The Trade Exhibition will be located in the Queenstown room at the Rydges Hotel. Please visit the booths during refreshment breaks in recognition of the generous support this conference has received from the sponsors.

Booth No	Company
1	Hologic
3	Reframe
4	Theramex
5	Getz Healthcare
6	Thomson Scientific Instruments
7/8	Amgen Australia
9/10	Eli Lilly

Hotel Check-Out

Please note that check out time is 10:00. Facilities are available for the storage of luggage.

SOCIAL EVENTS

Sunday 2 September
Welcome Reception - 17:30 – 19:00
Exhibition Area, Rydges Hotel
Substantial finger food, wine, beer and soft drinks will be served. One ticket is included in the full registration fee. Name badges must be worn. Extra tickets may be purchased from the registration desk.

Sunday 2 September
ECIC Bones & Brews Function 19:00 – late
Reds Bar, Rydges Hotel
An informal meet and greet for all members of the ANZBMS to interact, share their research and ideas, and join in some games with prizes to be won. Come meet fellow ANZBMS members, conference delegates and the members of the ANZBMS Early Career Investigator Committee (ECIC)

Tuesday 4 September
AGM and Conference Dinner
AGM - 18:00
Conference Dinner – 19.15
Skyline Queenstown
Dinner will be held at the Skyline Complex and will include a three-course dinner, drinks and entertainment by LA Social Club.
The Skyline Complex is accessed by Gondola leaving from Central Queenstown. The Gondola station is a 15 minute walk from the hotel. Buses will also be provided.
Admission is by ticket only. Dress code: Smart casual

Mobile Phones

Please ensure that all mobile phones are switched to silent mode during scientific sessions.

Refreshments

All refreshments will be served in the exhibition area at the Rydges Hotel. If you have requested a special diet please make yourself known to one of the waiting staff.

ANZBMS ANNUAL SCIENTIFIC MEETING 2-5 SEPTEMBER 2018 QUEENSTOWN NZ

Sunday 2nd September

8:00am		Registration Open
8:00am – 10:30am		Pre-conference satellite meeting DXA Workshop *Clancy's Room* a. Pitfalls in DXA/Orthopaedic DXA/TBS update – Nicholas Pocock b. Bone Density in unusual situations. Mark Cooper c. Sarcopenia. Best parameters – Gustavo Duque d. Small animal – Paul Baldock e. Questions/Discussions
10:30am – 10:40am		Introduction to ANZBMS Meeting, Professor Peter Croucher, ANZBMS President Coronet Room
10:40am – 12:30pm		Session 1 – Bone and its Environment Chairs: David Findlay and Michelle McDonald *Coronet Room*
10:40am – 11:10am	IS1	N-cadherin in osteolineage cells as modulator of bone mass and cancer growth. Roberto Civitelli (USA)
11:10am – 11:40am	IS2	Bone Vasculature and its role in bone remodelling Marie-Helene Lafage-Proust (France)
11:40am – 12:00pm	IS3	Vascular calcification: Disentangling the bone-cardiovascular disease nexus. Joshua Lewis (Edith Cowan University)
12:00pm – 12:15pm	OR1	Amgen-ANZBMS Outstanding Abstract – Clinical Presentation Zoledronate every 18 months for 6 years in osteopenic postmenopausal women: effects on fractures and skeletal endpoints. Ian Reid (University of Auckland)
12:15pm – 12:30pm	OR2	Amgen-ANZBMS Outstanding Abstract – Basic Presentation SLC37A2 is an endolysosomal transporter critical for osteoclast function during bone remodelling. Nathan Pavlos (The University of Western Australia)
12:30pm – 1:40pm		Lunch *Queenstown Room* Amgen Platinum Sponsor Symposium Prevention and treatment of glucocorticoid-induced osteoporosis. Kenneth Saag (USA) Clancy's Room

1:40pm – 2:55pm		Session 2 – Anabolic vs Anti-Resporptive Therapies Chairs: Jackie Centre and Dana Bliuc *Coronet Room*
1:40pm – 2:10pm	IS4	Anabolic vs anti-resorptive Therapies for Osteoporosis: How Should They Be Used? Mike McClung, (USA)
2:10pm – 2:30pm	IS5	Trials our patients need and some they don't. Andrew Grey (The University of Auckland)
2:30pm – 2:42pm	OR3	Continued fracture risk reduction after 12 months of romosozumab followed by denosumab through 36 months in the phase 3 FRAME (FRActure study in postmenopausal woMen with ostEoporosis) extension. Peter Ebeling (Monash University)
2:42pm – 2:54pm	OR4	A randomised alendronate-controlled trial of romosozumab: Results of the Phase 3 ARCH study (Active-contRolled fraCture study in postmenopausal women with osteoporosis at High risk). Kenneth Saag (USA)
2:55pm – 3:40pm		Afternoon Tea *Queenstown Room*
3:40pm – 5:30pm		Session 3 - Fracture Healing and 3D-Printing Chairs: Mike Rogers and Ayse Zengin *Coronet Room*
3:40pm – 4:00pm	IS6	Functional genomics and gene editing in bone: new tools and standardising outcomes. Aaron Schindeler (Westmead Children's Hospital)
4:00pm – 4:20pm	IS7	Conversion of Human Fibroblasts into Functional Osteoblasts by an Extrinsic Factor. Hala Zreiqat (University of Sydney)
4:20pm – 4:40pm	IS8	Periosteal progenitors and fracture healing. Brya Matthews (University of Auckland)
4:40pm – 4:52pm	OR5	Effect of sclerostin monoclonal antibody therapy on BMD is linked to osteocyte sensitivity - insights from a mechanobiological model of bone remodelling. Martin Madge (Queensland University of Technology)
4:52pm – 5:04pm	OR6	Intermittent PTH Increases Remodeling and Restores the Healing of Stress Fractures Following Bisphosphonate Treatment. Mahmoud Bakr (Griffith University)
5:04pm – 5:16pm	OR7	STAT3 signaling in heterotopic ossification following spinal cord injury. Kylie Alexander (Mater Research Institute)
5:16pm – 5:28pm	OR8	Timing of Vertebroplasty for Acute Painful Osteoporotic Fractures: Data from the VAPOUR Trial. Terry Diamond (St George Hospital)
5:30pm – 7:00pm		Welcome Reception and Plenary Posters *Queenstown Room and Wakatipu Room*

7:00pm		ECIC Networking Event *Red's Bar*

Monday 3rd September

8:30am – 9:50am		Session 4 – Osteoarthritis Chairs: Roberto Civitelli and Tania Crotti *Coronet Room*
8:30am – 8:50am	IS9	Circadian clock disruption in the pathogenesis of osteoarthritis. Raewyn Poulsen (The University of Auckland)
8:50am – 9:10am	IS10	Improving translation of pre-clinical osteoarthritis research to patients: disease phenotypes, endotypes and models. Chris Little (University of Sydney)
9:10am – 9:22am	OR9	Biochemical profiling of MRI detected bone marrow lesions in knee osteoarthritis patients: altered mineralisation of the subchondral bone matrix. Julia Kuliwaba (The University of Adelaide)
9:22am – 9:34am	OR10	Anti-Fractalkine Monoclonal Antibody Ameliorates Joint Destruction in Collagen-Induced Arthritis Model by Inhibiting Migration and Survival of Osteoclast Precursor Cells. Naoto Ishii (KAN Research Institute, Japan)
9:34am – 9:46am	OR11	Local unloading of subchondral bone through loss of articular surface congruity results in a focal increase in bone turnover. Megan Thomas (The University of Melbourne)
9:50am – 10:20am		Morning Tea *Queenstown Room*
10:20am – 11:30am		Christopher and Margie Nordin Young Investigator Award Finalists Poster Tour Chairs: Rory Clifton-Bligh and Sabs Ramchand Rachel Davey and Joshua Lewis *Wakatipu Room and Clancy's Room*
11:30am – 4:30pm		Lunch (*Queenstown Room*) and Activities Afternoon
4:30pm – 6:00pm		Roger Melick Young Investigator Award Finalists and Selected Orals Chairs: Natalie Sims and Julia Kuliwaba *Coronet Room*
		Roger Melick Young Investigator Award Finalists
4:30pm – 4:41pm	OR13	Anti-LRP6 antibody prevents myeloma induced bone disease and increases bone strength. Marija Simic (Garvan Institute of Medical Research)
4:41pm – 4:52pm	OR14	Clinical utility assessment of genetic profiling in fracture risk prediction: A decision curve analysis approach. Thao Ho-Le (The University of Technology Sydney)

4:52pm – 5:03pm	OR15	Optimization of TiO2 nanotube formation and dimensions on Ti6Al4V alloys influences osteogenic differentiation of human mesenchymal stromal cells. Jun Li (University of Otago)
5:03pm – 5:14pm	OR16	Patients on dialysis have markedly abnormal cortical hip parameters by dual-energy X-ray absorptiometry. Jasna Aleksova (Monash Health)
5:14pm – 5:25pm	OR17	Assessment of osteal macrophage and osteoclast distribution across the acute phase of bone loss in a mouse model of post-menopausal osteoporosis. Leena Batoon (Mater Research Institute and The University of Queensland)
5:25pm – 5:36pm	OR18	The impact of osteoporotic fracture type and subsequent fracture on mortality in TromsÃ¸, Norway. Dunia Alarkawi (Garvan Institute of Medical Research)
		Selected Orals
5:36pm – 5:47pm	OR19	High-fat Diets Induce Sclerostin Expression Via Enhanced Glucocorticoid Signalling in Osteocytes. Sarah Kim (ANZAC Research Institute)
5:47pm – 5:58pm	OR20	Longitudinal association between objectively measured sedentary time and physical activity with muscle strength, balance and falls in a cohort of Australian middle-aged women. Feitong Wu (Menzies Institute for Medical Research and The University of Tasmania)
6:00pm – 6:15pm		Short Break
6:15pm – 7:40pm		Session 5 - Exercise, Nutrition and Sarcopenia Chairs: Marie-Helene Lafage-Proust and Peter Croucher *Coronet Room*
6:15pm – 6:35pm	IS12	Sarcopenia: can nutrition make a difference? Sandy Iuliano (The University of Melbourne).
6:35pm – 6:55pm	IS13	Androgen deprivation as a model of osteosarcopaenic obesity. Mathis Grossmann (The University of Melbourne)
6:55pm – 7:15pm	IS14	Sarcopenia: Pharmacology of Today and Tomorrow. Gustavo Duque (The University of Melbourne and AIMSS)
7:15pm – 7:27pm	OR21	Cognitive decline is associated with an accelerated rate of bone loss and increased fracture risk in women 65 years or older in the longitudinal population-based Canadian multicentre osteoporosis study (CAMOS). Dana Bluic (Garvan Institute of Medical Research)
7:27pm – 7:39pm	OR22	Contribution of vitamin D insufficiency to fracture and post-fracture mortality in men. Sarah Molloy (Garvan Institute of Medical Research)

Tuesday 4th September

8:30am – 10:10am		Session 6 - Bone Cell Biology Chairs: Jill Cornish and Brya Matthews *Coronet Room*
8:30am – 9:00am	IS15	Multiple functions of connexins in bone and adipose tissue homeostasis. Roberto Civitelli (USA)
9:00am – 9:30am	IS16	Evaluation and management of bone fragility in Chronic Kidney Disease. Marie-Helene Lafage-Proust (France)
9:30am – 9:50am	IS17	The role of Eph/ephrin molecules in mesenchymal stem cell function and their influence on bone biology. Agnes Arthur (University of Adelaide)
9:50am – 10:10am	IS18	Using Big Data imaging to unravel the risks and/or benefits of cell-cell fusion in macrophages, osteoclasts and giant multinucleated cells. Dr Pei Ying Ng (University of WA)
10:10am – 10:55am		Morning Tea (*Queenstown Room*) and Poster Session (Even Poster Numbers) *Wakatipu Room and Clancy's Room*
10:55am – 12:35pm		Session 7 – Designing the skeleton Chairs: Mike McClung and Allison Pettit *Coronet Room*
10:55am – 11:15am	IS19	The use of post-mortem CT to assess skeletal trauma in fatal falls. Samantha Rowbotham (Monash University)
11:15am – 11:35am	IS20	Monoallelic *BMP2* variants predicted to result in haploinsufficiency cause craniofacial, skeletal, and cardiac features that overlap with those of chromosome 20p12 deletions. Tiong Tan (Murdoch Children's Research Institute)
11:35am – 11:47am	OR23	Adamts17 is involved in skeletal growth through the regulation of TGF-Î_ signaling via Microfibril biogenesis. Takeshi Oichi (The University of Tokyo)
11:47am – 11:59am	OR24	Long-term Outcomes of Osteogenesis Imperfecta in the Bisphosphonate Era. Andrew Feehan (Royal Children's Hospital, Melbourne)
11:59am – 12:11pm	OR25	Calcitriol may act on an alternate receptor during fetal development, since absence of calcitriol has different consequences than loss of the vitamin D receptor. Christopher Kovacs (Memorial University of Newfoundland)
12:11pm – 12:23pm	OR26	The Calcitonin Receptor attenuates the acidity of the osteocyte lacunae microenvironment during lactation in mice. Rachel Davey (The University of Melbourne)
12:23pm – 12:35pm	OR27	Bone corticalisation requires suppression of glycoprotein 130 signalling in osteocytes, and occurs by region-specific imbalances in bone formation and resorption. Emma Walker (St Vincent's Institute of Medical Research)

12:35pm – 1:45pm		Lunch *Queenstown Room* Eli Lilly Platinum Sponsor Symposium Osteoporosis: A patient's trajectory. Professor Gustavo Duque (The University of Melbourne and AIMSS) *Clancy's Room*
1:45pm – 3:15pm		Session 8 – Co-badged session with ANZORS: Orthopaedics and Biomechanics Chairs: Paul Anderson and Egon Perilli *Coronet Room*
1:45pm – 2:15pm	IS21	Biomaterials for Bone Healing and Regeneration in the Context of Biocompatibility. Buddy Ratner (USA)
2:15pm – 2:35pm	IS22	Coupling high-resolution bone imaging with micro finite element analysis to assess local bone adaptation – insights from the mouse tibia loading model. Peter Pivonka (Queensland University of Technology)
2:35pm – 2:55pm	IS23	Augmenting laboratory-based gait analysis with measurements of real-world physical activity. Dominic Thewlis (University of Adelaide)
2:55pm – 3:15pm	IS24	Wearables to assess knee joint replacement rehabilitation. Justin Fernandez (University of Auckland)
3:15pm – 4:00pm		Afternoon Tea (*Queenstown Room*) and Poster Session (Odd Numbered Posters) *Wakatipu Room and Clancy's Room*
4:00pm – 5:00pm		ECIC "Building Your Research Profile" Symposium *Coronet Room*
5:00pm – 6:00pm		Gondala ride to Skyline Restaurant
6:00pm – 7:00pm		ANZBMS AGM
7:15pm		Conference Dinner at Skyline Restaurant

Wednesday 5th September

8:30am – 10:20am		Session 9 - Selected Abstracts Chairs: Rory Clifton-Bligh and Rachel Davey *Coronet Room*
8:30am – 8:42am	OR28	Polara 1, a novel inhibitor of CYP24A1 activity, prevents vitamin D catabolism and enhances bone formation in mice. Paul Anderson (University of South Australia)
8:42am – 8:54am	OR29	Intestinal calcium absorption increases markedly during pregnancy and lactation despite absence of the vitamin D receptor (VDR) or calcitriol. Christopher Kovacs (Memorial University of Newfoundland)

8:54am – 9:06am	OR30	A longitudinal study of vitamin D status, bone quality and risk for fracture-related hospitalisation in older women. Kun Zhu (Sir Charles Gairdner Hospital)
9:06am – 9:18am	OR31	Low-trauma rib fracture in the elderly: risk factors and mortality consequence. Ha Mai (Garvan Institute of Medical Research)
9:18am – 9:30am	OR32	Pseurotin A suppresses osteoclastogenesis and prevents ovariectomy-induced bone loss by scavenging reactive oxygen species. Kai Chen (University of Western Australia)
9:30am – 9:42am	OR33	Post-fracture mortality: A latent class analysis of multimorbidities. Thao Ho-Le (The University of Technology, Sydney)
9:42am – 9:54am	OR34	Disruption of Glucocorticoid Signalling in Osteoblasts and Osteocytes Increases Skeletal Glucose Uptake in Mice Fed a High-fat Diet. Sarah Kim (ANZAC Research Institute)
9:54am – 10:06am	OR35	Osteoglycin: Co-ordination of bone/muscle cross talk through regulation of insulin action. Paul Baldock (Garvan Institute of Medical Research)
10:06am – 10:18am	OR36	Deterioration in Cortical and Trabecular Microstructure and Matrix Mineral Density Produce Bone Fragility in Patients with Chronic Renal Disease. Ali Ghasem-Zadeh (The University of Melbourne)
10:20am – 11:00am		Morning Tea *Queenstown Room*
11:00am – 12:40am		Session 10 - Protecting the value of bone research in a post-truth world. Chairs: Tim Cundy and Jeffrey Zajac *Coronet Room*
11:00am – 11:20am	IS25	Correcting the scientific record- a broken system? Mark Bolland (The University of Auckland)
11:20am – 11:40am	IS26	The design of clinical trials investigating steroid-induced osteoporosis. Kenneth Saag (USA)
11:40am – 12:00pm	IS27	Evidence-based medicine: ensuring it is robust, believable, relevant and has integrity. Ian Harris (University of NSW)
12:00pm – 12:12pm	OR37	Estimating potential treatment benefits for an initial fracture to prevent a subsequent fracture. Steve Frost (South Western Sydney Local Health District)
12:12pm – 12:24pm	OR38	Intravital imaging of osteoclasts in vivo reveals novel cellular dynamics which may underlie the therapeutic response to Denosumab withdrawal. Michelle McDonald (Garvan Research Institute)

12:24pm – 12:36pm	OR39	Effect of denosumab compared with risedronate on percentage change in lumbar spine BMD at 12 months in subgroups of glucocorticoid-treated individuals. Kenneth Saag (USA)
12:40pm		Meeting Close

Invited Speakers

IS1

N-cadherin in osteolineage cells as modulator of bone mass and cancer growth

Roberto Civitelli and Francesca Fontana
Division of Bone and Mineral Diseases, Washington University School of Medicine, St, Louis, MO, USA

We have shown that genetic ablation of *Cdh2* (N-cadherin gene) in osteolineage cells results in osteopenia and decreased osteoprogenitor number. However, others have shown that mice overexpressing *Cdh2* in osteoblasts are also osteopenic; an action linked to a negative effect of N-cadherin (Ncad) on Wnt signaling. We have also demonstrated that Ncad has different effects on osteolineage cells depending on their differentiation stage: it supports mesenchymal and progenitor stem cells but restrains mature osteoblast activity. Ncad is also involved in early post-natal skeletal growth; the latter action is independent of Ncad antagonism of Wnt signaling. Delaying *Cdh2* ablation in mice until after weaning results in higher trabecular bone mass; and lack of N-cadherin in osteolineage cells enhances the anabolic effect of Wnt stimulators without detrimental effects on osteoprogenitors. This, interference with Ncad in the adult skeleton may widen the therapeutic window of bone anabolic agents that depend on Wnt signaling. Osteoblast Ncad has also been proposed as a molecular dock for tumor cells to engraft and form the metastatic niche in bone. We find that *Cdh2* ablation in osteolineage cells in mice does not alter bone marrow stromal cells capability of engaging in direct cell-cell interactions with breast tumor cells, nor cancer cell dissemination to bone from primary tumors, intratibial growth and osteolysis. Surprisingly, however, subcutaneous tumors grow larger in *Cdh2* cKO; and intratibial injection of breast cancer cells produce higher number and size of lung metastases in mutant mice. Cell tracking experiments reveal the presence of Osx+ and Ncad+ double-positive cells in breast tumor stroma and, unexpectedly, in a small population of cells in the lungs and mammary fat pad from non-tumor bearing mice. RNAseq analysis show similarities between tumor stroma Osx+ cells and osteoblasts, including expression of cytokines and genes involved in extracellular matrix deposition and remodeling. Furthermore, in Osx+ cells of tumors growing in *Cdh2* cKO mice expression of PTEN is reduced, while MAPK target genes are up-regulated relative to tumors growing in wild type mice, patterns observed in the stroma of highly aggressive tumors. Thus, Osx+ cells with an osteogenic signature are resident in some extra-skeletal tissues frequent sites of metastasis and affect tumor growth. Further, and contrary to expectations, Ncad in Osx+ cells actually reduces cancer growth and metastasis, and in a cell-cell adhesion independent fashion, via modulation of pro-oncogenic signaling pathways.

IS2

Bone vasculature and its role in bone remodeling.

Marie-Helene Lafage-Proust
MH Lafage-Proust, B Roche, INSERM U1059, Université de Lyon, Saint-Etienne, France

All the functions of bone, including calcium/phosphate metabolism, endocrine secretions, locomotion and hematopoiesis, depend on a common blood supply. Not only bone blood vessels bring oxygen, nutrients and regulatory factors and remove metabolic waste products, as in any other organ, they also transport or bear bone precursor cells. Throughout life, these functional relationships are supported by a complex network of reciprocal signals in which vascular cells stimulate bone cells and bone cells, in turn, elicit cues to modulate blood vessels. The role of bone vessels is rather well described in bone modeling situations such as development, growth or fracture repair. In contrast, their functions in bone remodeling remain poorly understood.

In trabecular bone, Bone Multicellular Units BMU are separated from the marrow and the vessels by a thin canopy of cells which delineates the bone remodeling compartment (BRC). In cortical bone, remodeling secondary osteons constituting the Haversian canals are also centered by vessels Thus, remodeling BMUs are anatomically linked to a microvessel. Bone vessels bring osteoclast precursors at the front of the BMU while the osteoprogenitors, which give rise to the osteoblastic lineage, are recruited at the rear from circulating cells or differentiate from perivascular cells. The mechanisms which control the functional coupling between bone remodeling and vessels are not well known. For instance, we do not know whether the birth of BMU always necessitates sprouting of a new vessel (ie angiogenesis) or whether preexisting vessels are able to remodel their position in relation to the activated bone surface. Interestingly, JM Délaissé's team observed that the presence and spatial orientation of the vessels neighboring the BRC depended on the remodeling activity of the bone surface ( ). Furthermore, few information related to the tight spatial and temporal regulations that allow adequate guidance and regression of the capillary that accompanies the BMU throughout its lifespan, is available.

Kusumbe et al ( ) showed that osteoprogenitors are borne on the wall of a subtype of bone vessels which express both CD31 and endomucin. These vessels, close to the bone surfaces, are located at the interface between the arterial capillary and the venous sinusoid networks and are therefore referred to as "transitional vessels"( ). Flow cytometry analyses showed that the population of bone endothelial cells that express high levels of CD31 and endomucin, namely Type H cells, declines with age while no overall loss of endothelial cells occurs during ageing in the bone marrow. The H cell population can be expanded by activating endothelial HIF-1α or downregulating their Hippo/YAP/TAZ signaling pathways. Most interestingly, these genetic manipulations in endothelial cells only, increased transitional vessel density together with perivascular osteoprogenitors number, bone formation and bone mass. Furthermore, Cao's team demonstrated that PDGF-BB released by pre-osteoclasts also induced growth of type H vessels and stimulated bone formation in ovariectomised mice ( ), emphasising the coupling role of bone vessels in bone remodeling.

We used the intermittent administration of Parathyroid Hormone (int PTH) as a model to analyse vessel behaviour during bone anabolism. Blocking VEGF, a potent angiogenic agent, during int PTH treatment blunted PTH osteoanabolic properties in rodents. However, we found that int PTH relocated small bone vessels closer to the bone forming surfaces without significant changes in bone vessel density ( ) and induced microvessel morphological changes, suggesting that post angiogenesis, also referred to as "vascular maturation" may occur during bone anabolism ( ). Post angiogenesis entails an integrated series of vascular events including arteriovenous specification, vessel pruning and vessel coverage by mature pericytes. Bone marrow pericytes constitute a heterogeneous population which can express a number of markers including Nestin, PDGFR, SDF-1/ CXCL12 (CAR cells), Leptin receptor or NG2. Lineage tracing analyses revealed that the proportion of osteoblasts arising from the differentiation of Leptin receptor positive (Leptin R+) cells, found at the bone surface, increases with age ( ). Interestingly, it was recently shown that the expression of the transcription factor Ebf3 ( ) by Leptin R+ cells, dedicated these cells to supporting the hematopoietic niche and prevented them from differentiating into osteoblasts. Using Angiogenesis microarray, vessel immunofluorescence quantification, and Flow cytometry analyses at various time points, we found that int PTH did not expand the type H endothelial cell population but impacted the transitional vessels by reducing their coverage by Leptin R+ pericytes while increasing the number of "free" Leptin R+ detached from vessels. Int PTH upregulated transitional vessel expression of Collagen Type 18/Endostatin, while a same daily dose of continuously infused PTH did not. We also detected, under int PTH, a significant increase in bone expression of Pigment Epithelial Derived Factor (PEDF), an extracellular matrix-linked molecule which, as Collagen type 18, may exert potent antiangiogenic properties ( ).

In summary, a subset of vessels among the bone vasculature seem to be specifically involved in bone remodeling. These vessels may respond differentially according to the type of anabolic stimulus. A fine tuning of pro and antiangiogenic cues is probably critical in a time- and location-dependent manner to insure normal bone remodeling. Whether it is the nature of the vessel/pericyte cross-talk which determines the fate of pericytes remains to be further elucidated. Better understanding of the role of vessels in bone, and especially during bone anabolism in adults should help us to improve designing better drugs for treatment of bone fragility.

[1] Kristensen HB et al J Bone Miner Res. 2013 ;28:574-85
[2] Kusumbe A, et al . Nature, 2014; 507 325-328
[3] Acar M et al. Nature. 2015; 1;526(7571).
[4] Xie H et al. Nat Med. 2014 ;20:1270-8
[5] Prisby et al J Bone Miner Res. 2011 ;26(11):2583-96.
[6] Roche et al. J Bone Miner Res. 2014; 29:1608-18
[7] Zhou B et al Cell Stem Cell. 2014 7;15(2):154-68
[8] Seike M et al Genes and Development 2018,32:1–14
[9] Caire et al, ASBMR meeting 2018, in revision,

IS3

Vascular calcification: Disentangling the bone-cardiovascular disease nexus

Joshua Lewis
Edith Cowan University

Low bone mass and fractures have been linked with cardiovascular disease and vice versa. In addition to nutrient exchange, there is bi-directional signalling between the blood vessels and the skeleton that is essential to maintain both bone and vascular health. Perturbations of signalling during aging and chronic disease can lead to bone loss and vascular calcification. In vitro and in vivo animal studies have identified circulating promotors and inhibitors of vascular calcification produced by bone cells. After the skeleton, the vasculature is the next most calcified structure within the body in older men and women, and is particularly evident in the abdominal aorta. Low bone mass is associated with increased abdominal aortic calcification and cardiovascular disease risk. Advanced abdominal aortic calcification is associated with increased risk of fractures at a number of sites. It is essential to improve our understanding of bone-vascular interactions to address the potential risk-benefit for therapeutic agents to treat both skeletal deterioration and cardiovascular disease. This presentation will discuss the bone-cardiovascular disease nexus with particular emphasis on abdominal aortic calcification and its relationship to cardiovascular outcomes, bone mineral density and fractures in older women. The presentation will also discuss potential mechanisms underlying this nexus.

IS4

Anabolic vs anti-resorptive Therapies for Osteoporosis: How Should They Be Used?

Michael McClung, MD, FACP

Osteoporosis is a condition in which bone loss develops over years or decades, destroying the microarchitecture of both the trabecular and cortical compartments of bone resulting in impaired bone strength and increased risk of important fractures. The bone loss after menopause in women and with ageing in both men and women is due to an imbalance in bone remodeling such that resorption exceeds formation. The purpose of treating patients with osteoporosis is to strengthen the skeleton and to reduce the incidence of fracture. Over the past 25 years, several classes of therapeutic agents have been demonstrated to reduce fracture risk in older postmenopausal women with osteoporosis. All currently used therapies target and modulate the bone remodeling process, either by inhibiting bone resorption (anti-resorptive) or by activating bone formation (anabolic agents). Anti-resorptive agents include estrogen receptor agonists, bisphosphonates and RANK ligand inhibitors. All inhibit osteoclastic bone resorption and, secondarily, reduce bone formation. As a result, these agents are more appropriate called anti-remodeling agents. These drugs strengthen the skeleton by reducing or eliminating the overall remodeling imbalance and increase bone mineral density (BMD) by filling in the remodeling space and increasing tissue mineralization density. These drugs do not, however, correct the architectural disruption in trabecular bone although some agents may decrease the extent of cortical porosity. The only anabolic agents currently available are PTH receptor agonists, teriparatide and, in some countries, abaloparatide. These agents activate both remodeling-based and modeling-based bone formation but also activate bone resorption which limits the overall anabolic response. Teriparatide improves trabecular architecture by increasing trabecular thickness and number but also, at least transiently, increases cortical porosity. Sclerostin inhibitors are also anabolic agents that activate modeling-based bone formation, decrease bone resorption and in animal models, restore bone mass, structure and strength to normal levels. One of these agents, romosozumab, has completed Phase III fracture endpoint clinical trials and is undergoing regulatory review.

The notion of increasing bone formation while decreasing bone resorption by combining an anabolic and an anti-remodeling agent has theoretical appeal. However, studies using bisphosphonates and PTH receptor activators showed no significant advantage of combination therapy over monotherapy. The simultaneous use of denosumab and teriparatide resulted in larger gains in both spine and hip BMD during the first year of therapy than was achieved with either drug alone. However, the greater increase in BMD occurred despite a substantial decrease in markers of bone formation with combined therapy, raising the question of whether the salutary anabolic effect of teriparatide monotherapy on trabecular structure is realized when the two drugs are combined. The study was far too small to evaluate the effect of combination therapy on fracture risk. Even if the cost of combined therapy were not prohibitive, I see no justification in using anabolic and anti-remodeling agents in combination at this time.

Each available osteoporosis drug received regulatory approval based on placebo-controlled fracture endpoint studies. Differences in patient populations, duration of treatment and definitions of fracture endpoints in these studies obviate the ability to compare efficacy among the agents. Primarily because of cost and the need for daily subcutaneous injection, teriparatide has traditionally been used as a second line treatment in patients who failed bisphosphonate therapy. Recent studies with each of the three anabolic agents provides strong and consistent evidence that beginning therapy with an anabolic agent, followed by a potent anti-remodeling agent, in patients at moderate or especially high risk of fracture is more effective in reducing fracture risk than is beginning therapy with an anti-remodeling agent. Two studies were active comparator studies in high risk patients, all of whom had prevalent vertebral fractures at study entry. In the VERO trial (1), daily teriparatide therapy was shown to reduce vertebral and clinical fractures more effective than weekly risedronate therapy while the ARCH study demonstrated superiority of romosozumab over alendronate (2). These studies provide strong evidence to change the treatment paradigm in patients at high risk of fracture to initiate therapy with an anabolic agent for 12-24 months, followed by either a bisphosphonate or denosumab.

[1] Kendler DL et al. Lancet. 2017 Nov 9. pii: S0140-6736(17)32137-2.
[2] Saag KG et al. N Engl J Med. 2017;377:1417-27.

IS5

Trials our patients need (and some they don't)

Andrew Grey
University of Auckland

It is widely accepted that randomized clinical trials (RCTs) with 'hard' outcomes represent the most reliable evidence to inform clinical practice and thereby improve the health of patients. Oft-repeated limitations of RCTs include lack of external validity and practical issues that include high cost and resource consumption.

For skeletal health, the 'hard', clinically meaningful outcomes are fractures and falls. In the past 20 years, the conduct of RCTs has clarified the efficacy (or lack thereof) of a number of interventions for reducing the risk fracture. The research agenda during this time has been dominated by commercially sponsored programmes that have largely delivered short-term, placebo-controlled trials of drugs with varying configurations of fracture endpoints. As the speed of new drug development slows, a shift in focus and resourcing of clinical trials is both inevitable and desirable.

Consideration of key uncertainties faced by patients and their clinicians should drive the new research agenda. Areas of uncertainty are many, including the utility of systematic secondary prevention strategies, and the efficacy and safety of non-pharmacologic strategies such as exercise, falls prevention, and correction of vision abnormalities. In pharmacological treatment, comparative trials of initial therapy with the agents known to be superior to placebo in reducing fracture risk are needed, as are trials to determine the optimal means of applying individual agents, and trials to determine how best to integrate anti-resorptive and anabolic therapies. Some populations, such as the frail elderly, have not been adequately assessed in trials of current therapies.

Conducting RCTs that address these patient-important questions should be possible by better coordination of academic resources, adoption of 'point-of-care' trials with opt-out provisions, development of robust registry-based systems for collection of outcome data and redistribution of resources away from areas of investigation that are mature and/or very unlikely to yield important new findings.

IS6

Functional genomics and gene editing in bone: new tools and standardising outcomes.

A/Prof Aaron Schindeler
University of Sydney

A current limitation to understanding and treat genetic bone disease is a lack of tools for effective gene delivery and gene editing in bone. Our research program focuses on applying emerging genetic technologies in the context of bone.

Transplantation of healthy donor cells has been postulated as a strategy for treating brittle bone disease by supplying healthy osteoprogenitors, but engraftment levels are typically low. We attempted to improve engraftment efficiency by marrow ablation via irradiation. This approach failed to generate functional improvements in the Col1a2G610C mouse model, with the increased cell engraftment chiefly affected osteoclasts rather than osteoblasts. This led us to focus on alternative genetic approaches for restoring normal collagen levels in vivo.

CRISPR/Cas9 gene editing has emerged as an increasingly common method for disrupting gene function and engineering specific changes. We have validated a CRISPR gene editing system in cultured osteoblasts with utility in functional genomics assays. In addition to in vitro differentiation and mineralization outcomes, we report on an osteoblast xenograft model able to produce human-derived bone in nude mice.

Adeno-associated viruses (AAVs) have been successfully used to efficiently deliver genes to a variety of tissues, with different serotypes possessing differing cell tropisms. We tested a range of AAV serotypes, both natural and engineered, for their ability to transduce bone cells in vivo in a mouse fracture model, and in vitro in a cultured human osteoblast cell line (hFOB1.19). While the majority of AAV serotypes (including the conventional AAV-2) poorly transduced bone cells in healing fractures, we identified three AAV serotypes with high efficiency in bone have been targeted for future development.

Together, these technologies have significant potential for exploring bone gene function, patient diagnostics, and future gene therapy.

IS7

Conversion of Human Fibroblasts into Functional Osteoblasts by Extrinsic Factors.

Hala Zreiqat
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Head: Tissue Engineering & Biomaterials Research Unit, Faculty of Engineering and IT, The University of Sydney.

The success of reprogramming somatic cells such as fibroblasts into induced pluripotent stem cells (iPSCs) has been offering an exciting route for tissue repair and regeneration, as it allows patients to use their own cells for tissue repair and regeneration. However, the perspective of using iPSCs for tissue regeneration is compromised due to the complexity of iPSCs production and the fact of posing the risk of teratoma formation by the residual undifferented iPSCs upon transplantation. In order to surpass above-mentioned shortcomings of using iPSCs, our study aimed to directly reprogramming fibroblasts into functional osteoblasts for bone tissue regeneration. We identified one protein released by human osteoblasts, which is able to convert human fibroblasts into functional osteoblast-like cells. This pharmacological approach by using the protein compound to reprogram fibroblasts into osteoblasts will have critical therapeutic implications to the patients with bone loss-related diseases, including regeneration of critical-sized bone defect and repair of non-union bone fracture healing.

IS8

Periosteal progenitors and fracture healing.

Brya Matthews
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The periosteum is a major source of cells involved in fracture healing, but the identity of osteoprogenitors in the periosteum is undefined. A number of different markers and approaches have been utilized to identify osteoprogenitors in different settings. We have compared cell populations in periosteum to endosteum and bone marrow, and discovered that periosteum is greatly enriched for cells with colony forming potential in vitro, as well as enriched for the majority of putative stem cell markers evaluated. My research has focused on progenitors that express alpha smooth muscle actin (αSMA), and characterising the fate of these cells using lineage tracing.

To identify and trace αSMA+ cells we crossed αSMACreERT2 with Ai9 tdTomato reporter mice. In many instances this was combined with the Col2.3GFP reporter to identify mature osteoblasts. We have shown that αSMA-labelled cells contribute to a large proportion of osteoblasts, chondrocytes and fibrous tissue within a fracture callus. Global gene expression analysis identified numerous changes occurring in αSMA-labelled cells following fracture, including downregulation of Notch signalling.

In order to evaluate if the ✔SMA-labeled population in the periosteum contains long-term progenitors, tibia fractures were generated in αSMACre/Ai9/2.3GFP mice treated with tamoxifen 13, 6 or 2 weeks prior to fracture, or on the day of fracture, and contribution to osteoblasts and chondrocytes quantified in a 7 day fracture callus. The results indicate that the majority of osteoprogenitors involved in fracture healing express ✔SMA. A subset of these cells remain capable of contributing to osteoblasts after 3 months indicating long-term progenitor potential. Efforts are ongoing to try and identify subpopulations of cells that represent long term progenitors.

IS9

Circadian clock disruption in the pathogenesis of osteoarthritis.

Raewyn Poulsen
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The most well-known circadian clock is the light-sensitive clock present in the suprachiasmatic nucleus (SCN) of the hypothalamus that regulates daily rhythms in body temperature, hormone release and the sleep/wake cycle. However, clocks are also present in almost all other cells in the body. Cell clocks enable time-of-day specific control of cell activity. They also serve as a ”calendar”, regulating cell differentiation and the progression of cells through their lifecycle. Disruption to cell clocks has been implicated in a range of diseases including neurodegeneration, diabetes and cancer. Recently, we demonstrated that the chondrocyte clock is also altered in osteoarthritis. In vitro and in vivo studies indicate that this change in the clock causes disease-associated changes in chondrocyte phenotype and cartilage loss in animal models.

Cell clocks are “re-set” daily by environmental stimuli (light in the SCN; glucose, hormones, mechanical loading etc in peripheral tissues). Re-setting is important for synchronising the cell’s internal molecular clock with external time of day. In the SCN, N-methyl-D-aspartate receptors (NMDAR) are critical for clock re-setting. Chondrocytes also express NMDAR and there is a change in the type of NMDAR expressed by cells in osteoarthritis. We found that altered NMDAR sub-type expression in osteoarthritic chondrocytes causes disease-associated changes in cell phenotype and this is at least partially a result of changes in expression of core clock components. We also found that IL-1β and oxidative stress, two factors known to cause osteoarthritis-like changes in chondrocyte phenotype, induce similar changes in the chondrocyte clock as are observed in osteoarthritis. Preventing these changes in the clock mitigates the ability of these treatments to cause disease-associated changes in chondrocyte phenotype. Disruption of the chondrocyte clock may be a common mechanism contributing to induction of disease-associated changes in chondrocyte phenotype in response to multiple different factors.

IS10

Improving translation of pre-clinical osteoarthritis research to patients: disease phenotypes, endotypes and models.

Chris Little
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Osteoarthritis (OA) at its end stage is ultimately a diagnosis based on a collection of pathological changes in joint tissues with varying associated clinical symptoms. There may be many initiating causes and pathways to reach this final point of "joint failure", the emerging paradigm being that a single entity, OA is actually a collection of disease sub-types with similar end-stage pathology. The challenge is to identify patterns in this heterogeneity that will allow us to better phenotype the disease in question. In addition to clinically defined phenotypes (e.g. post-traumatic, metabolic), it is increasingly recognized that OA is a disease of the entire joint organ, that may be categorized by the relative involvement of or disease in the different joint tissues – e.g. degree of synovitis, bone erosion versus bone formation, greater or lesser cartilage loss. Pre-clinical research in OA, and particularly that using in vivo models, is increasingly embracing the study of the joint as an organ, and interpreting data in the context of the different OA phenotype that is being modeled. Critically, these pre-clinical models allow the bio-pathophysiology of the different OA phenotypes to be explored (ie. to define their "endotype"), and thus to potentially develop appropriately targeted therapies. There remain key unresolved issues that have important implications for understanding OA pathophysiology, endotyping and improved translation of pre-clinical findings to patients. I will present some of our most recent data demonstrating that:

• Structural joint disease & pain share common pathophysiological pathways, BUT the mechanisms involved are phenotype specific;

• What preceded/lead to "end-stage" OA may alter the molecular drivers of the chronic pain: "how you got there matters";

• The right pre-clinical models are predictive of therapeutic efficacy in patients but further work is needed to develop standardized outcomes and how they relate/scale to patient reported outcomes.

IS11

The social context of osteoarthritis.

Sharon Brennan-Olsen
AIMSS and The University of Melbourne

Background: In higher income countries (HIC), social disadvantage, and specific occupational exposures are associated with higher arthritis prevalence. Less is known about arthritis in low to middle income countries (LMICs), where two-thirds of the world's population resides. In LMICs there is a reduced ability to access or afford treatments including analgesic and anti-inflammatory pharmacotherapies, or arthroplasty for advanced disease. There exists less flexibility regarding working conditions/hours, and few if any options for early retirement, or social security 'safety nets'. Here, the social context of arthritis in HIC, and associations between occupational exposures and arthritis in LMICs, are reported.

Methods: Data from the World Health Organization's Study on global AGEing and adult health (SAGE) Wave 1 (2007-10) were extracted for adults (aged ≥50years) from Ghana, India, Russia and South Africa for whom detailed occupation data was available (n=21,389; 49.2% women). Arthritis was defined using a symptom-defined algorithm and self-reported doctor-diagnosis. Using a sex-specific, internationally comparable Job Exposure Matrix, occupational exposures were classified as: heavy physical work (HPW), kneeling/squatting (K/S), heavy lifting (HL), arm elevation (AE) and awkward trunk posture (ATP). Logistic regression models and excess exposure risk due to two-way interactions with other risk factors were explored.

Results: Doctor-diagnosed arthritis was associated with HPW (adjusted odds ratios [AOR] 1.12, 95%CI 1.01-1.23), ATP (AOR 1.23, 95%CI 1.12-1.36), K/S (AOR 1.25, 95%CI 1.12-1.38), and AE (AOR 1.66, 95%CI 1.37-2.00). Symptom-based arthritis was associated with K/S (AOR 1.27, 95%CI 1.08-1.50), HL (AOR 1.33, 95%CI 1.11-1.58), and AE (AOR 2.16, 95%CI 1.63-2.86). Two-way interactions suggested excess arthritis risk existed for higher body mass index, and higher income or education.

Conclusions: Minimization of occupational health risk factors is crucial for planning future healthcare delivery and ensuring sufficient health workforce capacity: significant concerns in an ageing world.

IS12

Sarcopenia: Can nutrition make a difference?

Sandra Iuliano
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Progression and severity of sarcopenia manifests with inadequate nutrition, in particular protein malnutrition. Additional to achieving protein adequacy, frequency of consumption and composition of proteins consumed may also alter the course to sarcopenia. Metabolic changes with aging increase protein requirement, which is higher than for healthy adults, and is suggested at 1.2 and up to 1.5g/kg body weight daily. How protein is distributed throughout the day may also promote muscle protein synthesis, with 20-30g protein per meal considered adequate. And finally, specific amino acids such as leucine are considered potent stimuli for muscle protein synthesis, even in those with sarcopenia, and so adequate consumption may potentially maintain muscle in old age. Within these parameters protein intake needs to be manipulated to fulfill these recommendations, whether through supplementation, fortification, appropriate food choices, or a combination of mechanisms to ensure an adequate intake. Provision of oral protein supplements is effective in the short term, but long-term sustainability remains unclear. Fortification improves nutritional density, so is feasible to increase protein content without adding to food volume. Animal sources of protein i.e. dairy, meats, eggs and seafood provide all essential amino acids, in particular leucine so consumption of these foods in line with recommendations may assist with slowing sarcopenia development. Those at high risk of protein inadequacy include isolated elderly in the community, and those in institutionalized care. Particular attention to menu planning for, and food provision to these vulnerable elderly is critical as sarcopenia risk is high and onset may be masked by other conditions or comorbidities. A better understanding of sarcopenia development and the role of protein nutrition in its prevention may help guide strategies required to curtail the burden of sarcopenia and its associated costs that would otherwise rise as the population ages.

IS13

Androgen deprivation as a model of osteosarcopaenic obesity.

Mathis Grossmann
The University of Melbourne

Androgens either directly or via aromatisation to estradiol regulate multiple somatic tissues including bone, muscle and fat. We use men with non-metastatic prostate cancer receiving androgen deprivation therapy (ADT) as a model of severe sex steroid deprivation with a temporarily defined onset to define clinical phenotypes associated with sex steroid deficiency. In this presentation I will describe work demonstrating that ADT is associated with accelerated loss of bone, loss of muscle mass and function, increased fat mass, and insulin resistance, leading to accelerated development of osteosarcopaenic obesity. It is possible that sarcopenia, increased adiposity and insulin resistance contribute not only to accelerated bone loss and increased fracture risk, but may also synergise in promoting frailty.

IS14

Sarcopenia: Pharmacology of Today and Tomorrow

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Originally described as “muscle wasting”, sarcopenia is now a disease which was recognised by the ICD-10 in 2015. Sarcopenia is defined as loss in muscle mass associated with a decline in muscle function and/or strength. It affects 25% of our Australian older population and is also associated with other diseases such as chronic kidney disease, COPD and heart failure. Pathophysiology includes a combination of changes in growth factors, inflammation, and fat infiltration of muscle fibres. Definition criteria for sarcopenia remain controversial with two major definitions using different criteria: FNIH and the European Consensus. Clinical outcomes associated with sarcopenia include functional decline, frailty and falls. Non-pharmacological approaches include vitamin D and protein supplementation, and physical activity. New pharmacological agents are being tested in phase II trials. However, the results have been limited. In this presentation, we will focus on current and future interventions to improve muscle mass and function in sarcopenic patients.

IS15

Multiple functions of connexins in bone and adipose tissue homeostasis.

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Connexin43 (Cx43), the major gap junction protein expressed in bone, modulates cortical modeling. Ablation of the Cx43 gene (Gja1) in the osteogenic lineage causes periosteal expansion and cortical thinning, driven by accentuated endocortical bone resorption, mimicking changes occurring in bone disuse and aging. Such changes are phenocopied by conditional replacement, in osteolineage cells, of one Gja1 allele with the oculodentodigital dysplasia Gja1G138R mutant, which lacks gap junction channel function, but retains "hemichannel" activity. Notably, mice heterozygous for a truncated Gja1 mutant, lacking the last 5 aminoacids at the C-terminus (Gja1D378STOP) also exhibit cortical thinning and expansion, but remindful of the epiphyseal flaring seen in craniometaphyseal dysplasia, whose recessive form has been linked to GJA1 mutations in humans. Since Gja1D378STOP cannot bind ZO-1, the accumulated results suggest that both the channel and the scaffolding / signaling functions are necessary for Cx43 action on cortical bone modeling. We also find that, at variance with Cx43, conditional ablation of the Cx45 gene (Gjc1) in osteolineage cells results in high trabecular bone mass and no cortical abnormalities. Furthermore, while Cx43-deficient and Gja1G138R –expressing bone marrow stromal cells exhibit accentuated support of osteoclast differentiation in co-cultures with macrophages, the opposite occurs with Cx45-deficient stromal cells, whose capacity to support osteoclastogenesis is decreased. Mechanistically, Cx43 inhibits osteoclastogenesis via up-regulation of osteoprotegerin, an inhibitor of the pro-osteoclastogenic factor, receptor activator of NFκB ligand (RANKL). By contrast, Cx45 stimulates RANKL expression by bone marrow stromal cells. Thus, Cx43 and Cx45 have distinct, compartment specific functions in regulating bone modeling and opposite actions on osteoclastogenesis. Recently, Cx43 and functional gap junctions have been detected in both brown adipose tissue (BAT) and white adipose tissue (WAT). Indeed, we confirmed expression of Cx43 in WAT and BAT; more to the point, ablation of Gja1 embryonically in mesenchymal multilineage cells leads to a "lean" phenotype, with decreased fat mass and body weight, but no alterations in food intake or energy consumption. Inactivation of Gja1 selectively in bone cells does not reproduce the lean phenotype; and absence of systemic alterations of metabolic markers (circulating fatty acids, cholesterol and glucose tolerance) imply that Cx43 regulates fat tissue via cell autonomous mechanisms. Thus, Cx43 plays an unexpected role in the regulation of fat homeostasis, favoring accumulation of WAT. Cx43 may represent a potential new pharmacologic target to modify bone structure and to counteract obesity.

IS16

Evaluation and management of bone fragility in Chronic Kidney Disease

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Chronic Kidney Disease (CKD) severely affects calcium/phosphate metabolism and bone health. As renal function declines, hypocalcemia, hyperphosphatemia and secondary hyperparathyroidism develop together with an increased risk for fracture and vascular calcifications, which both have a high impact on morbidity and mortality. In CKD stage 3 to 5, in dialyzed or kidney transplant (KT) patients, the risk for fracture is always higher than in age and sex-matched non uremic populations. However, a 30% decrease in fracture rate was observed in KT patients, probably due to the improvement of immunosuppressive therapy and the concomitant reduction in steroid burden.

Recent data contributed to promote DXA (Dual-Energy X-Ray Absorptiometry) for evaluating fracture risk in the CKD population. A meta-analysis gathering 13 cross-sectional studies including 1785 patients (stage 3 to 5d) showed that BMD at the lumbar and femoral sites is always lower in patients with fracture than without ( ). Longitudinal data reinforced these findings. 587 CKD patients (stage 3a-3b) and 2167 patients with normal renal function were followed for 11 years. The level of association between low BMD and fracture was identical in the two groups . Similar conclusions were drawn in a prospective study related to dialyzed patients . Thus, the latest KDIGO guidelines, in 2017, recommended the use of DXA in CKD patients . However, if DXA has a good specificity for the diagnosis of bone fragility, its sensitivity is poor, since 1/3 of the patients with fractures have a T score > 2.5. The FRAX score was designed to improve fracture prediction. It computes individual 10 year-risk for fracture by combining clinical data to femur DXA measurements. Among men and women with CKD, FRAX is able to discriminate fracture status but, unfortunately, performs no better than BMD alone probably because, the CKD factor was not included in the calculation procedure when the FRAX score was generated. High Resolution peripheral Computerized micro-Tomography (HR-pQCT) is able to measure BMD and structural parameters in both the trabecular an cortical compartments at the tibia and the wrist. Several studies suggest that HR-pQCT may be useful and exhibit better performance than DXA to predict fracture in the CKD population . However, this device is not widely available.

Evaluating bone turnover and mineralization is needed for the diagnosis of the type of renal osteodystrophy (ROD, ie histological bone lesions observed in CKD). This is critical for the management of ROD and when we consider giving an anti-osteoporotic drug to a CKD patient. A number of bone turnover markers, especially all the Collagen-derived markers, depend on Glomerular Filtration Rate (GFR) making their use problematic when dealing with patients with CKD. Thus, Bone alkaline Phosphatase (BAP) remains the only marker to be used when GFR is below 30ml/min. in a number of case BAP and PTH serum levels measurement allow a rather accurate evaluation of bone turnover. However, post-fracture modeling may strongly impact BAP serum levels for several weeks and thus become useless at a time when precisely it is critically needed. Further, BAP is a marker of bone formation which also bring indications on bone primary mineralization. However, its interpretation remains difficult when both remodeling and mineralization are affected concomitantly as in mixed uremic bone disease. Thus, histomorphometric analysis of anterior iliac crest biopsy remains the ultimate tool for the diagnosis of ROD. Bone biopsy is still recommended by the KDIGO guidelines, only when its results condition the therapeutic strategy. This procedure is usually performed with an 8mm-diameter trephine. However, this procedure is progressively forgone due to its invasiveness and cost as well as to the increasing lack of experts able to carry it out and availability of the trephine. We recently validated ROD diagnosis on halved bone samples, mimicking those obtained with a 4mm-diameter trephine, a procedure of increasing popularity that has not been endorsed yet as for the treatment for preventing fractures, there is no randomized clinical trial testing the efficacy and safety of anti-osteoporotic drugs in patients with late stages of CKD. Analyses of the data extracted from the pivotal studies related to patients from the general population whose GFR had been estimated from creatinine serum levels (MDRD, Cockcroft), concluded that bisphosphonates, teriparatide and denosumab were as safe and effective in CKD patients stage 2-3 as in the non uremic patients. Tight follow-up of calcium serum levels is recommended in patients under denosumab due to a higher risk of hypocalcemia within a median time of 71 days after the injection.

In conclusion, the evaluation and management of osteoporosis in CKD remains a challenge. Due to the major difficulties encountered when we take care of dialyzed patients with fractures, it would be necessary to detect bone fragility as early as possible, ie before CKD stage 4-5d, to be able to prevent fractures in an efficient and safe manner.

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IS17

The role of Eph/ephrin molecules in mesenchymal stem cell function and their influence on bone biology.

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Molecular signals are fundamental to preserve and maintain skeletal integrity. The largest family of receptor tyrosine kinases, the Eph receptors and ephrin ligands, comprised of the A and B-subclass, are well known for their role in contact-dependent repulsion, migration and differentiation. Over the last decade these molecules have been shown to influence the processes of skeletal developmental and homeostasis, stem cell niche maintenance, and aid in tissue repair in mouse models and in vitro assays using human mesenchymal stem cells (MSC). We have identified receptors and ligands of the B subclass that are expressed by both human and mouse mesenchymal stem cells (MSC), hematopoietic stem cells (HSC), in addition to progenitor and mature osteogenic and hematopoietic population. Furthermore, a number of these B subclass receptors and ligands are up-regulated in a mouse model of fracture repair and contribute to the repair process. With a specific focus on the ligand ephrinB1, where mutations in ephrinB1 in humans causes craniofrantonasal syndrome (CFNS), in addition to other skeletal deformities. We have demonstrated that ephrinB1 not only influences skeletal development, maintenance and repair by promoting osteochondral ossification, but is also implicated in maintaining the balance between osteoblasts and osteoclasts of 4 week old and in 6 month old mice. Human in vitro studies confirmed that both of these processes were mediated by the direct interaction between EphB2 and ephrinB1. Furthermore, our mouse and human studies indicate that EphB-ephrinB communication also contribute to the stromal support of the hematopoietic stem cell niche, particularly through EphB1/2 expressing haematopoietic stem/ progenitor cells and ephrinB1-expressing stromal populations. Collectively these studies illustrate the importance of Eph-ephrin communication not only in the functional response of MSC but also their contribution to the bone microenvironment.

IS18

Using Big Data imaging to unravel the risks and/or benefits of cell-cell fusion in macrophages, osteoclasts and giant multinucleated cells.

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To acquire phenotypes required for specialised functions, plant and animal cells generally undergo differentiation during asymmetric division. However, certain specialised cells can also acquire unique capabilities through the fusion of two or more cells, resulting in a mixture of cytoplasmic and possibly genetic material. Using a combination of Big Data and biophysical modelling, we focused on unravelling the phenomena behind macrophage fusion, which are precursors of osteoclasts and giant multinucleated cells. Through quantitative analysis, we find an unusual physiological scaling that, within the bounds of moderate assumptions, indicates that cells can grow larger at lower metabolic costs, potentially allowing energetic savings to be reallocated for other more energetic-intensive biological functions. Further analysis using unsupervised machine learning suggests that these emergent multinucleated cells phenotype self-organise their F-actin cytoskeleton by a phase separation process. These empirical data have remained obscure until now because of the relative size difference between the smallest and largest multinucleated cells spanning nearly four orders of magnitude, leading to fundamental challenges in data processing and visualisation. While our findings here are specific to the macrophage lineage, our technology-enabled discoveries offer insights on why certain cell types may develop through fusion rather than division.

IS19

The use of post-mortem CT to assess skeletal trauma in fatal falls.

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Victorian Institute of Forensic Medicine / Monash University

Falls are a form of vertical deceleration that may result in blunt force trauma (BFT) to the skeleton. In medico-legal cases where skeletonised remains exhibit BFT, and the circumstances of that death are unclear but suggest a fall may have been involved, it is necessary for anthropologist to understand if the exhibited BFT is possible to have resulted from the suggested fall. At present however, the trauma that results from fatal falls has been poorly documented. The aim of this study was to strengthen the medico-legal evidence base for analysing and interpreting the skeletal trauma that results from fatal falls through investigating the patterns and types of fractures that result from low- and high-energy falls using post-mortem computed tomography (PMCT). METHOD: Skeletal trauma was assessed from full-body PMCT scans, taken at the Victorian Institute of Forensic Medicine, for cases of low free falls (n = 145), falls involving stairs (n = 57) and high free falls (n = 95). Details of the extrinsic and intrinsic variables of each fall event were recorded from the National Coronial Information System database. Skeletal fracture patterns and fracture types for each of the three fall types were then investigated in the context of these variables using multiple logistic regression models. RESULTS: Findings indicate that, although skeletal trauma resulting from a fall is highly varied, there were actually significant fracture patterns and types characteristic of each fall type. Low free falls and falls involving stairs both exhibited distinct fracture patterns with anatomically associated fracture types. High free falls, in contrast, exhibited multiple fracture patterns and types across the full skeleton. DISCUSSION: These findings will augment the forensic anthropologist's interpretation of the mechanism of BFT in cases where skeletonised remains exhibit trauma and the circumstances of the death suggest a fall may have been involved.

IS20

Monoallelic BMP2 variants predicted to result in haploinsufficiency cause craniofacial, skeletal, and cardiac features that overlap with those of chromosome 20p12 deletions

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Keywords: Craniofacial, bone, bone morphogenetic proteins, congenital heart disease, skeleton

Bone morphogenetic protein 2 (BMP2) on chromosome 20p12 belongs to a gene superfamily encoding TGF-beta-signaling proteins involved in bone and cartilage biology. Monoallelic deletions of chromosome 20p12 are variably associated with cleft palate, short stature and developmental delay. Here we report a cranioskeletal phenotype due to monoallelic truncating and frameshift BMP2 variants and deletions in twelve individuals from eight unrelated families that share features of short stature, a recognizable craniofacial gestalt, skeletal anomalies and congenital heart disease. De novo occurrence and autosomal dominant inheritance of variants was observed across all reported families, including paternal mosaicism in two affected sisters who inherited a BMP2 splice-altering variant. Additionally, we observed similarity to the human phenotype of short stature and skeletal anomalies in a heterozygous Bmp2 knock out mouse model, suggesting that haploinsufficiency of BMP2 may be the primary phenotypic determinant in individuals with predicted truncating variants and deletions encompassing this gene. These findings demonstrate the important role of BMP2 in human craniofacial, skeletal, and cardiac development and confirm that individuals heterozygous for BMP2 truncating sequence variants or deletions display a consistent distinct phenotype characterized by short stature, skeletal and cardiac anomalies, without neurological deficits.

IS21

Biomaterials for Bone Healing and Regeneration in the Context of Biocompatibility.

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Hard materials for bone replacement can be traced back to the late 19th century. The criteria for acceptability for such materials has been appropriate mechanical properties, no toxic leachables and "biocompatibility." The words "osteoconductive," "osteoinductive" and "osteointegration" are widely used. After some 70 years of systematic study and materials development, there are still profound and unmet needs in the clinic for bone healing and regeneration. This talk will address a novel path to bone healing. It will focus on regeneration guided by the macrophage cell. We have fabricated hydrogel materials with uniform, interconnected 30-40 micron pores -- upon implantation the pores are infused by macrophages. The macrophages within the pores orchestrate a different healing process from that seen with non-porous materials or materials with non-optimal pore sizes. Macrophages may be biomechanically directed to a unique phenotype that encourages reconstructive healing (M2). Thus, the inflammatory process is exploited to generate improved healing – a new biocompatibility. Reconstructive (regenerative) healing has been noted subcutaneously, percutaneously and within heart muscle, cornea and bone. Results for healing in bone and other tissues will be presented.

IS22

Coupling high-resolution bone imaging with micro finite element 6 analysis to assess local bone adaptation – insights from the mouse tibia loading model.

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Bone tissue is able to respond to external mechanical loading by adapting its structure and mass to stresses and strains induced by this load [1]. Micro-CT is currently the gold standard when assessing the effect of mechanical loading on bone structure. Imaging is generally performed ex-vivo, after sacrificing the animal at the end of the mechanical loading period (end-point imaging). Bone quantities of the entire cross section are generally computed and used for statistical analyses to compare the loaded limb with the contralateral (control) limb. However, using this methodology the local bone adaptation response on the same limb cannot be assessed. To follow local bone adaptation over time, in-vivo, longitudinal imaging procedures have been developed in combination with image co-registration algorithms. However, these protocols present several downsides, such as: excessive exposure of the animal to high radiation and anaesthesia, and large amount of data accumulation.

The aim of the current study was to extend previously obtained whole cortical bone adaptation data [2] with respect to analysing local bone adaptation responses in different cortical bone regions. To do this a novel methodology based on image analysis was developed to quantify changes of cortical thickness due to different applied loads in female C57BL/6 mice based on end-point imaging data from the study of Sugiyama et al [2]. Furthermore, micro finite element analyses were performed in order to assess whether the local changes in cortical thickness are correlated with respective mechanical quanties such as stres, strain and strain energy density.

[1] Frost H *Anat Rec*, **275A(2)**:1081-101, 2003.
[2] Sugiyama T et al. *JBMR,* **27**: 1784-1793, 2012.

IS23

Augmenting laboratory-based gait analysis with measurements of real-world physical activity.

Dominic Thewlis
University of Adelaide

Gait analysis, in its current form, combines measurements of body segment motion, foot-floor reaction forces (ground reaction force), muscle activation patterns (electromyography), anthropometry, and medical imaging to quantify joint kinematics and dynamics. Our ability to measure the quantities essential for gait analysis has improved significantly over the past decade, yet two major issues remain: (1) uncertainty introduced by measurement and modelling errors; and (2) assumptions that laboratory-based measurements reflect the behaviour of a person in the real world. The combination of these two issues has led many to become sceptical of gait analysis as a clinical tool and in some instances the validity of research findings. The second of the issues highlighted will be the focus of this talk to demonstrate how wearable sensors, which can record data for extended periods of time, can provide new insight in orthopaedic procedures. Our approach uses a combination of high-fidelity laboratory-based measurements of gait in a highly controlled environment with wearable sensors to quantity physical activity patterns 'in the wild'. While many groups are looking to quantify detailed gait parameters using wearable sensors, our approach looks to accumulate a large volume of data with high compliance rates using wrist-worn sensors. This talk will present data from three orthopaedic examples: (1) the of change (or lack of) in behaviour patterns in patients following total hip replacement surgery; (2) how a combination of musculoskeletal modelling, gait analysis and wearable sensors is helping us to understand the postoperative demands following articular fracture of the knee; and finally, (3) an example of how gait analysis and physical activity monitoring can help guide postoperative goals following total knee replacement surgery.

IS24

Wearables to assess knee joint replacement rehabilitation

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The use of wearable technology has increased exponentially in clinical applications (Piwek, 2016). Coupled with patient motion analysis they have the potential to monitor and inform patient recovery following total knee joint replacement (TKR). In this study we aim to evaluate the use of small commercial inertial measurement units (IMUs) as a surrogate metric for knee joint loading following TKR.

Twelve patients (age 69 ± 10 years; weight 105 ± 27 kg; height 151 ± 50 cm) were monitored from pre-surgery through to 6 weeks post-recovery. Ethical approval was received from the University of Auckland human ethics committee. Gait analysis (Vicon) was conducted for each patient at baseline before surgery and after 6 weeks recovery collecting lower limb joint kinematics and kinetics. In parallel, IMU peak tibial accelerations 'tibial shock' were collected twice weekly. Wearables were strapped on the medial anterial tibial site to minimise soft tissue artifacts (Figure 1). For each patient we correlated the peak tibial acceleration at heel strike with their computed knee joint contact force (via an OpenSIM model) at baseline and after 6 weeks. The tibial shock vs knee joint force was interpolated between these 2 time points.

The IMU sensor revealed that most patients exhibited between 3Gs and 6Gs (30 ms-2 to 60ms-2) at heel strike. Correlations between peak tibial acceleration at heel strike and knee joint force ranged from R2 of 0.75 to 0.96 across all patients. This correlation changed between pre-surgery and 6 weeks post for each patient. During each patient's recovery timeline, some patients presented reduced peak tibial shock while others maintained consistent peak tibial shock pre and post-surgery.

The study revealed that IMU wearables may act as a useful surrogate measure to predict knee joint force on a patient-specific level following TKR.

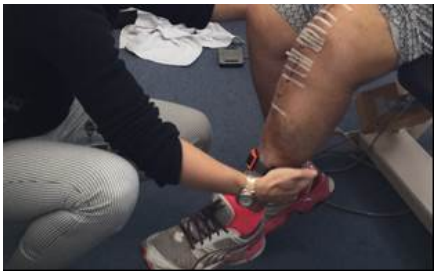

Figure 1: IMU sensor placement on patient.

Acknowledgements: The authors would like to thank the MedTech CoRE for seed funding.

Reference: Piwek, L., Ellis, D. A., Andrews, S., & Joinson, A. (2016). The rise of consumer health wearables: promises and barriers. PLoS Medicine, 13(2), e1001953.

IS25

Correcting the scientific record- a broken system?

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When errors occur in scientific papers, they should be promptly corrected. However, this often does not occur. A series of cases will be presented highlighting examples from research in the field of osteoporosis where the system for correcting errors failed to work as it ought to. Based on this experience, some different approaches to handling errors in papers will be discussed.

IS26

The design of clinical trials investigating steroid-induced osteoporosis.

Kenneth Saag
USA

Clinical trials in glucocorticoid-induced osteoporosis (GIOP) have had many similarities in inclusion/exclusion, subpopulations, glucocorticoid dosing strategies and other baseline characteristics. They have differed, however, in the comparator groups and corresponding statistical methods used. Past studies have variable reported on predominately surrogate outcomes including bone mineral density, bone turnover, but have not been powered to detect a fracture endpoint. This lecture will first examine what has been done in the past, but quickly focus on where the current gaps are in the GIOP literature and what types of future clinical trials would be optimal to fill those evidence gaps. Pragmatic trial design, ethical, and regulatory issues will also be considered.

IS27

Evidence-based medicine: ensuring it is robust, believable, relevant and has integrity.

Ian Harris
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There is disagreement in the interpretation of what makes practice evidence based that is centred on variation in the quality of evidence that is considered sufficient to guide practice. Furthermore, much of the evidence that is generated specifically to guide practice is of low quality.

There is a need for both an understanding of scientific methods (and therefore quality) and the application of scientific methods to improve the evidence based for medicine.

Each of the problems with the current practice of EBM and its solutions will be discussed;

1. Lack of science literacy. This results in low quality research being performed and inability for practitioners and the public to properly interpret the findings. Better training and knowledge requirements for doctors are required. Public education in science is also required.

2. Wasteful research. Funders or research and academics should be demanding requirements for research to minimise waste: to ensure important, answerable questions are prioritised; to improve methods, including study design, outcome reporting; to ensure feasibility and implementation.

3. Research is not integrated with practice. Regulatory and cultural changes are required for research to be part of practice instead of something 'other' than practice. Doing so will enhance the implementation of findings.

Oral Presentations

OR1 - AMGEN-ANZBMS OUTSTANDING ABSTRACT - CLINICAL PRESENTATION
Zoledronate every 18 months for 6 years in osteopenic postmenopausal women: Effects on fractures and non-skeletal endpoints

Ian Reid, Anne Horne, Borislav Mihov, Angela Stewart
University of Auckland

Bisphosphonates prevent fractures in patients with osteoporosis, but their efficacy in women with osteopenia is unknown. Most fractures in postmenopausal women occur in osteopenic individuals, so if pharmaceutical intervention is to impact significantly on total fracture numbers, therapies with efficacy in osteopenic postmenopausal women are needed.

We report a double-blind trial of 2000 osteopenic, postmenopausal women, randomly assigned to receive 4 infusions of either zoledronic acid (zol) 5mg, or normal saline at 18-month intervals. Each was followed for 6 years. Monthly vitamin D supplements were provided but not calcium supplementation. Women aged >65 years with hip T-scores between -1.0 and -2.5 were recruited.

Baseline age was 71 (SD 5) years and femoral neck T-score -1.5 (0.5). The primary endpoint of osteoporotic fracture (i.e. osteoporotic non-vertebral fractures plus morphometric vertebral fractures) occurred in 190 women in the placebo group (227 fractures) and in 122 women in the zol group (131 fractures), hazard ratio (HR) 0.63 (95%CI 0.50, 0.79; $P < 0.0001$). The number needed to treat to prevent one woman fracturing was 15. Non-vertebral osteoporotic fractures (HR 0.66, $P = 0.0014$), symptomatic fractures (HR 0.73, $P<0.0027$), vertebral fractures (odds ratio 0.45, $P = 0.0018$), and height loss ($P<0.0001$) were also reduced in the zol group.

There were 41 deaths in the placebo group and 27 in the zoledronate group (odds ratio 0.65, 95%CI 0.40, 1.05). Rate ratios for adverse events were: myocardial infarction 0.6 (0.3, 0.9), composite vascular endpoint 0.7 (0.5, 0.99), cancer 0.7 (0.5, 0.9), and breast cancer 0.6 (0.3, 0.98).

Conclusions: Zol prevents fractures in osteopenic older women, substantially broadening the target population for pharmaceutical intervention to prevent fractures. The beneficial effects seen on cancer and vascular disease are consistent with data from previous studies and suggest that zol should be formally trialled for the prevention of these conditions.

OR2 - AMGEN-ANZBMS OUTSTANDING ABSTRACT - BASIC PRESENTATION
SLC37A2 is an endolysosomal transporter critical for osteoclast function during bone remodelling

Nathan Pavlos 1, Guo Qiang 1, Pei Ying Ng 1, Jamie Tan 1, Nguyen Edward 1, Robert Parton 2
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2. The University of Queensland

During bone resorption the vectorial secretion of protons, ions and solutes by osteoclasts is dependent on the polarized expression of endolysosomal transporters and channels at the bone-apposed ruffled border membrane. While proton-pumping V-ATPases and chloride channels are fundamental requisites for extracellular acidification and the maintenance of electroneutrality, very little is known about the contribution of secondary active transporter systems. Here, we identify the SLC37 transporter family member Slc37a2 as a novel endolysosomal transporter critical to osteoclast bone resorption function. We show that Slc37a2 is robustly expressed in osteoclasts, localizes to the ruffled border and is required for bone resorption *in vitro*. Moreover, mice lacking *Slc37a2* (*Slc37a2*^{KO}) exhibit an intermediate form of osteopetrosis attributable to osteoclast dysfunction. Accordingly, micro-CT and 3-point bending reveal that bones from *Slc37a2*^{KO} mice exhibit profoundly elevated trabecular bone mass, increased mechanical strength and are protected against oestrogen-deprived bone loss. Using a series of histological, biochemical and cell biological approaches, we show that although the number of osteoclasts is expanded in *Slc37a2*^{KO} mice, their resorptive capacity is diminished, owing to disturbances in the maturation of the ruffled border membrane. Live imaging confirmed the altered resorptive behaviours of *Slc37a2*^{KO} osteoclasts. Further, we demonstrate that the Slc37a2 transporter localizes to a previously unappreciated network of dynamic tubular endolysosomes in osteoclasts. Consistent with this localization, *Slc37a2*^{KO} osteoclasts exhibit reduced expression of endolysosomal proteins at the ruffled border membrane, abnormal membrane turnover and reduced secretion of cathepsin K. Thus, our findings unmask Slc37a2 as a novel osteoclast endolysosomal transporter that is indispensable for bone resorptive function during physiological bone remodelling. We propose that Slc37a2 maintains

homeostatic regulation of the ruffled border membrane during bone resorption, in part, by controlling the integrity of a dynamic endolysosomal network. To our knowledge, this is the first ascribed physiological function of Slc37a2.

OR3

Continued fracture risk reduction after 12 months of romosozumab followed by denosumab through 36 months in the phase 3 FRAME (Fracture study in postmenopausal women with osteoporosis) extension

Peter Ebeling 1, Michael Lewiecki 2, Rajani V Dinavahi 3

1. Monash University
2. New Mexico Clinical Research and Osteoporosis Center
3. Amgen

Aim: Report efficacy and safety results from the final analysis of the FRAME1 study through 36 months.

Methods: In FRAME1 (NCT01575834), postmenopausal women with osteoporosis (T-score ≤–2.5 at total hip [TH] or femoral neck) received placebo or romosozumab SC monthly for 12 months, followed by open-label denosumab SC Q6M for 12 months. Here, we report results through 36 months after a further 12 months of open-label denosumab. Key endpoints included incidence of new vertebral, clinical, and nonvertebral fracture, and BMD.

Results: Of the 7,180 women enrolled, 5,743 (80%) completed the 36-month study (2,892 placebo-to-denosumab; 2,851 romosozumab-to-denosumab). Through 36 months, fracture risk reduction was observed for new vertebral, clinical, nonvertebral and other predefined fracture endpoints in patients who received romosozumab followed by 24 months of denosumab versus placebo followed by 24 months of denosumab (Table). At 36 months, BMD continued to increase in the romosozumab-to-denosumab group to 18.1% (lumbar spine) and 9.4% (TH) (Figure). The mean differences in BMD in patients who received romosozumab in the first 12 months compared with those initially on placebo remained significant over the entire 36 months ($P<0.001$). Adverse events were generally balanced between groups; no additional positively adjudicated cases of AFF or ONJ were reported since reporting the 24-month data.1

Conclusions: In postmenopausal women with osteoporosis, romosozumab followed by denosumab was well tolerated and reduced new vertebral, clinical and nonvertebral fracture risk versus placebo followed by denosumab for 24 months. BMD continued to increase in both groups. BMD in patients treated with romosozumab in the first 12 months remained significantly higher than in those who initially received placebo despite all patients receiving denosumab for 24 months, underscoring the important foundational effect of romosozumab. The sequence of romosozumab followed by denosumab will be a promising treatment regimen for postmenopausal women with osteoporosis.

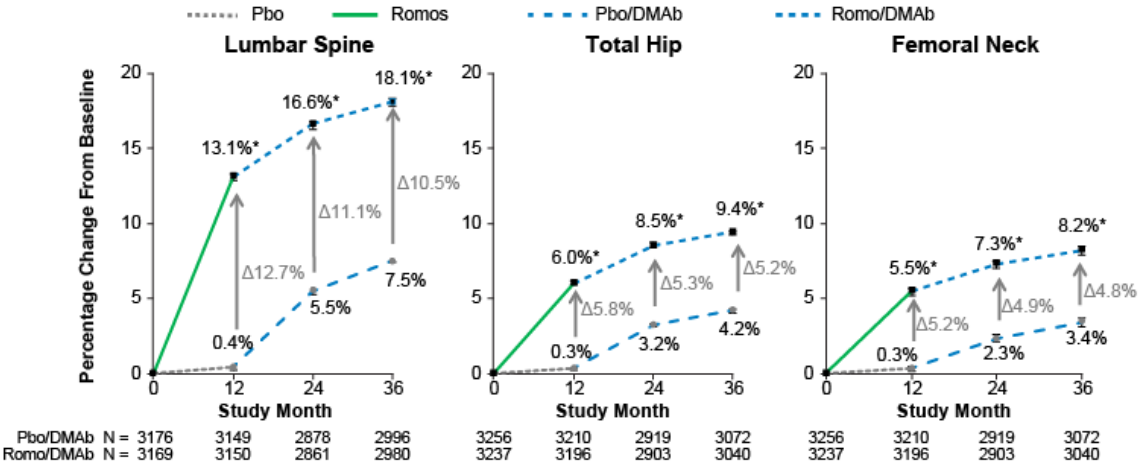
[1] Cosman *NEJM* 2016

Table. Summary of fracture endpoint results through month 36

Fracture Category	Incidence n/N1 (%) Pbo/DMAb N = 3591	Romo/DMAb N = 3589	RRR (%)	*P* Value
New vertebral fracture	94/3327 (2.8)	32/3327 (1.0)	66	<0.001
Clinical fracture	196/3591 (5.5)	143/3589 (4.0)	27	0.004
Nonvertebral fracture	176/3591 (4.9)	139/3589 (3.9)	21	0.039
Major nonvertebral fracture	138/3591 (3.8)	100/3589 (2.8)	27	0.015
Major osteoporotic fracture	147/3591 (4.1)	103/3589 (2.9)	30	0.006
Multiple new or worsening vertebral fracture	20/3327 (0.6)	2/3327 (<0.1)	90	<0.001
New or worsening vertebral fracture	94/3327 (2.8)	33/3327 (1.0)	65	<0.001
Clinical new or worsening vertebral fracture	26 (3591 (0.7)	5/3589 (0.1)	81	<0.001
Hip fracture	31/3591 (0.9)	18/3589 (0.5)	41	0.071

N = number of subjects randomized. n/N1 = number of subjects with fractures/number of subjects in the analysis set.
DMAb = denosumab; Pbo = placebo; RRR = relative risk reduction; Romo = romosozumab.

Figure. Percentage change from baseline in BMD



**P*<0.001 for group comparisons. Data are least square mean (95% CI).

Funding: Amgen Inc., Astellas, UCB Pharma.

OR4

A randomised alendronate-controlled trial of romosozumab: Results of the Phase 3 ARCH study (Active-controlled fracture study in postmenopausal women with osteoporosis at high risk).

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1. University of Alabama
2. Amgen
3. University of Florence
4. McGill University
5. University of Gothenburg and Sahlgrenska University Hospital

Aim: Report the efficacy and safety results of the ARCH study (NCT01631214).

Method: This multicentre, double-blind study enrolled postmenopausal women with osteoporosis and high fracture risk. Subjects were randomised 1:1 to romosozumab 210mg SC QM or alendronate (ALN) 70mg PO QW for 12 months, followed by open-label alendronate in both groups. Primary endpoints: subject incidence of new vertebral fracture through 24 months and clinical fracture through the primary analysis (PA). PA was performed when ≥330 clinical fractures had occurred and all subjects had completed the month 24 visit. Secondary endpoints: Nonvertebral fracture at the PA, BMD at the lumbar spine, TH and FN at months 12 and 24, and hip fracture.

Results: 4,093 women were randomised to romosozumab or ALN. 12 months of romosozumab prior to ALN versus ALN alone significantly reduced new vertebral, clinical, nonvertebral and hip fractures (Table). Treatment with romosozumab significantly increased BMD at all sites measured, at months 12 and 24 versus ALN alone (Table). Overall adverse events were balanced between groups. During the open label ALN period, 6 subjects (2 romosozumab; 4 ALN) were positively adjudicated for AFF and 2 subjects (1 romosozumab; 1 ALN) for ONJ. Cardiovascular serious adverse events (SAEs) were independently adjudicated; at 12 months the subject incidence was 2.5% in the romosozumab group versus 1.9% in the ALN group with cardiac ischemic events contributing 0.8% vs 0.3% and cerebrovascular events 0.8% vs 0.3%, respectively.

Conclusion: In postmenopausal women with osteoporosis at high risk of fracture, romosozumab followed by ALN significantly reduced new vertebral, clinical, nonvertebral and hip fracture risk versus alendronate alone, suggesting romosozumab followed by ALN leads to superior fracture risk reduction over ALN alone. An observed imbalance in cardiovascular SAEs compared with ALN, not seen in the previous placebo-controlled 7,180-patient FRAME study, is currently being evaluated.

Efficacy table

	Subject incidence of fracture (%)		**Relative Risk Reduction**	**Nominal *P* value**	**Adjusted *P* value[a]**
	ALN 70mg QW / ALN 70 mg QW N=2047[b]	Romo 210mg QM / ALN 70 mg QW N=2046[b]			
New vertebral Fx[c]	8.0	4.1	50%	P<0.001	P<0.001
Clinical Fx[d]	13.0	9.7	27%	P<0.001	P<0.001
Nonvertebral Fx[d]	10.6	8.7	19%	P=0.037	P=0.040
Hip Fx[d]	3.2	2.0	38%	P=0.015	NA[e]
	BMD percent change from baseline (%)[f]				
	ALN 70mg QW / ALN 70 mg QW	Romo 210mg QM / ALN 70 mg QW		Nominal P value	Adjusted P value
Lumbar spine	N=1757[g]	N=1750[g]			
Month 12	5.0	13.7		P<0.001	P<0.001
Month 24	7.2	15.3		P<0.001	P<0.001
Total hip	N=1829[g]	N=1826[g]			
Month 12	2.8	6.2		P<0.001	P<0.001
Month 24	3.5	7.2		P<0.001	P<0.001
Femoral neck	N=1829[g]	N=1826[g]			
Month 12	1.7	4.9		P<0.001	P<0.001
Month 24	2.3	6.0		P<0.001	P<0.001

[a]Based on a combination of Hochberg, fixed sequential, and group sequential testing procedures which included the primary and selected secondary endpoint comparisons to be compared to a significance level of 0.05. [b]N=number of subjects randomised. [c]Incidence of fracture through month 24. [d]Incidence of fracture through primary analysis. [e]Multiplicity adjustment not applicable for hip fracture because it was not part of sequential testing strategy. [f]Data are shown as least squares means based on ANCOVA models. [g]N=number of subjects with a baseline and ≥1 post-baseline value. ALN=alendronate, Fx=fracture, Romo=romosozumab.

Disclosure: This study was sponsored by Amgen Inc., Astellas, and UCB Pharmacy.

OR5

Effect of sclerostin monoclonal antibody therapy on BMD is linked to osteocyte sensitivity - insights from a mechanobiological model of bone remodeling

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2. Laboratoire MSME, Universite Paris-Est Creteil
3. University of Saskatchewan
4. Griffith University
5. Indiana University

Romosozumab is a monoclonal antibody that binds with high affinity to sclerostin, which inhibits the anabolic Wnt signaling. This antibody treatment is currently under phase 3 clinical trial and has already been shown to be highly effective in the treatment of osteoporosis (Keaveny et al. 2017). In this paper, we present a mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model of the action of romosozumab in post-menopausal osteoporosis.

This model allows to study the effects of drug dose and osteocyte sensitivity on changes in BMD via a comprehensive mechanobiological model of bone remodeling including osteocyte feedback. The anabolic feedback has been implemented using osteocyte derived sclerostin which interacts with the Wnt-beta catenin signaling pathway. Action of sclerostin in bone remodeling is taken into account via a proliferation term of osteoblast precursor cells. On the other hand, catabolic feedback has been linked to osteocyte-derived nitric oxide which regulates the RANKL/OPG ratio, acting on osteoclast precursors differentiation. The consistent description of sclerostin binding to the monoclonal antibody allows to simulate monthly injections of 70 mg, 140 mg or 210 mg, which reproduces drug-induced BMD gains of respectively 1.96 (1.85 +/- 0.75) %, 2.92 (2.85 +/- 0.81) % and 3.57 (3.76 +/- 1.08) % which are in good agreement with experimental findings (see values in brackets from Ishibashi et al 2017). Our numerical simulations show that osteocyte sensitivity plays an important role in changes of BMD due to the antibody treatment: a loss of osteocytes sensitivity of 20% would decrease bone gain by 72% and therefore dramatically hinders the efficiency of the treatment. Hence, the present model can help to progress towards more patient- and bone site- specific treatment options, by simulating the nonlinear relationship between dosage and BMD increase, as well as the influence of loss in osteocyte sensitivity during PMO or ageing.

OR6

Intermittent PTH Increases Remodeling and Restores the Healing of Stress Fractures Following Bisphosphonate Treatment

Mahmoud Bakr, Wendy Kelly, Athena Brunt, Bradley Paterson, Helen Massa, Nigel Morrison, Mark Forwood
Griffith University

Parathyroid hormone (PTH) and bisphosphonates (BP) including Alendronate (ALN) are known to have opposing effects on bone dynamics. ALN is an antiresorptive agent that decreases bone turnover. Consequently, prolonged doses of BP adversely delay the healing of stress fractures (SFx). The extent to which PTH can remain effective in the treatment of SFx in the presence of an ongoing BP treatment has not been tested. SFx was induced in 210 female Wistar rats during a single loading session. Rats were divided into seven equal groups (n=30), received either daily PTH (8 µg.100g-1.day-1) for 14 days, ALN (1 ug.kg-1.day-1) for the whole duration of the experiment, combined ALN-PTH with cessation or continuation of ALN after SFx induction or an equivalent vehicle (VEH) as a control. Ulnae were examined 2 or 6 weeks following SFx. Two Toluidine Blue and two TRAP-stained sections of the SFx were examined for histomorphometric analysis using Osteomeasure™ software. Intermittent PTH injections for 14 days increased SFx remodeling by improving the woven bone architecture, increasing osteoclast activation and basic multicellular unit (BMU) progression. Cessation of ALN after SFx induction in the Combined ALN-PTH was effective for increasing osteoclast parameters and erosion perimeter after two weeks ($P < 0.05$) as well as increasing the overall healing percentage at a SFx site after 6 weeks ($P= 0.048$). Furthermore, the combined ALN-PTH treatment was superior to daily PTH treatment only with regards to increasing cortical bone area. Woven bone parameters remained unaffected by the cessation or continuation of ALN. Our results show that intermittent short duration PTH injections effectively increase remodeling of SFx even after BP

treatment, provided that it ceases at the time of SFx. We hypothesize that an additional sequential ALN treatment after the cessation period could further improve the overall treatment outcomes.

OR7
STAT3 signaling in heterotopic ossification following spinal cord injury

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3. Inserm UMR-S-MD1197

Neurological heterotopic ossification (NHO) is a frequent complication of traumatic brain and spinal cord injuries (SCI), which manifests as abnormal ossifications in soft tissues near joints. NHO is debilitating, causing pain, joint ankylosis and vascular and nerve compression. NHO pathogenesis is unknown and the only effective treatment remains surgical resection although once resected NHO can re-occur. To further understand NHO pathogenesis, we developed the first animal model of NHO following SCI in genetically unmodified mice, which mimics most clinical NHO features. The combination of SCI and muscular damage/inflammation is required for NHO development, and inflammatory monocytes infiltrating the injured muscle play a critical role, as their in vivo depletion prevents NHO development. We recently confirmed the inflammatory cytokine Oncostatin M (OSM) as a key mediator NHO. OSM was shown to be expressed by both macrophages and osteoblasts around NHO and throughout injured muscle. Additionally, microCT analysis confirmed significantly reduced NHO in mice lacking the OSM receptor. In agreement with murine NHO, OSM was significantly higher in human NHO patients. Furthermore, conditioned media from macrophages isolated from patients' NHOs promoted mineralisation of patient muscle-derived stromal cells and this effect was inhibited by neutralising anti-OSM antibodies. A downstream signaling target of OSM/OSMR/gp130 interaction is the phosphorylation of the transcription factor STAT3 by JAK1/2 tyrosine kinases. Western blots from muscle lysates of SCI and SHAM-operated mice confirmed significantly higher STAT3 phosphorylation in injured muscles after SCI. We administered a JAK1/2 inhibitor for 7 days post-SCI to block STAT3 phosphorylation in vivo. This treatment significantly reduced NHO volumes at day+7 (52% reduction), and ongoing effects were seen up to day+21 (76% reduction). Immunohistochemistry at day+21 confirmed reduced bone deposition and reduced type 1 collagen. This data confirms that inhibition of JAK/STAT3 signaling after SCI is a valid therapeutic regime to reduce NHO development.

OR8
Timing of Vertebroplasty for Acute Painful Osteoporotic Fractures: Data from the VAPOUR Trial

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1. St George Hospital
2. Aged Care, Sutherland Hospital

Aim: To determine whether the timing of vertebroplasty affects outcome measures in acute painful osteoporotic fractures. Data from the VAPOUR Study – a randomized, blinded, parallel group, placebo-controlled trial.

Methods: Patients presenting with acute painful osteoporotic fractures were randomly assigned to receive either vertebroplasty (PV) or placebo. Entry criteria included fracture duration less than 6 weeks, positive MRI or SPECT-CT and Numerical Rating Scale (NRS) pain score >7/10. Outcome measure included (a) proportion of patients who achieved NRS pain score <4/10 at 14 days post intervention, (b) NRS pain and (c) Roland-Morris Low Back Pain and Disability Questionnaire (RDQ) scores recorded at 3, 14, 28 days, 3 and 6 months. Exploratory analyses were performed in cumulative subsets of patients according to time since fracture of less than 1, 2, 3, 4 weeks, or any duration.

Results: 120 subjects were enrolled (61 PV and 59 controls) over 3 years. Mean age was 80 years, 73% were females and 59% were inpatients at time of enrolment. Average duration of fracture at time of intervention was 2.6 weeks. The proportion of patients achieving NRS <4/10 at 14 days in the group < 2 weeks post fracture was 58% in PV and 18% in controls (P=0.0013); in the group < 4 weeks post fracture was 46% in PV and 20% in controls (P=0.0064); and in the group < 6 weeks post fracture was 44% in PV and 21% in controls (P=0.011). Mean reductions in NRS pain and RDQ from baseline favoured vertebroplasty at all-time points irrespective of the timing of the vertebroplasty.

Conclusion: Vertebroplasty is superior to a placebo in reducing back pain and disability in patients with painful osteoporotic vertebral fractures. This study demonstrates that within the critical 6 week time period post vertebral fracture, earlier intervention with vertebroplasty leads to greater reduction in pain compared to placebo.

OR9

Biochemical profiling of MRI detected bone marrow lesions in knee osteoarthritis patients: altered mineralization of the subchondral bone matrix

Julia Kuliwaba,Yea-Rin Lee, Dzenita Muratovic, David Findlay
The University of Adelaide

Subchondral bone marrow lesions (BMLs) associate with pain and structural progression of knee osteoarthritis (OA). Hence, emerging treatment approaches for OA (eg. anti-resorptives) are now targeting BMLs. However, the molecular basis for the distinctive pathology of BMLs remains unclear. Thus, the study aim was to examine the biochemical composition of BMLs for human knee OA.

Tibial plateaus (TP) collected from 24 knee-OA arthroplasty patients (11-men, 13-women; aged 52-77yrs) and 8 non-OA control cadavers (5-men, 3-women; aged 44-80yrs) were MRI-scanned (PDFS, T1) to identify BMLs. Four groups, each n=8, were assessed: OA-noBML, OA-BML1 (PDFS detected in medial-TP), OA-BML2 (PDFS+T1 detected in medial-TP), control. For each TP, subchondral trabecular bone was sampled from the medial (BML zone/noBML) and lateral compartment for Raman microspectroscopy analysis and quantitative backscattered electron imaging (qBEI).

Mineral-organic matrix ratios were similar between OA-noBML and control (medial and lateral). In contrast, phosphate/amide I was reduced in the medial BML zones, for both OA-BML1 ($p=0.02$) and OA-BML2 ($p=0.004$), compared to control. Also, phosphate/proline was lower for OA-BML1, compared to control ($p=0.02$). Within the OA-BML1 and OA-BML2 groups, phosphate/amide I (BML1:$p<0.0001$; BML2:$p=0.009$) and phosphate/amide III (BML1:$p=0.006$; BML2:$p=0.0005$) were lower in medial-vs-lateral. The qBEI bone mineralization density distribution (BMDD) for both OA-BML1 and OA-BML2 was shifted to low mineralization density ($p<0.03$) combined with an increased peak width ($p<0.04$), compared to control. There were no differences for mineral crystallinity, type-B carbonation, relative proteoglycan content, or hydroxyproline/proline.

Raman spectra of subchondral bone from knee OA patients without tibial BMLs and non-OA controls demonstrated a similar mineral-organic bone matrix composition. However, medial-tibial BML tissue from knee OA patients showed an altered Raman spectral bone signature, characterized by reduced mineralization with increased heterogeneity. These data support the concept of BMLs representing localized areas of active bone remodeling in response to microdamage accumulation [Muratovic et al. 2018].

OR10

Anti-Fractalkine Monoclonal Antibody Ameliorates Joint Destruction in Collagen-Induced Arthritis Model by Inhibiting Migration and Survival of Osteoclast Precursor Cells

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Objective: To elucidate the role of the fractalkine (FKN)-CX3CR1 pathway in joint destruction in rheumatoid arthritis, we examined the effect of treatment with anti-mouse FKN (mFKN) monoclonal antibody (mAb) on joint destruction and the migration of osteoclast precursors (OCPs) into the joint, using the collagen-induced arthritis (CIA) model.

Methods: DBA/1 mice were immunized with bovine type II collagen to induce arthritis, then treated with anti-mFKN mAb. Disease severity was monitored by arthritis score, and joint destruction was evaluated by soft X-ray and histological analysis. Plasma levels of joint destruction markers were assessed by ELISA. FKN expression on endothelial cells was detected by immunohistochemistry. Furthermore, bone marrow-derived OCPs were labeled with fluorescein and transferred to CIA mice; then, their migration to the joints was analyzed.

Results: Both prophylactic and therapeutic treatment with anti-mFKN mAb significantly reduced the arthritis and soft X-ray scores. Plasma levels of cartilage oligomeric matrix protein and matrix metalloproteinase-3 were decreased after treatment with anti-mFKN mAb. Histological analysis revealed that anti-mFKN mAb inhibited synovitis, pannus formation, and cartilage destruction, and that it suppressed bone damage, with marked reduction in the number of tartrate-resistant acid phosphatase+ osteoclasts. Anti-mFKN mAb strongly inhibited the migration of bone marrow-derived OCPs into the affected synovium.

Conclusion: Anti-mFKN mAb remarkably ameliorates arthritis and joint destruction in the CIA model, accompanied by reduced migration of OCPs into the synovium. These results suggest that inhibition of the FKN-CX3CR1 pathway could be a novel strategy for treatment of both synovitis and joint destruction in rheumatoid arthritis.

OR11

Local unloading of subchondral bone through loss of articular surface congruity results in a focal increase in bone turnover

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Bone remodeling is inhibited by high load environments such as that in joints of horses in training. However focal offsetting of remodeling inhibition is observed associated with micro-damage in the subchondral bone (SCB) of these animals either due to targeting of damaged areas by bone remodeling units or local unloading due to loss of articular surface congruity. This study aimed to test whether local unloading of the articular surface can stimulate focal bone remodeling.

An osteochondral defect was created arthroscopically in one surface of one midcarpal joint of six adult horses. Two weeks post-operatively the animals began an eight-week training program following which osteochondral samples were collected from the site directly opposing the defect and from an adjacent loaded site. Control samples were collected from the sham-operated contralateral limb. Undemineralised samples were imaged with microCT and backscattered scanning electron microscopy and stained with Masson's trichrome to measure osteoid. Cryosections from decalcified specimens were stained for TRAP to identify osteoclasts.

In the unloaded region opposing the defect in treated joints there was evidence of increased bone remodeling reflected by increased active surface (mean difference=1.28 mm-1, P=0.03), increased osteoclast numbers (mean difference=7.4 cells/mm2, P=0.03) and increased osteoid width (mean difference=3.64 um, P=0.04) with an overall decrease in bone volume fraction (median difference=-3.1%, P=0.04). In the adjacent SCB there was no change or the opposite effect (active perimeter: -0.66 mm-1, P=0.01 and osteoid area/bone area: mean difference= -0.61%, P=0.05).

There was a focal increase in SCB remodeling underlying the intact joint surface and confined to the unloaded region of bone in this equine model.

Local unloading of subchondral bone due to loss of articular surface congruity is a possible mechanism by which focal remodeling may be enabled at sites of subchondral bone injury in a high load environment.

OR12

Towards Cellular Epidemiology of Degenerative Diseases Using Geographic Information Systems, MultiSEM and Machine Learning Approaches

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The paucity of rapid throughput imaging technologies that allow for seamless bridging of structure-function relationships across length scales (10−2 to 10−9 m) has stymied the study of degenerative processes associated with ageing and disease. The combination of geospatial approaches using the Google Maps API and multibeam scanning electron microscopy (MultiSEM), have enabled cutting-edge cellular connectomics studies of ageing human tissues, from bone to brain [1-4].

As a proof-of-principle study to elucidate the relationship between bone and cell health in the human femoral neck, the Google JavaScript API was used to enable labelling of landmarks on a navigable map (**Figure 1-A, B**). A blinded observer marked blood vessel edges, viable and pyknotic (sick or dying) osteocytes. This coordinate-based mapping enabled testing of specific hypotheses related to bone health in terms of osteocyte viability, transport path distances, and network relationships. While initially adequate, this manual method is not conducive to the pipeline scalability necessary for the magnitude of current datasets (>10TB).

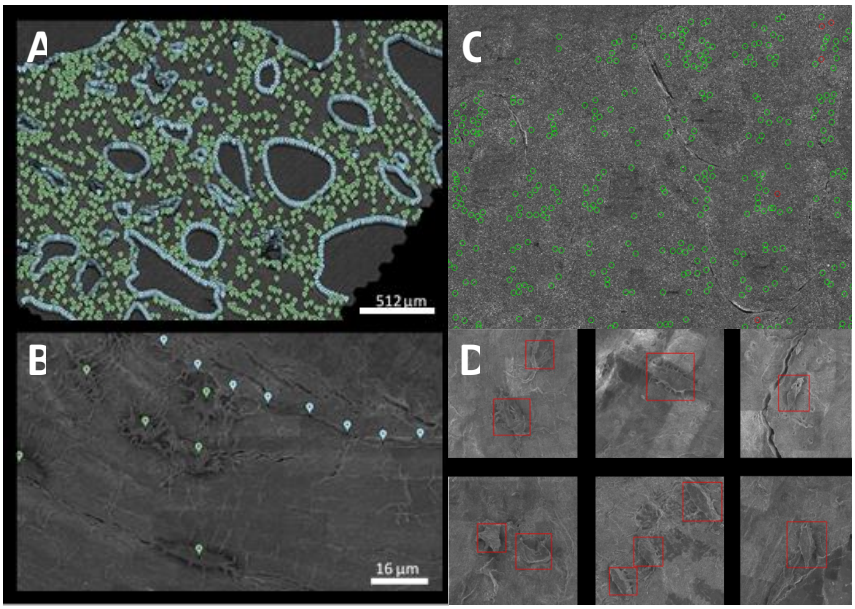

Figure 1. A, B - Manual pin dropping using the GoogleMaps API identifying key features such as osteocytes (green) and blood vessels (blue). **C** - Automatic detection using YOLO machine learning neural network (v2) detecting healthy (green) and unhealthy (red) osteocytes. **D** – Close up of individual osteocytes in bounding boxes predicted by version 1 of the YOLO algorithm.

Automated object detection algorithms such as the You Only Look Once (YOLO) neural network [5] facilitate rapid throughput diagnostic assessment of imaging datasets, also mitigating the effects of observer bias. Initially, YOLO was trained for automated osteocyte detection, using 629 annotated cells, which were further augmented to 106 examples through variation by rotation, scale and contrast. Unseen images were then processed with YOLO and automatically detected objects were identified by red bounding boxes (Figure 2-D). Post-hoc testing revealed >90% accuracy in identifying cells from new datasets. Latest testing (Figure 3-C) of the YOLO algorithm has proved successful in detecting osteocytes in a complete cross-section of the human femoral neck in <100hrs. This demonstrates that this technique achieve extremely high throughput classification of large datasets (>10TB) to investigate connectomics and network properties of degenerative diseases, as diverse as osteoarthritis and Alzheimer's disease.

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OR13

Anti-LRP6 antibody prevents myeloma induced bone disease and increases bone strength.

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2. Kids Research Institute
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Multiple myeloma (MM) is a plasma cell cancer that leads to bone destruction through both increased bone resorption and decreased bone formation. This bone loss leads to high fracture rates and increased mortality. Anti-resorptive treatments prevent further bone loss but patients continue to fracture. Therefore, new therapies which promote bone formation are required to rebuild skeletal structure and prevent fractures in patients with myeloma. LRP6 is Wnt ligand a co-receptor which drives Wnt stimulated differentiation of osteoblasts. Anti-LRP6 antibodies promote Wnt signaling and have been shown to increase bone mass, however they are yet to be explored in the setting of myeloma. We hypothesised that Anti-LRP6 treatment would prevent bone loss through increasing bone formation, and improve resistance to fracture in myeloma.

Mice injected with eGFP expressing 5TGM1 murine myeloma cells demonstrated a 23% reduction in vertebral bone volume of total volume (BV/TV) compared to naïve controls ($p<0.05$). This loss in bone volume was associated with a 34% reduction in the maximum load required to failure ($p\leq0.001$). Anti-LRP6 treatment (100mg/kg weekly intravenously) prevented the structural bone loss seen in 5TGM1-burdened mice, increasing vertebral bone volume by 33% ($p<0.01$). This in turn increased the maximum load to failure by 32% ($p<0.01$), almost reaching equivalent strength to naïve controls in this model. Similar increases in BV/TV were shown in the femur. Anti-LRP6 treatment reduced osteoclast activity on the endocortical surfaces of the femur, but did not alter bone formation or tumour growth. Although Anti-LRP6 treatment did not reduce tumour burden, it did prevent the development of myeloma induced bone disease, improving bone structure and strength. This study defines a therapeutic strategy superior to current approaches, which will reduce fractures and enhance quality of life in patients with myeloma.

OR14

Clinical utility assessment of genetic profiling in fracture risk prediction: A decision curve analysis approach

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Aims: Genetic profiling is emerged as a promising tool for assessing the risk fracture in asymptomatic individuals. This study used a decision curve analysis approach to determine the utility of genetic profiling in prediction of fracture.

Methods: The study involved 2188 women and 1324 men aged 60 years and above who have been followed up to 20 years. Bone mineral density (BMD) and clinical risk factors were obtained at baseline. The incidence of fracture and mortality was recorded during the period. A weighted genetic risk score (GRS) was constructed for each individual from sixty-two BMD-associated genetic variants. Four models were considered: Model I (Base model) included only clinical risk factors (CRF); Model II (Garvan model) included CRF and femoral neck BMD; Model III (Garvan+GRS) included CRF, femoral neck BMD and GRS; and Model IV (Base+GRS) included CRF and GRS. Decision curve analysis was used to evaluate the clinical net benefit in terms of true positives and false positives of predictive models.

Results: In women, for risk threshold above 0.15, the Base+GRS model had a significantly higher net benefit than the Base model; however, for threshold below 0.15, there was no significant difference in net benefit between the two models. The Garvan model yielded the highest net benefit; adding GRS into the Garvan model did not substantially improve the net benefit. In men with fracture risk greater than 0.15, the Base+GRS model resulted in a better net benefit than the Base model. Interestingly, the Garvan+GRS model did improve the net benefit over and above the Garvan model.

Conclusion: Genetic profiling can provide additional prognostic information to that obtained from clinical risk factors, particularly in women at higher risk of fractures. However, in the presence of BMD in a predictive model, GRS does not further improve net clinical benefit.

OR15

Optimization of TiO2 nanotube formation and dimensions on Ti6Al4V alloys influences osteogenic differentiation of human mesenchymal stromal cells

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In order to obtain bioactive bone-implant interface and enhanced osteogenesis, various approaches have been developed to modify surface physico-chemical properties of bio-inert titanium and titanium alloys [1] [2]. One promising strategy involves fabricating highly ordered nanotubular structures by electro-chemical anodization [3]. However, few studies have applied this onto Ti6Al4V alloys most commonly adopted for bone-interfacing orthopedic implants. In this study, we optimized nanotube (NT) fabrication conditions on Ti6Al4V in ethylene glycol based electrolyte and investigated osteogenic differentiation capacity of human mesenchymal stromal cells (hMSCs) on anodized NT surfaces.

Anodized Ti6Al4V surfaces were characterized by scanning electron microscopy (SEM) to measure NT morphology and distribution. Computational Fluid Dynamics (CFD) was employed to simulate electrolyte flow profiles under various stirring conditions, and their correlation to NT formation. Polished Ti6Al4V disks (240 grit) were anodized at 20, 40 and 60 volts under optimal electrolyte flow conditions for comparison of NT surface-controlled osteogenic differentiation and mineralization potential of hMSCs over 21 days culture in osteogenic media (alpha-MEM, 10 nM dexamethasone). Polished disks were used as controls.

Ti6Al4V surfaces anodized at 20, 40 and 60 volts resulted in NT with average diameters 39, 83 and 104 nm, respectively **(Fig 1)**. Hydrodynamics (shear stress) significantly influenced uniformity of NT formation, i.e. parallel shear flow impaired mature NT formation whereas uniform perpendicular shear flow promoted homogeneous NT distribution. After 1 day culture, hMSCs showed filopodia-like cellular extension on anodized samples and demonstrated sheet-like morphology on controls. At D21, significantly higher expression of osteocalcin, collagen I and greater mineralized deposits were observed on V20 NT surfaces (Ø39 nm) relative to V40 and controls **(Fig. 1)**.

Our study provides for the first time a clear view about the hydrodynamic effect to NT formation on Ti6Al4V alloys and the potential application of anodization to enhance bone- implant surface modification.

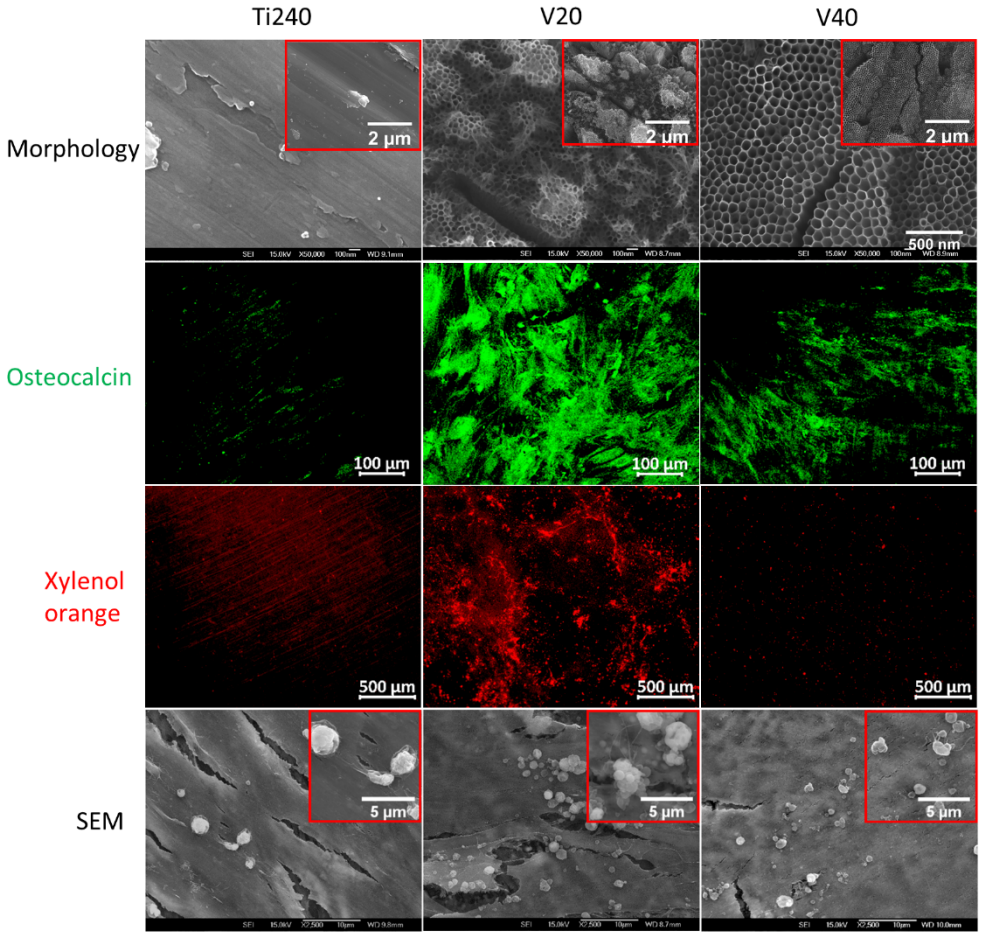

Figure 1: Nanotube morphology of 20 and 40 volt anodized samples, and osteogenic differentiation analysis of hMSCs after 21 days culture. Osteogenic differentiation was evaluated by fluorescent staining of osteocalcin, xylenol orange staining of mineral deposits and SEM/EDS for mineralized nodules. Few cells existed on V60 samples and were excluded from osteogenic differentiation assay.

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OR16

Patients on dialysis have markedly abnormal cortical hip parameters by dual-energy X-ray absorptiometry

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Background: Patients with chronic kidney disease (CKD) have fracture rates above the general population and higher post-fracture mortality, yet bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) is less predictive of fractures in this population. HR-pQCT scans indicate a reduction in cortical thickness and increased cortical porosity in patients on dialysis, but it has limited accessibility and high expense. Cortical assessment of the hip using DXA has not been performed in this population.

Aim: To assess cortical parameters using BMD and advanced hip analysis (Lunar iDXA) in dialysis patients awaiting transplantation.

Methods: Normal ranges were first determined from 4,298 femur scans of women (mean age 72±7 years) and men (mean age 73±7 years) enrolled in the Dubbo Osteoporosis Epidemiology Study (DOES), and then compared with 65 women (age 46±13 years) and 90 men (age 49±13 years) receiving dialysis.

Results: Compared with older patients from DOES, women receiving dialysis had T-scores of -1.52±1.33 vs. -1.27±1.11 (non-significant), but significantly lower mean cortical thickness at the femoral neck (FN) of 2.53 ± 1.52 mm vs. 5.13±1.79 ($p<0.001$), and calcar of 3.26±1.20 mm vs. 3.82±1.2 ($p<0.001$). Buckling ratios (higher values indicate FN instability) were 8.01±4.57 vs. 3.97±1.68 ($p<0.001$). Men receiving dialysis had lower T-scores (-1.38±1.3 vs. -0.49±1.17, $p<0.001$), FN cortical width 2.91±1.98 mm vs. 5.84±2.32 ($p<0.001$) and calcar: 3.67±1.0 mm vs. 4.43±1.92 ($p<0.001$) compared with DOES men. Buckling ratios were 8.43±6.32 vs. 3.94±1.67 ($p<0.001$). Differences at the femoral shaft were non-significant in both men and women.

Conclusion: Stage 5D CKD has a profoundly adverse effect on cortical bone structure and strength. Cortical parameters measured non-invasively by DXA are markedly reduced in patients with CKD, even when compared to significantly older men and women, and should be assessed prospectively for utility in fracture prediction in this population.

OR17

Assessment of osteal macrophage and osteoclast distribution across the acute phase of bone loss in a mouse model of post-menopausal osteoporosis

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Increased osteoclast activity and reduced osteoblast function are downstream mechanisms driving bone loss in osteoporosis. Altered macrophage function could contribute to osteoporosis pathobiology through amplifying systemic immune activation, ultimately increasing osteoclastogenesis. Evidence that osteal macrophages (osteomacs) promote osteoblast-mediated bone formation raise the hypothesis that altered osteomac biology contribute to osteoporosis-associated bone loss and/or suppressed bone formation. We established an ovariectomy (OVX)-induced model of post-menopausal osteoporosis in 16-week-old C3H/HeJ mice (high baseline bone mineral density). Bone loss was confirmed 4 weeks post-OVX with significantly reduced trabecular bone/total volume (-46%), decreased trabecular thickness and increased trabecular separation. Cortical thickness was also significantly reduced (-8.15%). Bone marrow (BM) macrophage subset frequency was assessed weekly for 4 weeks using flow cytometry. No difference in frequency of BM F4/80+ macrophages, CD169+ macrophages and Ly6Chi monocytes was observed at any week post-OVX.

Similarly, no significant change in osteoclast precursor (CD3-Ter119-B220-CD115hiCD11b-Ly6Chi) number was seen, although there was a trend towards an increase one week post-OVX. Immunohistochemical analysis suggested a trend towards increased osteoclast frequency at one and two-weeks post OVX on trabecular and endocortical bone surfaces. However, osteoclast frequency on periosteal surfaces was not altered at these timepoints. 4 weeks post-OVX, osteoclast frequency was not different between sham and OVX mice. This minimal change in osteoclast frequency was unexpected. Preliminary analysis of total endocortical osteomac and osteoblast frequency shows no significant difference between sham and OVX mice at one or two weeks post-OVX. These support that the significant reduction in bone volume herein is not mediated by alterations in osteoclast or macrophage frequency, or that these changes are a transient event not captured in the time-course assessed. Assessment of altered macrophage and osteoclast functional efficiency and/or profile is underway to improve understanding of the cellular mechanisms leading to bone loss in this model of post-menopausal osteoporosis.

OR18

The impact of osteoporotic fracture type and subsequent fracture on mortality in TromsÃ, Norway

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Non-hip non-vertebral fractures (NHNV) account for two-thirds of all osteoporotic fractures and are associated with subsequent fracture. However, mortality risk following NHNV is controversial.

This study examined the contribution of: 1) initial fracture type over and above other factors on mortality risk, and 2) subsequent fracture on post-fracture mortality.

The Tromsø Study is a prospective population-based cohort with repeated health surveys since 1974 in Tromsø, Norway. Women and men aged 50+ years were followed from 1994 to 2010. All incident non-vertebral fractures were registered during that period. Hip fractures were analysed separately and NHNV fractures were classified as proximal or distal. Self-reported co-morbidities including diabetes, stroke and heart disease and lifestyle factors including smoking, physical activity, general health and education level were collected. Multivariable Cox models were used to quantify mortality risk after accounting for potential confounders. Incident and subsequent fractures were analysed as time-dependent.

Of 5,214 women and 4,620 men, 1,549 (30%) and 504 (11%) sustained a fracture, followed by 589 (38%) and 254 (51%) deaths over 10,523 and 2,820 person-years, respectively. There were 403 (26%) subsequent fractures in women and 68 (13%) in men. Hip fracture was associated with a two-fold increase in mortality risk (HR 2.04, 95% CI: 1.72-2.41 in women, and 2.40, 1.93-3.05 in men). NHNV proximal fracture was associated with 40% and 70% increased mortality risk in women and men (HR 1.41 95%CI 1.14-1.73) and (HR 1.71 95%CI 1.28-2.29), respectively. NHNV distal fractures were not associated with mortality. Subsequent fracture was associated with 24% increased mortality risk in women (HR 1.24 95%CI 1.04-1.49) while in men it was not significant (HR 1.09 95%CI 0.76-1.56).

This study highlights the impact of proximal NHNV as well as hip fractures on mortality risk. Subsequent fracture also contributes to excess mortality post-fracture, highlighting the importance of early intervention.

OR19

High-fat Diets Induce Sclerostin Expression Via Enhanced Glucocorticoid Signalling in Osteocytes

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ANZAC Research Institute

We have previously demonstrated that abrogating glucocorticoid signalling in osteoblasts and osteocytes (11βHSD2-tg mice) attenuates high-fat diet (HFD)-induced bone loss in mice, suggesting that these deleterious effects are mediated through increased glucocorticoid action in these bone cells. Here, we aim to investigate the mechanism behind these adverse effects.

Gene expression was analysed in long bone extracted from 11βHSD2-tg mice and their wild-type (WT) littermates fed either chow (fat: 14% total-energy) or a HFD (fat: 43% total-energy) for 18 weeks. While circulating glucocorticoid levels were similar in all groups, skeletal mRNA expression of the glucocorticoid-activating enzyme, 11β-hydroxysteroid dehydro¬genase type 1 (11βHSD1) was increased in both WT and 11βHSD2-tg mice fed a HFD compared to animals on normal chow (fig. 1A). However, expression of the downstream glucocorticoid target gene, GILZ, was higher only in WT mice fed a HFD (fig. 1B). As a consequence of disrupted glucocorticoid signalling, GILZ expression remained unchanged in 11βHSD2-tg animals fed the HFD (fig. 1B). Importantly, mRNA and protein expression of sclerostin, a known glucocorticoid target, were 15- and 8-fold higher in WT mice on HFD, respectively, but remained unchanged in HFD-fed tg animals (fig. 1C&D). These observations are consistent with locally augmented glucocorticoid signalling in HFD-fed WT mice, leading to increased sclerostin expression.

To investigate whether enhanced glucocorticoid signalling in bone is a direct effect of fatty acids present in the HFD (e.g. oleic, linoleic, linolenic, palmitic or stearic acid), we developed an in vitro bone granule culture where bone cells are maintained in their natural environment. We found that only palmitic acid significantly induced 11βHSD1 and GILZ mRNA expression in bone granules (fig. 1E).

Together our data suggests that high-fat feeding in mice, particularly palmitic acid, promotes 11βHSD1 expression and activity in bone, and thus expression of sclerostin through glucocorticoid signalling in osteoblasts and osteocytes.

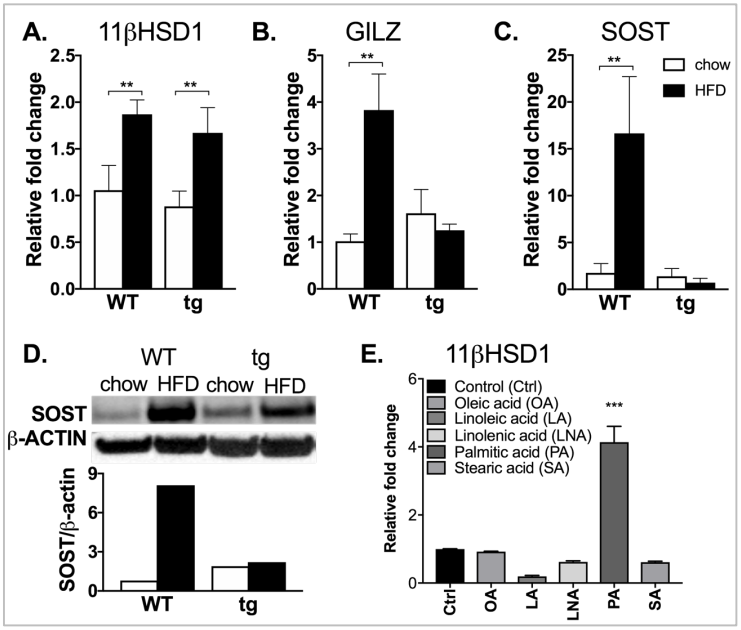

Figure 1

OR20

Longitudinal association between objectively measured sedentary time and physical activity with muscle strength, balance and falls in a cohort of Australian middle-aged women

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Aims: Exercise and strength training can improve muscle strength and balance and reduce falls in older people. However, the potential long-term effects of sedentary time and physical activity on muscle strength, balance and falls in middle-aged women are yet to be examined. This analysis aimed to describe longitudinal associations between sedentary time, physical activity and these outcomes in middle-aged women.

Methods: 308 women aged 36-57 years were followed for 5.3 years. Linear mixed-effects was used to examine associations of sedentary time and time spent in light and moderate to vigorous physical activity (MVPA) (by Actigraph GT1M accelerometer) with changes in lower limb muscle strength (LMS) and balance (timed up and go [TUG], functional reach [FRT], lateral reach [LRT], and step tests [ST]). Log-binomial regressions was used to examine the association of sedentary time and these activities with one-year retrospective incidence of falls at follow-up (n=239).

Results: After adjusting for confounders and MVPA, sedentary time was detrimentally associated with falls incidence (relative risk= 1.28/unit, 95%CI: 1.01, 1.63) but was not associated with LMS or balance measures. One standard deviation increase in MVPA (28 minutes) was detrimentally associated with FRT (β=-0.69 cm/SD, 95%CI: -1.37, -0.01) but no other outcomes. This association was attenuated and no longer statistically significant after further adjustment for sedentary time. Light physical activity was not associated with any outcomes.

Conclusions: This the first study to show that reducing sedentary time in middle-age could have long-term benefits for the prevention of falls, independent of MVPA. This supports providing advice to reduce sedentary time as part of health promotion messages for maintaining musculoskeletal health in younger women.

Table Log binomial regression model for associations of Sedentary Time and Time Spent in Light Physical Activity and in MVPA with 1-yr retrospective incident falls after 5.3 years (n=239)

	Unadjusted model	Adjusted model	Adjusted model
	(RR, 95%CI)	(RR, 95%CI) [a]	(RR, 95%CI) [b]
Sedentary time	**1.32 (1.06, 1.65)**	**1.32 (1.05, 1.66)**	**1.28 (1.01, 1.63)**
Light physical activity	1.02 (0.83, 1.25)	1.03 (0.83, 1.28)	1.06 (0.85, 1.32)
MVPA	0.82 (0.64, 1.04)	0.82 (0.63, 1.06)	0.91 (0.69, 1.19)

RR, relative risk; CI, confidence interval; MVPA, moderate to vigorous physical activity.
RR represent the change in the outcome for a standard deviation-unit increase in time spent sedentary (86 minutes), time spent in light physical activity (65 minutes) and MVPA (28 minutes).
[a] Adjusted for age, weight, height, menopausal status, calcium intake, serum 25-hydroxyvitamin D levels, and education level.
[b] further adjusted MVPA for sedentary time and light physical activity; adjusted sedentary time for MVPA.

OR21

Cognitive decline is associated with an accelerated rate of bone loss and increased fracture risk in women 65 years or older in the longitudinal population-based Canadian multicentre osteoporosis study (CAMOS)

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Garvan Institute of Medical Research

Cognitive deficit and osteoporosis are common among older people with some studies suggesting a causal link. However, longitudinal studies that assess the complex relationships among cognitive decline, bone loss and fracture risk independent of ageing are lacking.

This study aimed to determine the association between: 1) cognitive decline and bone loss, and 2) significant cognitive decline (≥3 points) on Mini Mental State Examination (MMSE) (baseline (Y0) - Year 5 (Y5)) and incident fracture risk (Y5 - 15).

A cohort of 2443 women and 844 men aged ≥65 years from population-based Canadian Multicentre Osteoporosis Study (CaMos) was followed from 1997 to 2013. Association between bone loss and cognitive decline was estimated from 3 measurements over 10 years using mixed-effects models. Fracture risk was estimated using Cox Proportional Hazards models. Models were adjusted for age, education and co-morbidities.

Over 95% of participants had normal cognition at baseline (MMSE≥24). The annual % change in MMSE was similar for both genders [women -0.33 (IQR:-1.00 to +0.02) and men -0.33 (IQR:-0.72 to 0.00)]. After accounting for potential confounders, cognitive decline was significantly associated with bone loss (0.13%/year MMSE loss for each 1%/year BMD loss; 95% CI: 0.10 to 0.33). Approximately 13% of participants experienced significant cognitive decline by Y5.

In this group, fracture risk was increased significantly only in women [HR, 1.45 (95% CI: 1.00 to 2.10)]. After further adjustment for bone loss the magnitude decreased only slightly although was no longer significant [HR, 1.39 (95% CI, 0.93-2.06)].

In summary, this study showed a significant association between cognitive decline and bone loss. Fracture risk was also increased in women with significant cognitive decline, which was only marginally explained by bone loss. Further studies are needed to determine mechanisms that link these common conditions.

OR22

Contribution of vitamin D insufficiency to fracture and post-fracture mortality in men

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Background: Approximately one-third of all fragility fractures occur in men, and men with a fracture have an increased risk of mortality. The effect of vitamin D on fracture remains controversial. This study sought to define the relationship between vitamin D status and fracture and post-fracture mortality in elderly men.

Methods: This study was part of the ongoing Dubbo Osteoporosis Epidemiology Study (DOES) which is designed as a longitudinal population-based prospective study. The study involved 413 men aged 60 years and older as at 1989-1990. Fracture incidence was ascertained from X-ray reports from 1990 to 2017. Mortality was ascertained from funeral lists and obituary reports. Bone mineral density (BMD) was measured by dual X-ray absorptiometry. The DiaSorin assay was used to measure serum concentrations of 25-hydroxyvitamin D (25(OH)D). Statistical analysis was completed using Cox's proportional hazard model, logistic regression models, and t tests. Adjustments were made for age, BMI, and comorbidities.

Results: Among 413 men (average age: 72 years), 225 (55%) were vitamin D insufficient (25(OH)D levels ≤74mmol/L). During the follow-up period, 104 men (25%) sustained a fragility fracture. There was no statistically significant association between vitamin D insufficiency and fracture risk (HR 1, 95%CI 0.8 to 1.2; P=0.16). Among 104 men with a fracture, 85 (82%) died during the follow-up period. Each SD decrease in 25(OH)D was associated with 1.3 increase in post-fracture mortality (HR 1.25; 95%CI 1.02 to 1.54). Advancing age was also a significant predictor of fracture risk (OR 1.3; 95%CI 1.1 to 1.6) and mortality risk (OR 3.19; 95%CI 1.7 to 6.1; P<0.001).

Conclusion: In elderly men, the contribution of vitamin D insufficiency to fragility fracture was negligible. However, there was an inverse association between vitamin D and post-fracture mortality.

OR23

Adamts17 is involved in skeletal growth through the regulation of TGF-Î_ signaling via Microfibril biogenesis

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Aims: Autosomal recessive ADAMTS17 mutations cause Weill-Marchesani-like syndrome in humans in which the major signs and symptoms are short stature and eye abnormalities. Furthermore, ADAMTS17 has been reported to link to variant height in humans. Recently, some ADAMTS proteins have been reported to participate in the structural and regulatory roles of Microfibrils which contain FIBRILLIN-1 as a major component. Here, we investigated the function of Adamts17 in skeletogenesis using genetic approaches in mice.

Methods: We created Adamts17-flox mice in which the exon 4 was flanked by loxP sites. By mating adamts17-flox mice with CAG-Cre mice or Col2a1-Cre mice, we generated CAG-cre;Adamts17flox/flox (Adamts17 KO) mice and Col2a1-Cre;Adamts17flox/flox (Adamts17 cKO) mice.

Results: Immunohistochemistry revealed that Fibrillin-1 and Adamts17 were colocalized in the extracellular matrix of the growth plate. Both Adamts17 KO mice and Adamts17 cKO mice developed postnatal dwarfism and radiographical analyses revealed that longitudinal lengths of long bones of both KO and cKO mice were significantly shorter than controls, indicating Adamts17 plays an important role in skeletogenesis. Histological analyses revealed that the length of hypertrophic zone of Adamts17 KO mice was significantly shorter than controls. TUNEL and EDU assays revealed no significant differences in apoptosis and proliferation, respectively. We investigated the activity of TGF-β signaling in vivo because microfibrils have been reported to be involved in storage and regulation of the TGF-beta superfamily.

Phosphorylation and subsequent nuclear translocation of Smad2 were markedly increased in the hypertrophic zone of KO mice compared to WT mice. When si-ADAMTS17 was transfected to MG63 osteosarcoma cell lines, Fibrillin-1 matrix assembly was inhibited. Primary costal chondrocyte cultures and metatarsal organ cultures from each genotype revealed that TGF-beta signaling was abnormally activated and terminal differentiation was inhibited in KO mice.

Conclusion: Adamts17 is involved in skeletal growth through the regulation of TGF-β signaling via Microfibril biogenesis.

OR24

Long-term Outcomes of Osteogenesis Imperfecta in the Bisphosphonate Era

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Background: Bisphosphonates have been used for treatment of bone fragility disorders for over 25 years to increase bone mineral density (BMD). Anecdotally, bisphosphonate-treated Osteogenesis Imperfecta (OI) has a different trajectory to the natural history of untreated OI, with minimal published evidence to support this clinical observation.

Aims: To describe functional outcomes of a cohort of adults with OI, stratified according to severity and treated with bisphosphonates as children, including fracture incidence before and after puberty, mobility and BMD outcomes of this cohort, compared to adults with OI who were never treated as children.

Methods: All participants completed four questionnaires: a study specific questionnaire addressing fracture and treatment history, WHOQOL-BREF (quality of life), SF-36 (musculoskeletal function) and IPAQ (physical activity), and medical records were reviewed.

Results: Fifty-two adults with OI (80% response rate) completed the questionnaires; 33 of whom were treated with bisphosphonates in childhood. The childhood treated cohort had higher lumbar spine BMD than the adult treated cohort (z-score -0.5 at mean age 21.3 years versus -2.1 at mean age 40.9 years; p=0.005). There were less post-pubertal fractures in severe forms of OI in the childhood treated cohort compared to the adult treated cohort. In less severe OI, childhood treated individuals had higher levels of physical activity and physical functioning than adult treated individuals. Incidence of scoliosis was not different between cohorts. There were no differences in quality of life scores between the two cohorts.

Conclusions: Improvements in BMD correlate with reduction of post-pubertal fracture rates in severe OI but do not appear to influence the prevalence of scoliosis. Results suggest that treatment with bisphosphonates at an earlier age improves physical activity, particularly in less severe forms of OI but may not alter quality of life.

OR25

Calcitriol may act on an alternate receptor during fetal development, since absence of calcitriol has different consequences than loss of the vitamin D receptor

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Calcitriol may act on an alternate receptor during fetal development, since absence of calcitriol has different consequences than loss of the vitamin D receptor

Vitamin D receptor (Vdr) null fetuses have normal serum minerals, PTH, and skeletal morphology/mineralization. But Vdr null fetuses also have increased serum calcitriol, placental calcium transport, and placental expression of PTHrP and TRPV6.

We examined Cyp27b1 null fetal mice, which do not make calcitriol, to determine if loss of calcitriol has the same consequences.

Cyp27b1 null and WT females (first-degree relatives) were mated to Cyp27b1+/- males. This generated Cyp27b1 null and Cyp27b1+/- fetuses from null dams, and Cyp27b1+/- and WT fetuses from WT dams.

Fetal and maternal serum were collected; intact fetuses were ashed; ash mineral content was measured by atomic absorption spectroscopy; tibiae and placentas were harvested; placental gene expression was assessed by qPCR. 45Ca and 51Cr-EDTA were administered by intracardiac injection to pregnant dams and radioactivity transported to the fetuses was measured after 5 minutes. Statistical analysis used ANOVA with Tukey's for multiple comparisons. Animal ethics approved these studies.

Calcitriol was undetectable in null fetuses, which also had (vs. littermates) normal serum calcium (2.22±0.10 vs. 2.29±0.07 mM), phosphorus (2.10±0.11 vs. 2.14±0.12 mM), FGF23 (49±8 vs. 53±7 pg/mL), PTH, skeletal ash weight (17±0.4 vs. 17±0.4 mg), and ash calcium (74.5±1.0 vs. 76.2±1.5 mg/g) and phosphorus. Placental calcium transport (104±5 vs. 111±9%), and placental expression of Pthrp (0.85±0.32 vs. 1.00±0.86) and Trpv6 (0.95±0.39 vs. 1.00±0.25), were no different between null and WT fetuses. Tibial lengths and morphology were normal.

In summary, loss of calcitriol did not alter fetal mineral or bone homeostasis, including placental calcium transport and placental gene expression. Vdr nulls differ by displaying increases in placental calcium transport and expression of TRPV6 and PTHrP. These findings confirm that calcitriol and VDR are not required during fetal development, but imply that high levels of calcitriol upregulate placental function in Vdr null fetuses by acting on an alternate receptor.

OR26

The Calcitonin Receptor attenuates the acidity of the osteocyte lacunae microenvironment during lactation in mice

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The physiological role of calcitonin, and its receptor, the CTR, has long been debated. We previously provided the first evidence for a physiological role of the CTR to limit bone loss during lactation in mice by inhibiting osteocytic osteolysis. The expression of CTR in bone during pregnancy and lactation, the mechanism by which it inhibits osteocytic osteolysis and its role in restoring bone mineral to the osteocyte lacunae post-lactation has yet to been determined.

CTR gene expression was determined in whole bone, including bone marrow, of female control mice at the end of pregnancy (E18) and lactation (P21). CTR mRNA was upregulated by 12-16 fold at the end of pregnancy and lactation compared to virgin controls ($P<0.05$). CTR mRNA levels returned to virgin control levels 21 days post-weaning.

To study in vivo acidification by osteocytes, lactating (P14 days) Global-CTRKO and littermate control mice were injected with a pH indicator dye, acridine orange intravenously. 30 mins post-injection, osteocyte lacunae in the tibia were imaged by confocal microscopy. In preliminary analyses, a red shift in emission spectra consistent with lower pH was observed in the osteocyte lacunae of lactating Global-CTRKO compared to controls ($P<0.05$, n=3-8/group).

To determine whether the CTR is required for the replacement of mineral within the lacunae post-lactation, lacunae area was determined in the femur of control and Global-CTRKO mice 3 weeks post-weaning. Comparison of the largest 20% of lacunae by area showed that lacunar area did not differ between Global-CTRKOs and controls post-lactation (mean ± SE (μm2): control: 86.1 ± 2.9; Global-CTRKO: 85.3 ± 1.7, n=6-9/group).

In conclusion, the CTR exerts its inhibitory actions on osteocytic osteolysis during lactation by attenuating the acidity of the lacunae microenvironment, however the CTR is dispensable for replacement of bone mineral within lacunae by osteocytes post-lactation.

OR27

Bone corticalisation requires suppression of glycoprotein 130 signalling in osteocytes, and occurs by region-specific imbalances in bone formation and resorption

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The mechanisms that control bone corticalisation (the coalescence of trabeculae to form thick cortical bone during development) are unknown. Recently, it has been shown that this process requires the intracellular protein SOCS3, and in female mice lacking SOCS3 in osteocytes (Dmp1Cre.Socs3f/f) metaphyseal corticalisation is delayed until after 12 weeks of age, when longitudinal bone growth has ceased. This therefore provides a model to study this process. Furthermore, since SOCS3 provides negative feedback for a subset of receptors, including leptin receptor (LepR) and glycoprotein 130 (gp130 / Il6st), the model also provides a way to identify the specific cytokine receptor that delays corticalisation.

To identify cellular activities mediating corticalisation, we quantified bone resorbed and formed from 12 to 16 weeks of age in female Dmp1Cre.Socs3f/f mice using co-registration of in vivo micro-CT images. In regions where cortical bone formed, bone formation was significantly (10-fold) greater than bone resorption. In contrast, in the central region that became trabecular, bone resorption was significantly greater (2-fold higher) than formation. This indicates that two processes occur during corticalisation: (1) bone formation between bone struts at the periphery, and (2) resorption of trabeculae in the marrow space.

To identify which SOCS3-dependent receptor must be suppressed for corticalisation, we sought to rescue the Dmp1Cre.Socs3f/f phenotype by suppressing gp130 (using the Il6st-flox transgene) or LepR (using Lepr-flox). The corticalisation defect was fully rescued by gp130 deletion. At 12 weeks of age, cortical porosity was significantly less in Dmp1Cre.Socs3f/f.Il6stf/f mice compared with Dmp1Cre.Socs3f/f controls (60% less in male, and 77% less in female, $p<0.001$, n=8-10/group). No rescue was observed with LepR deletion.

In conclusion, cortical and trabecular structures form by formation-mediated closure of cortical pores at the periphery, and by resorption of trabeculae in the central metaphysis. This process requires suppression of gp130 signalling in osteocytes by SOCS3.

OR28

Polara 1, a novel inhibitor of CYP24A1 activity, prevents vitamin D catabolism and enhances bone formation in mice

Paul Anderson
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CYP24A1 is the enzyme which exclusively catabolises vitamin D, both in the kidney and in peripheral tissues. This enzyme frustrates the activity of active vitamin D (1,25(OH)D3), and under several conditions, such as chronic kidney disease (CKD), is inappropriately elevated, and can also result in the catabolism of 25(OH)D resulting in vitamin D-deficiency, contributing to the development of mineral and bone disorders (MBD). We have assessed the effects of targeting CYP24A1 activity either genetically or with specific novel pharmacological inhibitors to establish evidence for direct and indirect actions of CYP24A1 in regulation bone mineralisation. Firstly, we have shown that increased CYP24A1 mRNA expression in bone is a significant negative determinant of bone quality (Bone surface/bone volume) in elderly human bone biopsies (n=111). Secondly, the specific deletion of CYP24A1 expression in mature osteoblasts in mice, using the osteocalcin-Cre promoter, significantly increases bone volume and strength, at least in young mice.

Thirdly, CYP24A1 inhibition is highly effective in restoring normal bone by enhancing bone mineralisation in a model of X-linked hypophosphatemic rickets (Hyp mice), a similar phenotypic disorder to CKD. Lastly, we have developed a novel inhibitor of vitamin D catabolism (Polara 1) which prolongs the half-life of 1,25(OH)2D in HEK293 cells; elevates the intestinal vitamin D-responsive genes, in in vitro and in vivo settings; and prevents bone loss either directly or indirectly due to low dietary calcium without causing hyerpcalcemia in mice. Our data suggest that targeting vitamin D catabolism has merit as a therapy in disorders where vitamin D activity is lacking and where elevated CYP24A1 activity is a primary or contributing cause.

OR29

Intestinal calcium absorption increases markedly during pregnancy and lactation despite absence of the vitamin D receptor (VDR) or calcitriol

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Intestinal calcium absorption (CaAbs) and calcitriol increase during pregnancy and lactation. Does calcitriol explain increased CaAbs during these times?

We investigated whether calcitriol is required by comparing absence of calcitriol (Cyp27b1 nulls) to absence of its receptor (Vdr nulls) in pregnant and lactating mice.

WT and null sisters were raised on a calcium and lactose-enriched "rescue" diet. At pre-pregnancy, day 18.5 of pregnancy, and day 10 of lactation, mice were fasted overnight and 0.5 microCi 45Ca was administered by oral gavage. After 10 min, blood was collected by cardiac puncture. Duodena were snap frozen for gene expression analysis by qPCR.

Compared to pre-pregnancy, Vdr null mice showed a 2.5-fold increase ($p<0.05$) of CaAbs during pregnancy and 2.1-fold increased during lactation; achieved increases were similar to WT at all time points. Cyp27b1 nulls showed a marked 9.2-fold increase in CaAbs during pregnancy with no difference from corresponding WT value; lactation has not yet been assessed.

Duodenal expression of Trpv6 and S100g increased 4-fold ($p<0.01$) and 2.4-fold ($p<0.01$) respectively in WT during pregnancy but did not change in Vdr nulls; pregnancy values were 6 and 5-fold fold higher in WT than respective Vdr null ($p<0.01$). Pmca1 showed no differences between time points or genotypes. Ncx did not change in WT but decreased 0.35-fold during pregnancy in Vdr nulls ($p<0.01$).

In summary, CaAbs increased >2-fold during pregnancy and lactation in Vdr nulls, and 9-fold during pregnancy in Cyp27b1 nulls. WT and Vdr null mice differed in duodenal gene expression patterns. None of the changes explained increased CaAbs in Vdr nulls.

In conclusion, CaAbs upregulates during pregnancy without calcitriol, since neither absence of VDR or calcitriol blunted the increase. The persistence of increased CaAbs during lactation indicates that placental factors cannot explain it. The mechanisms that explain this reproductive increase in CaAbs are unknown.

OR30

A longitudinal study of vitamin D status, bone quality and risk for fracture-related hospitalisation in older women

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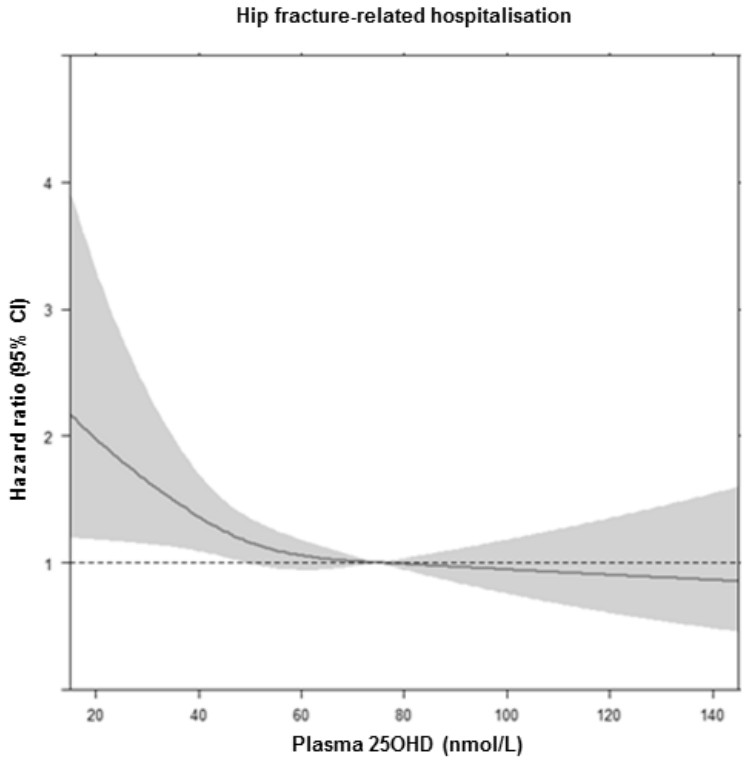
Aim In the Perth Longitudinal Study of Aging in Women Study, we examined the association between plasma 25-hydroxyvitamin D (25OHD) with bone quality and 14.5-year fracture-related hospitalisations.

Methods The study participants were 1,348 women aged 70-85 years at baseline (1998). Assessments included plasma 25OHD concentration using LC-MS/MS at baseline, hip DXA bone mineral density (BMD) at year 1, trabecular bone score (TBS) at year 5, and fracture-related hospitalizations over 14.5 years obtained by record linkage. Covariates adjusted in the analyses included seasonality, baseline age, body mass index, physical activity, calcium intake, smoking and alcohol consumption, and for fracture related models, fracture history.

Results The mean plasma 25OHD was 66.9±28.2 nmol/L, and 28.5%, 36.4% and 35.1% women had levels <50, 50-75 and ≥75 nmol/L, respectively. Women with 25OHD ≥75 nmol/L had significantly higher total hip and femoral neck BMD at year 1 (3.3-3.9%) and TBS at year 5 (2.0%) than those with 25OHD <50 nmol/L (all P<0.05). Generalized additive regression models showed that the values for total hip and femoral neck BMD and TBS were higher with increasing plasma 25OHD up to 100 nmol/L. In the Cox regression analyses, compared with women who had plasma 25OHD <50 nmol/L, those with 25OHD levels at 50-75 and ≥75 nmol/L had significantly lower risk for hip fracture (hazard ratio (95% CI): 0.60 (0.40-0.91) and 0.61 (0.40-0.93), respectively) and any fracture-related hospitalisation (hazard ratio (95% CI): 0.75 (0.58-0.97) and 0.69 (0.53-0.90), respectively). Restricted cubic spline regression models showed that plasma 25OHD below 50 nmol/L was associated with higher risk of hip fracture-related hospitalisation (Figure) and below 75 nmol/L any fracture-related hospitalisation.

Conclusion Our study suggests maintaining serum 25OHD levels to at least 50 nmol/L and ideally up to 75 nmol/L could confer benefit for the long-term prevention of fracture-related hospitalisation in community-dwelling older women.



OR31

Low-trauma rib fracture in the elderly: risk factors and mortality consequence

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Increased osteoclast activity and reduced osteoblast function are downstream mechanisms driving bone loss in osteoporosis. Altered macrophage function could contribute to osteoporosis pathobiology through amplifying systemic immune activation, ultimately increasing osteoclastogenesis. Evidence that osteal macrophages (osteomacs) promote osteoblast-mediated bone formation raise the hypothesis that altered osteomac biology contribute to osteoporosis-associated bone loss and/or suppressed bone formation. We established an ovariectomy (OVX)-induced model of post-menopausal osteoporosis in 16-week-old C3H/HeJ mice (high baseline bone mineral density). Bone loss was confirmed 4 weeks post-OVX with significantly reduced trabecular bone/total volume (-46%), decreased trabecular thickness and increased trabecular separation. Cortical thickness was also significantly reduced (-8.15%). Bone marrow (BM) macrophage subset frequency was assessed weekly for 4 weeks using flow cytometry. No difference in frequency of BM F4/80+ macrophages, CD169+ macrophages and Ly6Chi monocytes was observed at any week post-OVX. Similarly, no significant change in osteoclast precursor (CD3-Ter119-B220-CD115hiCD11b-Ly6Chi) number was seen, although there was a trend towards an increase one week post-OVX. Immunohistochemical analysis suggested a trend towards increased osteoclast frequency at one and two-weeks post OVX on trabecular and endocortical bone surfaces. However, osteoclast frequency on periosteal surfaces was not altered at these timepoints. 4 weeks post-OVX, osteoclast frequency was not different between sham and OVX mice. This minimal change in osteoclast frequency was unexpected. Preliminary analysis of total endocortical osteomac and osteoblast frequency shows no significant difference between sham and OVX mice at one or two weeks post-OVX. These support that the significant reduction in bone volume herein is not mediated by alterations in osteoclast or macrophage frequency, or that these changes are a transient event not captured in the time-course assessed. Assessment of altered macrophage and osteoclast functional efficiency and/or profile is underway to improve understanding of the cellular mechanisms leading to bone loss in this model of post-menopausal osteoporosis.

OR32

Pseurotin A suppresses osteoclastogenesis and prevents ovariectomy-induced bone loss by scavenging reactive oxygen species

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The maintenance of bone homeostasis is achieved by the delicate balance between osteoblastic bone formation and osteoclastic bone resorption. Estrogen deficiency-induced bone loss, due to enhanced osteoclast activity, plays an essential role in osteoporosis in postmenopausal women. Growing evidence shows that intracellular reactive oxygen species (ROS) accumulation is a critical factor in the development of osteoporosis by triggering osteoclast formation and function. Pseurotin A (PA) is a bioactive secondary metabolite originally isolated from Aspergillus fumigatus that has been shown to exert a wide range of potential therapeutic applications due to its antioxidant activity, immunosuppressive activity, and antibacterial activity. However, its effects on osteoclasts remain unknown. In this study, the effect of PA treatment on bone loss in ovariectomized (OVX) mice was assessed. Results showed that ovariectomy resulted in increased ROS level in the bone marrow microenvironment in vivo, with dramatically increased numbers of osteoclasts on the bone surface, and significant bone loss; whereas PA supplementation could effectively prevent these OVX-induced changes. In vitro analyses were then performed to demonstrate that PA inhibited osteoclast formation, osteoclast-specific gene expression, and resorptive activity in a dose-dependent manner. These effects were achieved by suppressing receptor activator of nuclear factor-κB (RANKL)-induced mitogen-activated protein kinases (ERK, p38, and JNK) and NF-κB pathway, thus leading to the downregulation of NFATc1, the master transcriptional regulator of osteoclastogenesis. In addition, PA was further found to significantly attenuate the RANKL-induced intracellular ROS production in vitro. Collectively, our findings demonstrated for the first time, to our knowledge, that PA may be a novel alternative therapy for osteoclast-related bone disease such as osteoporosis by acting as a ROS scavenger.

OR33

Post-fracture mortality: A latent class analysis of multimorbidities

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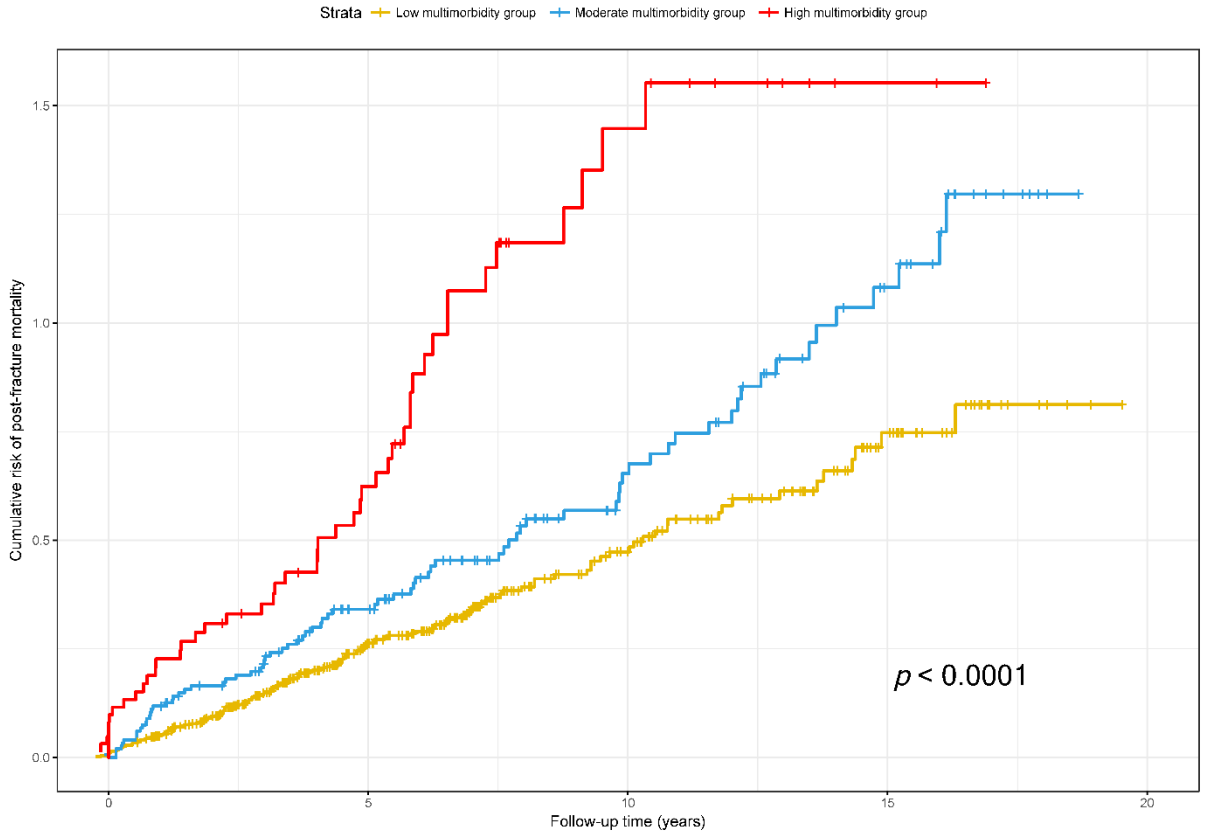
Aims: Osteoporotic patients commonly suffer from other chronic diseases (i.e. multimorbidity), but the relationship between multimorbidity (presence of ≥ 2 diseases) and mortality is unclear. We sought to define the pattern of multimorbidity and its impact on the risk of post-fracture mortality.

Methods: This study involved 890 women and 244 men with a fracture. During over 20 years of follow-up, health status of the individuals had been recorded: osteoarthritis, cardiovascular disease, type II diabetes, cancer, rheumatoid arthritis, neurological illnesses, and mental illnesses. The diseases were ascertained at baseline. We used a latent class analysis (LCA) to define the pattern of co-occurrence of diseases within an individual. The relationship between the LCA-derived clusters of diseases and post-fracture mortality was assessed by the Cox's proportional hazard model.

Results: The prevalence of multimorbidity was 38% in women and 35% in men. Multimorbidity was associated with increased risk of post-fracture mortality (HR 2.4, 95%CI: 1.68-3.38). The LCA identified three clusters of patients: low multimorbidity group (68%), moderate multimorbidity group (20%), and high multimorbidity group (12%). The 5-year risk of post-fracture mortality among the high multimorbidity group was 45%, which was 1.64-fold higher than the moderate multimorbidity group, and 2.3-fold higher than the low multimorbidity group. The increased risk of death was mainly seen among individuals with cardiovascular disease (HR 1.6, 95%CI: 1.2-2.3), type II diabetes (HR 1.8, 95%CI: 1.0-3.2), rheumatoid arthritis (HR 1.8, 95%CI: 1.1-2.9) and neurological illnesses (HR 2.74, 95%CI: 1.2-6.3). The proportion of post-fracture mortality attributable to multimorbidity in women and men was 33% and 28%, respectively.

Conclusions: These prospective data suggest that more than one-third of men and women with a fracture have multimorbidity, and that the coexistence of morbidities account for one-third of post-fracture mortality. The data emphasize the need for a wholistic management of patients with a fracture.



OR34

Disruption of Glucocorticoid Signalling in Osteoblasts and Osteocytes Increases Skeletal Glucose Uptake in Mice Fed a High-fat Diet

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Background: The skeleton's contribution to whole-body glucose homeostasis is undefined at present. We have previously found that abrogating glucocorticoid signalling specifically in osteoblasts and osteocytes, by overexpressing 11β-HSD2 (11β-HSD2-tg mice), improves glucose homeostasis in mice fed a high-fat diet (HFD). Here, we aimed to elucidate the underlying mechanism(s).

Methods: Eight-week old 11β-HSD2-tg mice and WT littermates were fed either standard chow or HFD (14% vs. 43% of energy from fat, respectively) for 8 weeks (n=12-14/group). Insulin tolerance tests were performed, and glucose uptake into insulin-sensitive tissues (skeletal muscle, white adipose tissue (WAT)) and bone was measured using radiolabelled glucose tolerance tests (2g glucose/kg *i.p.* containing 1μCi of 3H-deoxyglucose and 10μCi of U14C-glucose/mouse).

Results: WT mice developed fasting hyperglycaemia and insulin resistance when fed a HFD (Figure 1A). In contrast, HFD-fed 11β-HSD2-tg mice did not develop insulin resistance, and remained comparable to chow-fed mice. Fasting insulin concentrations did not differ between groups of mice (Figure 1B).

Overall, glucose uptake was highest in skeletal muscle, followed by WAT and bone. In muscle and WAT, no significant differences due to diet or genotype were observed (Figures 1C&D). In bone, remarkably, glucose uptake was increased ~3-fold in HFD-fed 11β-HSD2-tg mice ($p<0.01$, Figure 1E), relative to chow-fed tg mice. In WT mice, there was no difference in bone glucose uptake between chow- and HFD-fed mice.

Conclusion: Unlike WT mice, 11β-HSD2-tg mice maintain normal glycaemia and insulin sensitivity when challenged with HFD. Our results show that 11β-HSD2-tg mice adapt to HFD feeding by markedly increasing their glucose uptake into bone, without any changes in plasma insulin concentrations. This improved skeletal insulin sensitivity may protect 11β-HSD2-tg mice from the deleterious effects of chronic HFD feeding. Our results establish the skeleton as an important insulin-sensitive organ and suggest that altered skeletal glucose uptake can influence whole-body glucose homeostasis.

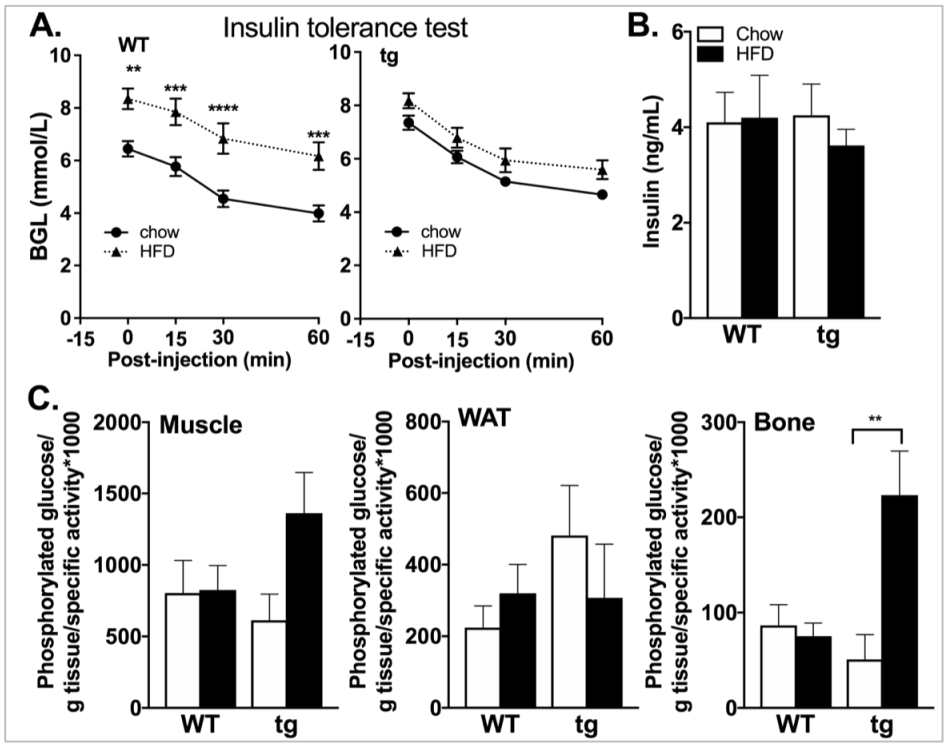

Figure 1

OR35

Osteoglycin: Co-ordination of bone/muscle cross talk through regulation of insulin action

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Introduction: Bone mass must be matched to prevailing energy status/weight. In addition to direct loading-bearing effects, we show that osteoblasts monitor energy balance and secrete osteoglycin (Ogn) a circulating proteoglycan to modulate bone mass through the control of insulin signalling; reduced insulin activity increasing glucose availability to bone cells. We also show that Ogn is expressed by muscle during exercise, highlighting a novel bone/muscle crosstalk pathway.

Aim: To examine the role of osteoglycin in regulating bone mass, glucose balance and energy homeostasis through its production by osteoblasts and muscle.

Methods: Osteoglycin deficient (Ogn-/-) mice were made using Crispr/Cas9; osteoblast Raptor null (RptorOB-/-) mice were made using Osterix-Cre, to disrupt energy sensing via mTORC1. 10- 16 week old mice were assessed for bone, metabolic and glycaemic changes, as well as serum Ogn and tissue-specific Ogn expression. Wild type mice were exercised on a treadmill for 90 minutes, and muscle osteoglycin protein production measured.

Results: Ogn-/- and RptorOB-/- mice displayed opposing phenotypes in a number of systems. RptorOB-/- mice showed increased serum Ogn levels, coincident with a lean phenotype. Greater Ogn was associated with improved insulin signalling, reduced fasting glucose, improved glucose tolerance, increased metabolic rate and reduced bone mass and bone formation rate. In wild type mice, exercise was associated with an increase in Ogn secretion from muscle, with Ogn the 10th largest exercise-induced change in secretion. In RptorOB-/- mice, Ogn expression was increased 3-fold in bone tissue, with no change in skeletal muscle.

Conclusion: Osteoglycin stimulates the insulin signalling pathway. In bone, osteoblasts sense positive energy balance and suppress osteoglycin secretion to promote tissue-specific glucose influx and bone accrual. During exercise, muscle increases osteoglycin secretion to promote insulin-mediated uptake in muscle cells. Osteoglycin represents a mechanism whereby tissue-specific energy portioning is coordinated between bone and muscle.

OR36

Deterioration in Cortical and Trabecular Microstructure and Matrix Mineral Density Produce Bone Fragility in Patients with Chronic Renal Disease

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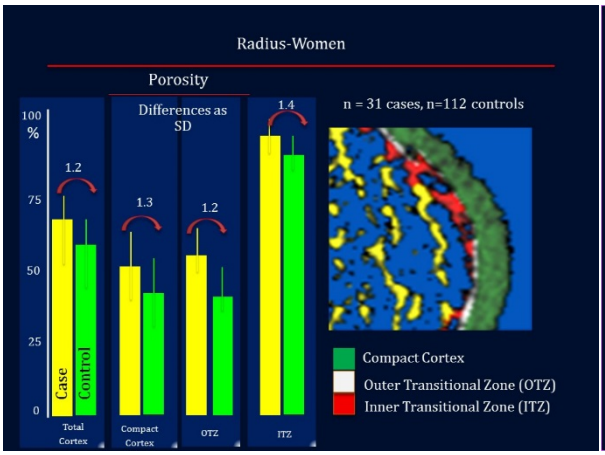
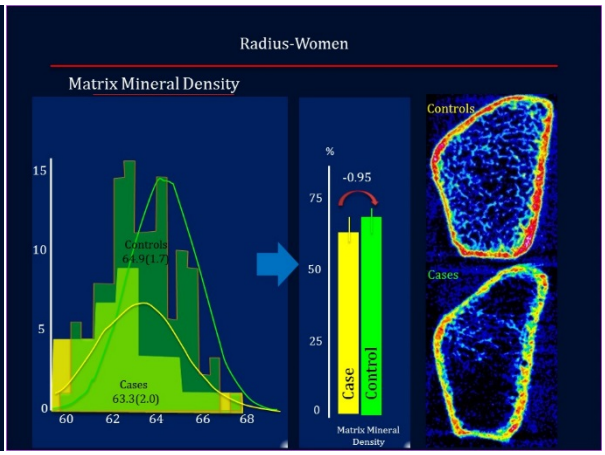
Introduction: Identifying patients with chronic kidney disease at risk for fractures is an unmet need. Bone mineral density (BMD) measurement lacks sensitivity as most persons having fractures have osteopenia or normal BMD. Remodeling markers are not helpful because their renal clearance is impaired. However, high porosity is a consistent finding in CRF in animal models of renal disease. Loss of bending strength is a 7th power function of porosity so a small increase in porosity with modest bone loss disproportionately increases fracture risk. Unbalanced trabecular remodeling erodes trabeculae but high trabecular density is reported in some animal models, perhaps due to errors in segmenting cortical from trabecular bone using threshold based image analyses.

Methods: One hundred and one (52 male) subjects with CKD (79 transplants, 22 dialysis) and 188 healthy age-sex matched controls (76 males) had measurements of bone microarchitecture using HR-pQCT. Distal radius image of 31 cases women (59 years, 22-81) were analysed by StrAx 1.0 software for cortical porosity and matrix mineral density and compared with 112 sex and age-matched controls (57 years, 26-79).

Results: Compared to controls, women with CKD had 1.2, 1.34, 1.38, and 1.17, SD higher distal radial total, compact cortex, outer and inner transitional zone porosity, respectively, and 1.8 SD lower trabecular number and 1.6 SD greater separation and 0.95 SD lower matrix mineral density. Limited data in men show 1.1 and 1.3 SD lower total vBMD and trabecular number, respectively and 1.2 SD higher trabecular separation (all p<0.001).

Conclusion: Patients with chronic kidney disease have severe deficit in mineralized bone matrix volume due to high cortical porosity and reduced matrix mineral which may compromise material stiffness. Studies are needed to determine whether these measurements identify patients who come to sustain fractures and whether treatments can be targeted to these abnormalities.





Fig(a). Higher cortical porosity on total cortex, compact cortex, inner and outer transitional zones of distal radius in CKD cases in comparison to age/sex matched controls. Fig(b). Axial view of distal tibia with different cortical zones and trabecular compartment

Impaired trabecular compartment, higher cortical porosity and lower matrix mineral density of distal radius bone in CKD case in comparison to age/sex matched controls

Keywords: Osteoporosis, CKD, Bone Microstructure, Cortical Porosity and Matrix Mineral Density

OR37

Estimating potential treatment benefits for an initial fracture to prevent a subsequent fracture

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Sustaining a low-trauma fracture has been shown to increase the risk of a subsequent fracture. Importantly, initiation of appropriate treatment at the time of the initial fracture has been shown to reduce risk of re-fracture. This study attempted to address the following question: for an individual who has sustained a low-trauma osteoporotic fracture, given their current sex, site of initial fracture, and BMD - what are the potential treatment benefits to prevent a subsequent fracture?

Elderly women (n=1368) and men (n=878) over the aged 60+ in 1989 from the Dubbo Osteoporosis Epidemiology Study were followed to identify the incidence of low-trauma fracture, and re-fracture. Of these, 950 who sustained an incident low-trauma fracture. Potential treatment benefit was estimated by a meta-analysis of published trials investigating the effectiveness of bisphosphonates to reduce re-fracture, and presented as absolute risk reduction, and numbers needed-to-treat to prevent a single refracture.

Among these 950 elderly women and men with an initial low-trauma-fracture, subsequent fractures were: 250 hip fractures, 429 clinical vertebral fractures, 161 major fractures and 514 minor fractures. Overall, 70% of the study participants have died following fracture over a median follow-up period of 9-years (IQR, 4 to 16 years). Meta-analysis of published studies (n = 4) of the effectiveness of the bisphosphonates, alendronate, risedronate, and zoledronic acid to prevent subsequent fracture – summary relative risk from a random effects Bayesian model = 0.69 (95% Credible Interval = 0.52 to 0.88). Summary of potential treatment benefits to avoid re-fracture, based on site of initial fracture, sex, and femoral neck T-score, and NNT to prevent a single subsequent fracture are presented in the Table below.

Using data from the Dubbo Osteoporosis Study, among women and men who had sustained an initial low-trauma fracture, we have described the absolute risk of a subsequent fracture, and estimated the potential treatment benefits, for prevention of subsequent fracture.

Table. Summary of potential treatment benefits with bisphosphonate treatment following and initial low-trauma fracture.

Initial site of fracture	*FN BMD T-score*	*RD with treatment per 1000*	*NNT*
Hip fracture			
Women	*-3.5 or lower*	*81 fewer (31 - 126 fewer)*	*13*
	-2.5 to -3.5	*89 fewer (34 - 138 fewer)*	*12*
	-1.5 to -2.5	*70 fewer (27 - 109 fewer)*	*15*
Men	*-3.5 or lower*	*59 fewer (23 - 92 fewer)*	*17*
	-2.5 to -3.5	*68 fewer (26 - 105 fewer)*	*16*
	-1.5 to -2.5	*48 fewer (18 - 74 fewer)*	*21*
Vertebral fracture			
Women	*-3.5 or lower*	*99 fewer (38 - 153 fewer)*	*11*
	-2.5 to -3.5	*84 fewer (32 - 130 fewer)*	*12*
	-1.5 to -2.5	*68 fewer (26 - 105 fewer)*	*15*
Men	*-3.5 or lower*	*88 fewer (34 - 137 fewer)*	*12*
	-2.5 to -3.5	*73 fewer (28 - 114 fewer)*	*14*
	-1.5 to -2.5	*56 fewer (21 - 87 fewer)*	*18*
Major fracture			
Women	*-3.5 or lower*	*87 fewer (33 - 135 fewer)*	*12*
	-2.5 to -3.5	*105 fewer (40 - 163 fewer)*	*10*
	-1.5 to -2.5	*69 fewer (26 - 107 fewer)*	*14*
Men	*-3.5 or lower*	*74 fewer (28 - 115 fewer)*	*14*
	-2.5 to -3.5	*93 fewer (36 - 144 fewer)*	*11*
	-1.5 to -2.5	*55 fewer (21 - 85 fewer)*	*19*
Minor fracture			
Women	*-3.5 or lower*	*88 fewer (34 - 136 fewer)*	*12*
	-2.5 to -3.5	*56 fewer (21 - 87 fewer)*	*18*
	-1.5 to -2.5	*45 fewer (17 - 70 fewer)*	*23*
Men	*-3.5 or lower*	*78 fewer (30 - 121 fewer*	*13*
	-2.5 to -3.5	*45 fewer (17 - 70 fewer*	*23*
	-1.5 to -2.5	*33 fewer (13 - 52 fewer*	*31*

Note: RD = (absolute) risk difference with treatment. Rates of fracture within 3-years, and estimated treatment benefit of relative risk reduction by 31% of new low-trauma fracture (RR = 0.69, 95% Credible Interval, 0.52 to 0.88). Therefore, number the needed-to-treat (NNT) to reduce a single fracture are also for a 3-year period. Major fractures included: pelvis, distal femur, proximal tibia, three or more simultaneous ribs, and proximal humerus. Minor fractures included all remaining osteoporotic fractures, excluding those of the face, head or digits.

OR38

Intravital imaging of osteoclasts in vivo reveals novel cellular dynamics which may underlie the therapeutic response to Denosumab withdrawal

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The Anti-RANKL treatment Denosumab (Dmab) has been proven an effective agent for the treatment of low bone mass. However upon treatment withdrawal patients frequently experience rapid bone loss and fracture. We hypothesised that a rebound in osteoclast activity occurs following removal of RANK inhibition. Using a novel intravital imaging methodology, we imaged the intact endocortical surface of the tibia in live mice, producing the first real time visualisation of osteoclast dynamics in vivo. This revealed a cellular plasticity and an alternative cell fate to apoptosis, both previously un-appreciated from in vitro or static histological studies, and importantly provided a mechanism for the Denosumab withdrawal rebound.

We showed that multi-nucleated LysM+Blimp-1+Osteosense+ osteoclasts form syncytial networks on bone and for the first time documented osteoclasts undergoing cell fission in vivo, which was shown to be morphologically distinct to apoptosis. Daughter cells were then observed to re-fuse with parent cells or other nearby osteoclasts, a process we have termed osteoclast recycling. Following osteoprotegerin-Fc fusion protein (OPG-Fc) treatment to mimic Dmab, small round LysM+Blimp-1+ cells (recycling osteoclasts) accumulated. Critically, 3-4 weeks following OPG-Fc withdrawal, recycling osteoclasts had re-fused to form networks of active osteoclasts, co-incident with subsequent loss in bone microarchitecture. Further, these recycling osteoclasts re-fused to form large active osteoclasts following re-injection into tibia of naïve mice.

These data demonstrate that intravital imaging of the endosteal bone surface in the tibia can be used to study osteoclast dynamics in vivo, and that in addition to apoptosis, osteoclasts can recycle their cellular constituents. Osteoclast recycling not only provides a new paradigm for understanding the behaviour of these cells in vivo, but the rapid re-fusion of these cells following withdrawal of RANK inhibition explains the paradoxical acceleration of bone loss and fractures observed upon discontinuation of Denosumab.

OR39

Effect of denosumab compared with risedronate on percentage change in lumbar spine BMD at 12 months in subgroups of glucocorticoid-treated individuals

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Aim: Explore the effects of denosumab and risedronate on lumbar spine (LS) BMD in predefined subgroups to determine whether these factors affected the treatment effect.

Methods: A phase 3, randomised (1:1), double-blind, active-controlled study to evaluate denosumab 60mg Q6M versus risedronate 5mg daily in glucocorticoid(GC)-treated individuals for 24 months. Eligible subjects: adults ≥18 years receiving GC therapy (≥7.5 mg prednisone daily or equivalent) for ≥3 months or <3 months prior to screening (GC-continuing [GC-C] and GC-initiating [GC-I], respectively). All subjects <50 years were required to have a history of osteoporotic fracture. GC-C subjects ≥50 years were required to have a LS, total hip or femoral neck T score ≤–2.0; or a T-score ≤–1.0 with a history of fracture. Subjects received daily calcium and vitamin D. Percentage change from baseline in LS BMD at 12 months was determined in both subpopulations and in seven predefined subgroups that may influence treatment effect.

Results: A total of 795 subjects (505 GC-C and 290 GC-I) enrolled in the study. Baseline characteristics were balanced between treatment groups. Denosumab resulted in significantly greater gains in LS BMD at 12 months compared with risedronate for both the GC-C and GC-I subpopulations (Table). Results from all subgroups consistently demonstrated a greater increase in LS BMD at month 12 with denosumab compared with risedronate. A significant quantitative interaction was observed only in the gender analysis in the GC-I subpopulation. However, non-significant qualitative interaction testing indicated that there was no evidence that the direction of the denosumab effect differed by gender in this subpopulation.

Conclusion: Denosumab consistently increased LS BMD more than risedonate at 12 months across seven different subgroups of GC-treated individuals. Denosumab has the potential to become another treatment option for patients newly initiating or continuing GC who are at increased risk for fracture.

Table. Difference in lumbar spine BMD percentage change from baseline at month 12 (GC-C and GC-I subpopulations)

	GC-C subpopulation (N = 230 RIS / 228 DMAb)		**GC-I subpopulation (N = 133 RIS / 128 DMAb)**	
	Difference (DMAb – RIS)	**Interaction *P* value Quantitative (Qualitative)**	**Difference (DMAb – RIS)**	**Interaction *P* value Quantitative (Qualitative)**
Overall subpopulation	2.2 (1.4, 3.0)*	N/A	2.9 (2.0, 3.9)*	N/A
Gender				
Female	2.4 (1.5, 3.3)*	0.31	3.7 (2.5, 4.9)*	0.018
Male	1.3 (–0.3, 2.9)		1.2 (–0.4, 2.8)	(0.50)
Race				
Caucasian	2.1 (1.2, 2.9)*	0.46	2.9 (1.9, 3.8)*	0.68
Not Caucasian	2.5 (0.2, 4.8)*		3.0 (–0.4, 6.3)	
Age Group				
< 60 years	1.9 (0.8, 3.1)*	0.57	2.7 (0.6, 4.8)*	0.57
≥ 60 years	2.3 (1.2, 3.5)*		3.1 (2.0, 4.2)*	
Baseline LS T-score				
≤ –2.5	2.4 (0.9, 3.9)*	0.40	3.8 (1.5, 6.0)*	0.081
> –2.5	2.0 (1.1, 2.9)*		2.5 (1.5, 3.5)*	
Baseline LS T-score				
≤ –1.0	2.2 (1.3, 3.2)*	0.55	3.5 (2.2, 4.9)*	0.18
> –1.0	1.3 (–0.2, 2.9)		2.0 (0.7, 3.2)*	
Geographic Region				
Europe	2.0 (1.0, 3.0)*	0.46	3.2 (2.1, 4.3)*	0.60
Non-Europe	2.5 (1.1, 3.8)*		2.6 (0.8, 4.3)*	
Menopausal status				
Premenopausal	1.7 (–0.7, 4.1)	0.47	6.3 (1.9, 10.7)*	0.37
Postmenopausal	2.4 (1.4, 3.4)*		3.5 (2.2, 4.8)*	
Baseline GC daily dose				
7.5 - < 10 mg	2.9 (1.7, 4.1)*	0.37	3.1 (0.9, 5.4)*	0.98
≥ 10 mg	2.0 (1.0, 3.1)*		2.9 (1.8, 4.0)*	

N = Number of randomised subjects with a baseline and ≥ 1 postbaseline lumbar spine BMD measurement
*DMAb compared with RIS $p < 0.05$

Plenary Poster Presentations

Plenary Poster P1
Musculoskeletal Changes in a Mouse Model of Inflammatory Bowel Disease

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2. Vixtoria University and Macquarie University

Background/Aims: Osteoporosis and sarcopenia commonly associate with inflammatory bowel disease (IBD). However, studies on the mechanisms, prevention, and treatment of IBD-related osteosarcopenia are scarce, mainly due to the lack of animal models. Chemically-induced IBD models are inadequate due to a short timeframe of intestinal inflammation. The Winnie mice (MUC2 mutant) develop spontaneous chronic colitis after 6 weeks of age closely resembling symptoms and pathophysiology of IBD. We therefore hypothesised that Winnie mice are osteosarcopenic and have significant changes in muscle and fat volumes, bone volume and volumetric density (vBMD).

Methods: Winnie mice and their age-matched controls (C57BL/6; n ≥5 per group) were scanned cross-sectionally at the level of mid-thigh using pQCT (180µm resolution with a slice thickness of 2.4mm) before the development of IBD (week 6), and after disease development (weeks 14 and 21).

Results: At week 6, compared to controls, Winnies had smaller body cross-section (312±40.2 vs 353.8±14.1; p=0.034) and consequently smaller muscles (686.7±93.6 vs 781.6±29.5; p=0.037); but with no difference in vBMD or bone volume (p>0.5). Muscle volume as percentage of the body was not significantly different (p=0.6).

At week 14, Winnie mice show smaller body cross-section area (384.6±13.5 vs 451.6±11.9; p<0.001), muscle volume (842.6±28.9 vs 988.9±27.28; p<0.001), vBMD (436.8±20 vs 493.7±14; p=0.002) and bone volume (6.9±0.3 vs 8.6±0.6; p<0.001) compared to controls.

After skeletal maturity (week 21), Winnie mice demonstrated 39% smaller body cross-section area (277.4±43.9 vs 453.4±19.1; p<0.001), 38% lower muscle volume (611.8±91.1 vs 986.2±38.5; p<0.001) and 21% reduced vBMD (383.25±22.8 vs 483.5±11.5; p<0.001), 34% reduced bone volume (5.6±0.8 vs 8.4±1; p=0.003), 63.3% lesser fat volume (16±2.2 vs 43.7±4; p<0.001) and 40% lower fat percentage (2.4±0.1 vs 4±0.3; p<0.001) compared to controls.

Conclusion: This is the first study demonstrating the occurrence of osteosarcopenia in a mouse model of IBD. Further exploration of the mechanisms explaining this musculoskeletal phenotype is warranted.

Plenary Poster P2
The association between fractures sustained during childhood and bone measures in young adulthood

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The association between fractures during childhood and peak bone mass is unknown. This study aimed to describe the association between fractures sustained at different stages of growth with bone measures in early adulthood.

We followed 201 young adults (mean age (SD) = 25.5 (0.7) years) from birth to 25 years. At age 8, 16 and 25, fractures were self-reported and confirmed by X-ray. Areal bone mineral density (aBMD) at the lumbar spine (LS), hip and total body (TB) was measured by DXA. Total, trabecular and cortical volumetric BMD, microarchitecture measures at the distal radius and tibia were obtained by High Resolution Peripheral Quantitative Computerised Tomography (HRpQCT) at 25 years old. Multivariable linear regression was used to analyse the association of the occurrence of pre-pubertal (occurring before age 8), pubertal (between age 8 to 16) and post-pubertal (between age 16 to 25) fractures with all bone measures.

During the 25 years, 99 participants had at least one fracture. At age 25, pre-pubertal fractures were significantly associated with decreased aBMD, vBMD and most microarchitecture measures at tibia, and also associated with increased outer transitional porosity and suboptimal trabecular measures at radius. Pubertal fractures had no association with any bone measure at age 25. Post-pubertal fractures were associated with trabecular number at the tibia. Pre-pubertal fractures had no association with aBMD at age 8, but were inversely associated with aBMD at LS, hip and TB at 16 and 25 years.

Pre-pubertal fractures are not associated with BMD at that stage but are associated with later bone measures (both DXA and HRpQCT). Fractures occurring at other age periods are inconsistently associated with bone measures in young adulthood. This implies that the pre-pubertal fractures may lead to a later deficit in bone.

Plenary Poster P3
A Novel Collagen Scaffold for Augmenting Rotator Cuff Repair - An in vivo Rat Study

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Surgical repair of rotator cuff tears have high failure rates (20-70%), often due to a lack of biological healing. Augmenting repairs with extracellular matrix-based scaffolds is a common option for surgeons, although to date, no commercially available product has proven to be effective. In this study, a novel collagen scaffold was assessed for its efficacy in augmenting rotator cuff repair.

The collagen scaffold was assessed in vitro for cytocompatability and retention of tenocyte phenotype using alamarBLUE assays, confocal imaging and real-time PCR. Immunogenicity was assessed in vitro by the activation of pre-macrophage cells. In vivo, using a modified rat rotator cuff defect model, supraspinatus tendon repairs were carried out in 46 animals. Overlay augmentation with the collagen scaffold was compared to unaugmented repairs. At 6- and 12-weeks post-op the repairs were tested biomechanically to evaluate repair strength, and histologically for quality of healing.

The collagen scaffold supported human tenocyte growth in vitro, with cells appearing morphologically tenocytic and expressing higher tendon gene markers compared to plastic controls. No immunogenic responses were provoked compared to suture material control. In vivo, augmentation with the scaffold improved the histological scores at 12 weeks (8.37/15 vs. 6.43/15, p=0.0317). However, no significant difference was detected on mechanical testing.

While the collagen scaffold improved the quality of healing of the tendon, a meaningful increase in biomechanical strength was not achieved. This is likely due to its inability to affect the bone-tendon junction. Future materials/orthobiologics must target both the repaired tendon and the regenerating bone-tendon junction.

Plenary Poster P4
Profilin1 deficiency in osteoclasts causes osteolytic developmental bone deformities due to increased osteoclast movements and differentiation

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Profilin1 (Pfn1), an evolutionarily conserved actin-binding protein, is an important regulator of cytoskeletal remodeling, cell adhesion, motility, and even proliferation in various cells. However, its roles in bone cells such as osteoblasts, osteocytes and osteoclasts are remained unclear. We previously reported that osteoclast-specific Pfn1-conditional knockout (cKO) mice grew slightly shorter after birth. At about 4 weeks, skeletal deformities became significant, especially at zygomatic arches, nasal bone in the skull, and long bones in the extremities with decreased bone mineral density, metaphyseal bone volume and diaphyseal cortical bone thickness. Histologically, tartrate-resistant acid phosphatase-positive osteoclasts were increased in the Pfn1-cKO mice compared to wild-type control mice. However, preliminary our trials failed to demonstrate the alterations in Pfn1-deficient cells in standard in vitro osteoclastogenesis assays, using bone marrow cells as well as the RAW264.7 cells. Therefore, in this study, we addressed the same issues, by focusing on the maturity of the osteoclasts, number of the nuclei, shapes of the processes, in addition to their behaviors on live-imaging analysis. The analysis indicated that Pfn1 KO and knockdown increased the differentiated osteoclast size potentially by promoting the podosome-belt formation. The migratory movements of the immature osteoclasts tend to be increased in the Pfn1-deficient cell culture. These data suggest that Pfn1 suppresses the migratory function of the pre-osteoclasts, which may lead to the appropriate regulation of the osteoclast differentiation. Since the roles of the osteoclasts in skeletal disorders tend to be discussed based only on their number and their size, our study may open new area of osteoclast biology and pathology, which remain incompletely understood.

Plenary Poster P5
Abdominal aortic calcification predicts cardiovascular events and mortality in kidney and simultaneous pancreas-kidney transplant recipients

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Aim: To investigate whether abdominal aortic calcification (AAC) at time of kidney or simultaneous pancreas-kidney (SPK) transplantation predicts future cardiovascular (CV) events and patient survival.
Background: AAC is a predictor for CV events in the general population, but whether AAC at time of transplantation predicts incident CV outcomes after transplantation in kidney transplant recipients remains uncertain.

Methods: This prospective cohort study assessed 413 kidney and 213 SPK recipients undergoing their first transplant without known CV disease. Within 4 weeks of transplant, lateral spine radiographs of the abdominal aorta were scored for AAC using the Kaupilla 24-point scale. The primary outcomes were incident CV events, comprising of myocardial infarction, stroke or peripheral vascular disease, and all-cause mortality.

Results: The mean age was 44 ± 12 years with 277 (44%) having any AAC. After a median follow up of 65 months (IQR 29-107 months), 47 transplant recipients experienced 1 or more CV events and 60 died from any cause. In restricted cubic spline regression, a linear dose response was seen with increasing AAC scores (Figure). After adjustment for age, diabetes, gender, dialysis vintage and transplant type, each 1 point increase in AAC was associated with a 7-9% increase in the relative hazards for CV events and all-cause mortality (HR 1.07 95%CI [1.02-1.12], P=0.005 and HR1.09 95%CI [1.04-1.12], P<0.001, respectively). Transplant recipients with AAC ≥8 had 3.3-3.5 times the relative hazards for CV events or death vs. recipients without any AAC (HR 3.46 [1.53-7.82] and HR 3.36 [1.71-6.60], respectively.

Conclusions: In kidney and SPK transplant patients AAC predicted cardiovascular events and mortality. This test may identify transplant recipients for whom intensive interventions could reduce their cardiovascular risk.

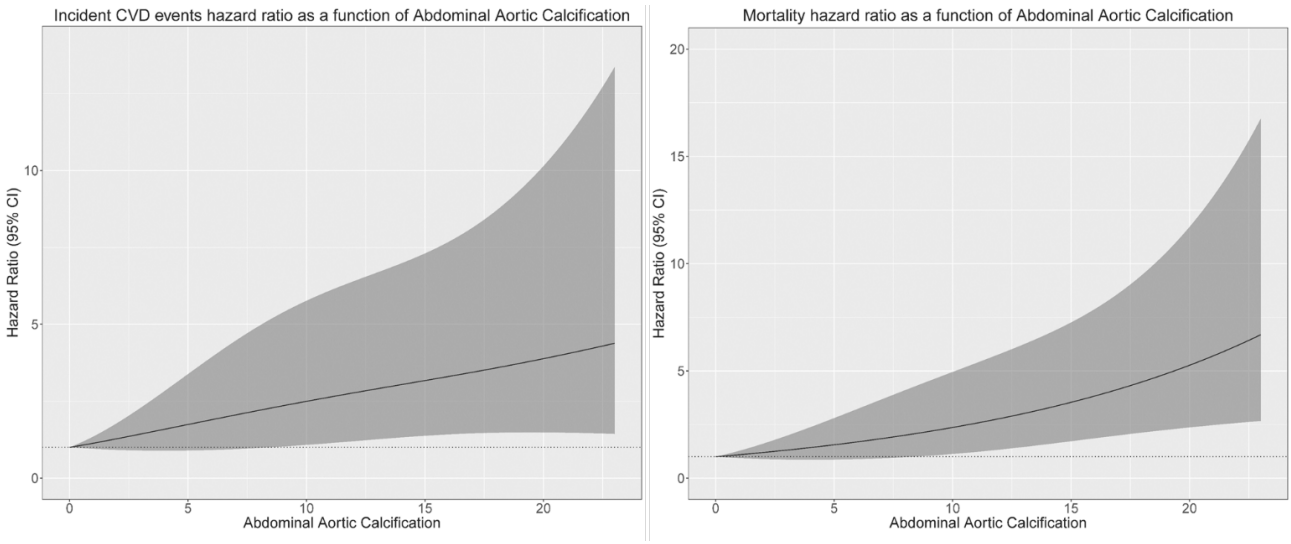

Figure. Multivariable-adjusted restricted cubic spline regression for the association between AAC24 scores (range 0-24) and a) cardiovascular events and b) all-cause mortality. Adjusted for age, gender, smoking history, type of kidney transplant (kidney only or simultaneous pancreas-kidney), dialysis vintage and diabetes.

Plenary Poster P6
Inflammation and bone mineral density: Geelong Osteoporosis Study

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Aim: Osteoporosis is a disease state possibly underpinned by systemic inflammation. Thus, we aimed to determine whether serum interleukin 6 (IL-6), a marker of systemic inflammation, is associated with bone mineral density (BMD) in a population-based sample of men.

Method: This cross-sectional study utilised data from 1143 men aged 20-96 years (median 61.5, IQR 44.5-75.5) participating in the Geelong Osteoporosis Study. Serum IL-6 was measured following an overnight fast using an enzyme-linked immunosorbent assay (ELISA; R&D Systems). BMD (g/cm2) was measured at the PA-spine, femoral neck, total body and forearm using dual energy absorptiometry (Lunar). Anthropometric measurements and socio-economic status (SES) were determined and information on medication use and lifestyle was obtained via questionnaire. IL-6 values were natural log transformed (ln-IL6) and associations between ln-IL6 and BMD were tested using Pearson's correlation. Multivariable regression models were developed to test the association between ln-IL-6 and BMD after adjusting for age and weight.

Results: Ln-IL-6 (median 1.9 mg/ml, IQR 1.2-3.2) was positively correlated with age ($r=0.52$, $p<0.001$) and negatively with BMD at the femoral neck ($r=-0.27$, $p<0.001$), total body ($r=-0.16$, $p<0.001$), distal-forearm ($r=-0.17$, $p<0.001$) and mid forearm ($r=-0.19$, $p<0.001$) sites. After adjustments, ln-IL-6 was associated with decreased BMD at the femoral neck (β -0.014, se ± 0.006, $p=0.015$), total body (β -0.010 ± 0.004, $p=0.010$), distal (β -0.010 ± 0.003, $p=0.050$) and mid forearm (β -0.009 ± 0.003, $p=0.011$), but not spine BMD (β -0.010 ± 0.010, $p=0.237$). These associations persisted after further adjustment for smoking, physical activity, SES, alcohol consumption, medications (statins, antidepressants, non-steroidal anti-inflammatory drugs) and arthritis.

Conclusion: These population-based data suggests IL-6 is independently associated with BMD in adult men. This supports an aetiological role for inflammatory activity in the pathophysiology of osteoporosis.

Plenary Poster P7
FRAME study: The foundation effect of rebuilding bone with one year of romosozumab leads to continued lower fracture risk after transition to denosumab

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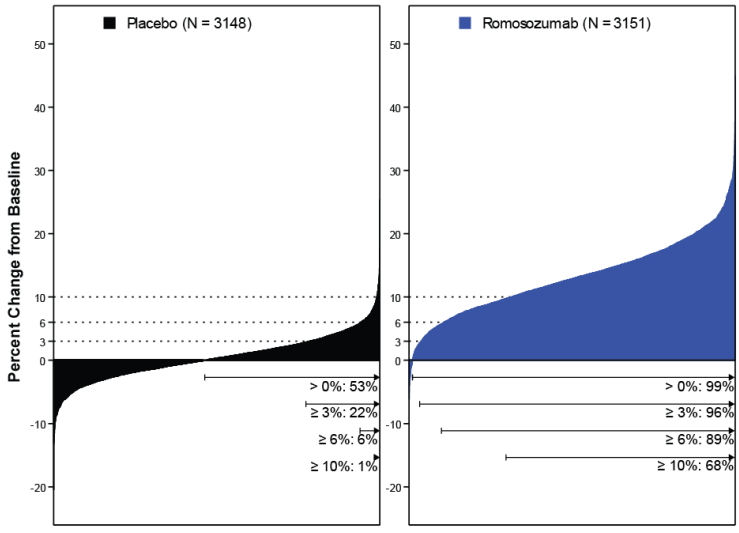
Aim: Post hoc analysis to characterise the BMD gains during the FRAME study and the effect of building bone with romosozumab on fracture risk reduction after transition to denosumab.

Methods: Subjects in FRAME (NCT01575834) were randomised to receive romosozumab 210 mg QM or placebo for 12 months, all subjects then received denosumab 60 mg Q6M for 12 months. Mean change from baseline in BMD T-score, percent of subjects with a BMD gain and subject incidence of fractures in the second year of the study were assessed.

Results: There were 7,180 subjects in the study (N=3,589 romosozumab, N=3,591 placebo). At month 12, mean change from baseline in lumbar spine BMD T-score was 0.88 for romosozumab and 0.03 for placebo; at month 24 the mean change from baseline was 1.11 for romosozumab-to-denosumab and 0.38 for placebo-to-denosumab. At the total hip, the mean changes were 0.32 for romosozumab and 0.01 for placebo at month 12, with month-24 changes of 0.45 for romosozumab-to-denosumab and 0.17 for placebo-to-denosumab. 99% of subjects in the romosozumab group showed some increase in BMD at month 12, with 89% achieving ≥6% gains in lumbar spine BMD (figure). Romosozumab during the first year led to relative risk reductions in fractures between groups during the second year, despite both groups receiving denosumab in year two, with reductions of 81% for vertebral fractures (p<0.001), 32% for clinical fractures (p=0.052) and 39% for major osteoporotic fractures (p=0.034).

Conclusions: Romosozumab resulted in substantial T-score increases after one year; upon transition to denosumab, gains in both groups were similar, resulting in unprecedented BMD gains after treatment with romosozumab-to-denosumab. One year of romosozumab before transition to denosumab, substantially reduced fracture rates during year two. These data support the clinical benefit of rebuilding the skeletal foundation with romosozumab treatment before transition to denosumab.

Figure. Percent Change in Lumbar Spine BMD From Baseline to Month 12 by Individual Subject



N = Number of subjects with values at baseline and at least one post-baseline visit at or before month 12.
Data are percent change in BMD from baseline to month 12 by individual subject at the lumbar spine. N's included subjects with baseline and ≥1 postbaseline measurement; missing data were imputed by last observation carried forward. X-axis represents each individual subject. Dotted horizontal lines reflect 3%, 6%, and 10% BMD gain from baseline to month 12. Lines with arrowheads below the X-axis represent the percent of subjects with the indicated BMD gains (> 0%, ≥ 3%, ≥ 6%, and ≥ 10%).
BMD, bone mineral density.

Funding: Amgen Inc., UCB Pharma, and Astellas Pharma.

Plenary Poster P8
Runx3 is protective for articular chondrocyte.

Kosei Nagata
The University of Tokyo

Runx3 plays an important role in chondrocyte proliferation and differentiation cooperating with Runx2. In this study, we evaluated the role of Runx3 on articular cartilage in osteoarthritis (OA). By producing instability in mouse knee joints (OA model), we attempted to determine the involvement of Runx3. Runx 3 was strongly expressed both in the superficial zone (SFZ) and in the deep zone (DZ) in articular cartilage but its expression attenuated with progress of OA. Performing OA model at 8 weeks of age, the progression of OA was significantly promoted in Runx3 cartilage-specific knockout (cKO) mouse, in particular, the collapse of the SFZ was remarkable. There was no difference in TUNEL stain between Runx3-cKO and control mice, although Runx3 was reported to be an apoptosis inducer. Primary chondrocyte of Runx3-cKO mouse decreased expression of chondrocyte markers including Col2a1, Aggrecan, and Sox9. After separating SFZ chondrocyte and DZ chondrocyte, Runx3-cKO SFZ chondrocyte decreased Prg4 expression, although DZ chondrocyte did not. Runx3 overexpressed primary chondrocyte increased expression of cartilage markers and Prg4. The PRG4 promoter assay identified RUNX3 as a potent inducer of PRG4 expression. There was no catabolic difference of Runx3 cKO chondrocyte in the dimethylmethylene blue assay. These findings suggest Runx3 is important for expression of cartilage marker in articular cartilage and it may act protectively on articular cartilage through expression of Prg4.

Plenary Poster P9
Evaluation of invasive oral procedures and events in women with postmenopausal osteoporosis treated for up to 10 years with denosumab: Results from the phase 3 FREEDOM open-label Extension

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2. Mercy Health
3. University of Wisconsin School of Medicine and Public Health
4. Columbia University

Aim: Positively adjudicated ONJ in the denosumab clinical trial program is rare. Completion of the 7-year FREEDOM Extension study (EXT) permitted an in-depth assessment of risk factors and observed invasive oral procedures and events (OPEs).

Methods: In FREEDOM, women received denosumab 60mg or placebo Q6M for 3 years. Patients who missed ≤1 dose of investigational product and completed the year-3 visit were eligible to participate in the 7-year open-label EXT.

Women who reached the EXT year-3 visit were asked to chronicle their history of invasive OPEs since the start of the EXT through year 2.5, as well as oral events in the prior 6 months. The oral event questionnaire was repeated every 6 months until the end of the EXT.

Results: During the EXT, the overall ONJ rate was 5.2 per 10,000 patient-years. Majority (79%; 3,591/4,550 patients) participated in the survey. Over the EXT, 1,621 (45.1%) reported at least one invasive OPE; the incidence of five individual OPEs were similar between groups. There were 12 confirmed cases of ONJ among women who participated in the survey (11 had OPE and one did not) and one additional case did not complete the survey. ONJ incidence was 0.7% in women reporting invasive OPEs and 0.05% in women reporting no invasive OPEs. Of the 12 ONJ cases, one outcome was unknown, one was ongoing and 10 resolved with treatment.

Conclusions: Nearly all cases of ONJ observed occurred after a reported invasive OPE, yet while invasive OPEs were common in this group of denosumab treated women, ONJ incidence was low. The actual number of invasive OPEs may be underestimated due to limited capture of OPEs in medical charts and possible recall bias in patients with events that occurred in the first 2.5 years of the EXT.

Study funded by Amgen Inc,.

Table: Invasive OPEs during the EXT for patients who completed at least one oral event questionnaire.

	7-year FREEDOM Extension		
	Cross-over (N = 1731)	Long-term (N = 1860)	All (N = 3591)
Age at EXT baseline in years, mean (SD)	74.3 (4.9)	74.4 (4.8)	74.3 (4.8)
Any invasive oral procedure or event, n (%)	795 (45.9)	826 (44.4)	1621 (45.1)
Scaling or root planing	503 (29.1)	531 (28.5)	1034 (28.8)
Tooth extraction	434 (25.1)	458 (24.6)	892 (24.8)
Dental implant	100 (5.8)	112 (6.0)	212 (5.9)
Natural tooth loss	72 (4.2)	75 (4.0)	147 (4.1)
Jaw surgery*	16 (0.9)	17 (0.9)	33 (0.9)

N = Number of patients who received ≥1 dose of investigational product in the EXT and responded to ≥1 oral event questionnaire related to the EXT
n = Number of patients with an OPE
*Collected in the oral event questionnaire every 6 months; therefore, jaw surgery in the first 2.5 years of the EXT was not captured

Plenary Poster P10
Denosumab treatment in women with osteoporosis rapidly prevents deterioration in trabecular microstructure at the distal tibia

Ego Seeman 1, Bin Zhou 2, Ji Wang 2, Arkadi Chines 3, Yifei Shi 3, Andrea Wang 3
1. Austin Health
2. Department of Biomedical Engineering Columbia University
3. Amgen

Aim: Denosumab treatment rapidly reduces bone remodelling and resorptive activity of osteoclasts. Individual Trabecula Segmentation (ITS) distinguishes individual trabeculae and quantitates plate and rod microstructure (Liu JBMR 2008). Here, we quantify the effect of densoumab treatment on trabecular plate-rod microstructure using ITS analyses and whole bone stiffness using finite element (FE) analyses.

Methods: Post-hoc analysis assessed postmenopausal women with low bone mass randomised to placebo or denosumab 60 mg Q6M from a phase 2 study. Subjects were required to have baseline lumbar spine or total hip BMD T-score between –2.0 and –3.0. Effect of treatment on microstructure and whole bone stiffness was quantified using ITS and FE analyses at baseline and 12 months. Individual ITS and FE parameters at the distal tibia were expressed as a percent change from baseline (LS mean with 95% CI) at month 12 using an ANCOVA model adjusting for age, BV/TV and baseline parameter value.

Results: Analysis included 164 subjects (82 placebo, 82 denosumab). Baseline characteristics were similar between groups: mean (SD) age 61 (6) years, BMI 27.1 (4.7) kg/m2, years since menopause 13 (7) and BMD T-scores -2.4 (0.4) at the lumbar spine and -1.2 (0.7) at the total hip. At the distal tibia, denosumab treatment prevented deterioration in or improved all microstructural parameters compared with placebo (Table). Denosumab treatment increased plate number and thickness compared with baseline and prevented the increase in rod number and thinning seen in the placebo group. Plate to rod ratio, a marker of microstructural integrity, decreased significantly in the placebo group compared with baseline and was preserved in the denosumab group. Trabecular stiffness also increased in the denosumab group relative to placebo.

Conclusions: In postmenopausal women with low BMD, denosumab treatment prevents the rapid deterioration of trabecular microstructure at the distal tibia, resulting in preservation of bone strength.

Plenary Poster P11
Microstructural Properties of the Proximal Sesamoid Bones of Racehorses in Training

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University of Melbourne

Proximal sesamoid bone (PSB) fractures are common exercise-related injuries of racehorses that may be fatal. PSB fracture is due to the accumulation of microdamage with repeated high-speed exercise and risk factors include longer racing career, greater cumulative race distance and higher exercise intensity. To understand how PSBs respond to race training we used microCT to evaluate the bone microstructure of PSBs from horses at different stages of their racing careers. Lateral and medial PSBs from a single forelimb of 64 Thoroughbred racehorses were examined post-mortem with microCT (70 kVp, 200 μA, 0.5 mm Aluminium filter and 24 μm3 voxel). Bone volume fraction (BVTV) and bone material density (BMD) were evaluated for individual PSBs. Linear regression models, adjusting for clustering at the horse level, were fitted to identify associations between the BVTV or BMD and horse characteristics (age, sex, length of training, and performance rating). Higher BVTV was associated with medial PSBs compared with lateral PSBs ($P<0.001$), longer racing career ($P=0.001$), and with poorer race performance ($P<0.02$). BMD was higher in raced horses compared to unraced horses ($P<0.001$), horses in active training compared with resting horses ($P=0.002$) and in castrated males compared with intact males ($P<0.001$). Higher PSB BVTV was associated with poorer race performance in the current study and PSB fracture in previous studies consistent with a response to the accumulation of bone microdamage or the cyclic loading that produces microdamage. Higher BMD is associated with active training or racing status and accumulated training and racing over time which may be due to the lower remodelling rates induced by training. PSB fracture may be due to higher rates of damage accumulation and lower rates of bone repair associated with intense cyclic loading. Detailed histomorphometry will be required to elucidate bone modelling and remodelling of PSBs in response to training.

Plenary Poster P12
Analyses of Tmem161a function in bone metabolism using the exchangeable gene trap mutagenesis show significant bone ingrowth

Takuya Nagai, Tomohisa Sekimoto, Syuji Kurogi, Taro Funamoto, Takuya Tajima
University of Miyazaki

We have constructed a knockout mice library with bone metabolism abnormality using the exchangeable gene trap mutagenesis to identify novel genes involved in bone metabolism. We describe the Transmembrane161a (Tmem161a) trap mice which have strong bone. In the past report, this gene may play a role in protection against oxidative stress, but there is no data in bone metabolism.

These mice were prepared by electroporating trap vector pU-21T into KTPU8 ES cells.

Trap vector was inserted in intron 1, we confirmed that Tmem161a was null by RT-PCR. Tmem161a expression in femur was observed in articular cartilage, proliferative zone of growth plate and osteoblasts by X-gal staining. Compared with wild type, bone structure of trap mice femur was dense in μCT and there were significant differences in bone morphometric analysis (bone mineral density, cortical bone analysis and trabecular bone analysis). In biomechanical strength analysis, trap mice indicated significant strength.

In HE staining, cortical bone thickness was thick and primary trabecular bone was also large. We performed realtime PCR on the mRNA extracted from the femur of 8 month mice and confirmed increased expression BMP4 and Runx2. Increased expression of Runx2 and Osterix and increased calcification were observed in primary osteoblast culture isolated 2-4 days mice calvaria. Similar results were obtained in cell lines knocked out Tmem161a by CRISPR/Cas9 system.

It is evident in Tmem161a knockout mice increased bone mass and strength. It seems that Tmem161a is involved in osteoblast function. There is a possibility that Tmem161a is a novel gene involved in bone metabolism, and we would like to apply it for drug discovery in the future.

Plenary Poster P13
The association of breastfeeding, maternal smoking, birth weight and maternal diet with bone density and microarchitecture in young adulthood: a 25-year

Yi Yang, Feitong Wu, Tania Winzenberg, Graeme Jones
Menzies Institute for Medical Research, University of Tasmania

We have previously shown that early life exposures are associated with bone mass assessed by densitometry at age 8 and 16. This study aimed to describe whether these associations persisted to young adulthood and extended to microarchitecture.

There were 201 participants followed from birth to age 25. Outcomes were areal BMD at the spine, hip and total body (by DXA) and trabecular and cortical bone measures (by High Resolution Peripheral Quantitative Computerised Tomography (HRpQCT)) at the radius and tibia. Exposures were breastfeeding status, maternal smoking, birth weight and diet (by Food Frequency Questionnaire) during the third trimester of pregnancy.

In participants born prematurely, there were beneficial associations between breastfeeding and volumetric BMD (vBMD), cortical porosity and trabecular microarchitecture at radius or tibia at age 25. Low birth weight was associated with better spine aBMD and trabecular measures in those born prematurely. Maternal meat intake during pregnancy was associated with higher total vBMD and lower cortical porosity. Maternal smoking and intake of other foods and of protein, fat, carbohydrate, calcium, magnesium and phosphorus were not associated with any bone measures. In participants born at term, the only associations present were those between smoking during pregnancy and suboptimal trabecular microarchitecture and higher cortical porosity.

There were long-term beneficial associations of breastfeeding, low birth weight, meat intake during pregnancy and vBMD as well as bone microarchitecture in participants born prematurely. Maternal smoking was an early life marker for suboptimal trabecular bone and porosity in those children born at term. This study suggests that early life exposures could have long-term effects bone development until the time of peak bone mass.

Table 1. Standardised coefficients for the association of breastfeeding and bone measures at age 25 in participants stratified by period of gestation [a]

	Born at term (n=138) β^b (95%CI)	Born prematurely (n=58) β^b (95%CI)	All participants (n=201) β^b (95%CI)
Areal BMD			
Spine(g/cm^2)	0.01 (-0.04, 0.05)	0.05 (-0.04, 0.14)	0.02 (-0.02, 0.05)
Hip(g/cm^2)	0.01 (-0.04, 0.06)	0.07 (-0.02, 0.15)	0.03 (-0.01, 0.07)
Total body(g/cm^2)	0.01 (-0.03, 0.04)	0.04 (-0.03, 0.10)	0.01 (-0.01, 0.04)
Volumetric density			
Radius			
Tt.vBMD(mg HA/cm^3)	-0.05 (-0.40, 0.29)	**0.84 (0.22, 1.46)**	0.14 (-0.16, 0.44)
Ct.vBMD (mg HA/cm^3)	0.05 (-0.30, 0.39)	**0.65 (0.01, 1.30)**	0.16 (-0.14, 0.46)
Tb.vBMD (mg HA/cm^3)	0.10 (-0.21, 0.41)	**0.58 (0.01, 1.14)**	0.23 (-0.03, 0.50)[c]
Tibia			
Tt.vBMD(mg HA/cm^3)	0.05 (-0.31, 0.40)	**0.73 (0.20, 1.26)**	0.19 (-0.10, 0.48)
Ct.vBMD (mg HA/cm^3)	0.02 (-0.32, 0.36)	**0.63 (0.05, 1.20)**	0.14 (-0.14, 0.43)
Tb.vBMD (mg HA/cm^3)	0.17 (-0.18, 0.52)	0.43 (-0.14, 1.00)	0.23 (-0.06, 0.52)
Microarchitecture			
Radius			
Porosity (%)			
Total cortex	-0.05 (-0.40, 0.29)	-0.65 (-1.30, 0.10)[c]	-0.16 (-0.46, 0.14)
Compact-appearing cortex	-0.06 (-0.38, 0.26)	-0.45 (-1.10, 0.19)	-0.12 (-0.40, 0.17)
Outer transitional zone	-0.07 (-0.42, 0.27)	-0.52 (-1.09, 0.05)[c]	-0.15 (-0.44, 0.14)
Inner transitional zone	0.04 (-0.29, 0.37)	**-0.71 (-1.31, 0.11)**	-0.14 (-0.43, 0.15)
Tb.N(mm^{-1})	0.04 (-0.29, 0.38)	0.46 (-0.07, 0.98)[c]	0.17 (-0.11, 0.45)
Tb.Th(mm)	0.07 (-0.23, 0.36)	0.45 (-0.19, 1.09)	0.17 (-0.10, 0.44)

Tb.Sp(mm)	-0.22 (-0.58, 0.13)	-0.42 (-0.89, 0.04)[c]	**-0.31 (-0.60, -0.03)**
Tb.BV/TV(%)	0.06 (-0.23, 0.35)	**0.61 (0.02, 1.19)**	0.20 (-0.06, 0.46)
Tibia			
Porosity (%)			
Total cortex	-0.02 (-0.37, 0.32)	**-0.61 (-1.19, -0.03)**	-0.14 (-0.43, 0.15)
Compact-appearing cortex	-0.03 (-0.35, 0.30)	-0.43 (-1.08, 0.23)	-0.11 (-0.39, 0.18)
Outer transitional zone	-0.02 (-0.35, 0.32)	-0.43 (-1.14, 0.29)	-0.09 (-0.39, 0.20)
Inner transitional zone	-0.19 (-0.55, 0.18)	**-0.66 (-1.23, -0.10)**	-0.29 (-0.59, 0.01)[c]
Tb.N(mm^{-1})	0.20 (-0.12, 0.52)	**0.73 (0.15, 1.31)**	**0. 31 (0.04, 0.58)**
Tb.Th(mm)	-0.02 (-0.36, 0.32)	-0.24 (-0.87, 0.39)	-0.05 (-0.34, 0.25)
Tb.Sp(mm)	-0.29 (-0.63, 0.05)[c]	-0.49 (-1.09, 0.11)	**-0.33 (-0.62, -0.04)**
Tb.BV/TV(%)	0.14 (-0.20, 0.49)	0.42 (-0.13, 0.98)	0.21 (-0.08, 0.49)

Tt.vBMD, total volumetric bone density; Ct.vBMD, cortical volumetric bone density; Tb.vBMD, trabecular volumetric bone density; Ct.Th, cortical thickness; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Tb.BV/TV, trabecular bone volume fraction. HA:hydroxyapatite. BMD:bone mineral density. β: coefficient; 95%CI: 95% confidence interval.
Bold denotes statistical significance.
[a] Adjusted for sex, weight, height. [b] standardised coefficients
[c] $p<0.1$

Table 2. Standardised coefficients for the association between early life factors and bone measures at age 25 in participants [a]

	Smoking in utero[b]		Birth weight [d]	
	Born at term β (95%CI) (n=138)	Born prematurely β (95%CI) (n=58)	Born at term β (95%CI) (n=138)	Born prematurely β (95%CI) (n=58)
Areal BMD				
Spine(g/cm^2)	-0.02 (-0.06, 0.02)	0.04 (-0.03, 0.11)	0.02 (-0.03, 0.08)	**0.08 (0.01, 0.14)**
Hip(g/cm^2)	-0.03 (-0.07, 0.02)	0.02 (-0.05, 0.08)	0.02 (-0.04, 0.08)	0.01 (-0.06, 0.08)
Total body(g/cm^2)	-0.01 (-0.05, 0.02)	0.02 (-0.03, 0.06)	0.01 (-0.04, 0.05)	0.04 (-0.01, 0.09)[c]
Volumetric density				
Radius				
Tt.vBMD(mg HA/cm^3)	-0.08 (-0.41, 0.26)	0.03 (-0.48, 0.54)	-0.06 (-0.53, 0.41)	0.25 (-0.27, 0.76)
Ct.vBMD (mg HA/cm^3)	-0.02 (-0.35, 0.32)	0.09 (-0.43, 0.61)	-0.04 (-0.51, 0.44)	0.16 (-0.36, 0.69)
Tb.vBMD (mg HA/cm^3)	-0.24 (-0.55, 0.07)	0.01 (-0.44, 0.46)	0.09 (-0.35, 0.52)	0.41 -(0.04, 0.85)[c]
Tibia				
Tt.vBMD(mg HA/cm^3)	-0.26 (-0.60, 0.08)	-0.16 (-0.60, 0.28)	0.36 (-0.13, 0.84)	0.33 (-0.10, 0.76)
Ct.vBMD (mg HA/cm^3)	-0.21(-0.54, 0.13)	-0.15 (-0.61, 0.32)	0.36 (-0.10, 0.83)	0.15 (-0.31, 0.62)
Tb.vBMD (mg HA/cm^3)	**-0.37 (-0.71, -0.03)**	0.03 (-0.43, 0.48)	0.09 (-0.39, 0.57)	**0.51 (0.08, 0.95)**
Microarchitecture				
Radius				
Porosity (%)				
Total cortex	0.01 (-0.33, 0.35)	-0.09 (-0.61, 0.44)	0.06 (-0.41, 0.54)	-0.16 (-0.67, 0.35)
Compact-appearing cortex	0.12 (-0.20, 0.43)	-0.09 (-0.60, 0.42)	-0.02 (-0.47, 0.42)	-0.20 (-0.65, 0.26)
Outer transitional zone	0.22 (-0.12, 0.55)	-0.14 (-0.59, 0.31)	-0.05 (-0.53, 0.42)	-0.39 (-0.87, 0.09)
Inner transitional zone	0.24 (-0.10, 0.56)	-0.18 (-0.66, 0.31)	0.02 (-0.44, 0.48)	-0.47 (-0.97, -0.03)[c]
Tb.N(mm^{-1})	**-0.36 (-0.68, -0.04)**	-0.04 (-0.46, -0.38)	-0.05 (-0.51, 0.41)	0.25 (-0.17, 0.66)
Tb.Th(mm)	-0.06 (-0.35, 0.24)	0.14 (-0.36, 0.64)	0.25 (-0.16, 0.67)	0.25 (-0.25, 0.76)
Tb.Sp(mm)	**0.38 (0.04, 0.72)**	0.07 (-0.30, 0.44)	-0.13 (-0.61, 0.35)	-0.35 (-0.71,0.01)[c]
Tb.BV/TV(%)	-0.19 (-0.48, 0.10)	0.04 (-0.43, 0.50)	0.06 (-0.35, 0.47)	0.37 (-0.10, 0.83)
Tibia				
Porosity (%)				
Total cortex	0.20 (-0.13, 0.54)	0.15 (-0.32, 0.61)	-0.35 (-0.82, 0.12)	-0.13 (-0.60, 0.35)
Compact-appearing cortex	0.17 (-0.15, 0.48)	0.36 (-0.15, 0.87)	-0.36 (-0.81, 0.08)	-0.17 (-0.69, 0.34)
Outer transitional zone	0.22 (-0.10, 0.55)	0.12 (-0.44, 0.68)	-0.33 (-0.79, 0.12)	-0.15 (-0.72, 0.41)
Inner transitional zone	**0.43 (0.07, 0.78)**	0.15 (-0.31, 0.61)	-0.24 (-0.75, 0.27)	-0.41 (-0.86, 0.04)[c]

Tb.N(mm^{-1})	-0.24 (-0.57, 0.08)	-0.03 (-0.50, 0.44)	0.18 (-0.28, 0.63)	0.25 (-0.23, 0.72)
Tb.Th(mm)	-0.14 (-0.47, 0.20)	0.11 (-0.38, 0.60)	0.01 (-0.47, 0.48)	0.20 (-0.30, 0.69)
Tb.Sp(mm)	0.33 (-0.01, 0.66)[c]	-0.05 (-0.52, 0.43)	-0.08 (-0.55, 0.40)	**-0.47 (-0.93,-0.01)**
Tb.BV/TV(%)	**-0.38 (-0.71, -0.05)**	0.03 (-0.41, 0.47)	0.16 (-0.31, 0.64)	0.39 (-0.04, 0.82)

Tt.vBMD, total volumetric bone density; Ct.vBMD, cortical volumetric bone density; Tb.vBMD, trabecular volumetric bone density; Ct.Th, cortical thickness; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Tb.BV/TV, trabecular bone volume fraction. HA:hydroxyapatite. BMD:bone mineral density. β: coefficient; 95%CI: 95% confidence interval.
Bold denotes statistical significance.
[a] Adjusted for sex, weight, height; [b] yes *vs.* no during any trimester;
[c] $p<0.1$
[d] Birth weight <=2500g *vs.* >2500g (the reference group).

Association of maternal nutrient intake during the third trimester of pregnancy and bone measures at age 25 :
Protein, Fat density, Carbohydrate, Calcium, Magnesium, Phosphorus
Food intake: Meat, Fish, vegetable, milk

I did the analysis of the former maternal nutrients and food intake with bone measures in young participants. However, there only meat intake was associated with bone measures. As time is not enough for me, I did not list the result as a table.

Plenary Poster P14
Microstructural Decay in Spinal Cord Injury

Ali Ghasem-Zadeh, Andrew Nunn, Maya Panisset, Xaio-Fang Wang, Roger Zebaze, Mary P.Galea
University of Melbourne

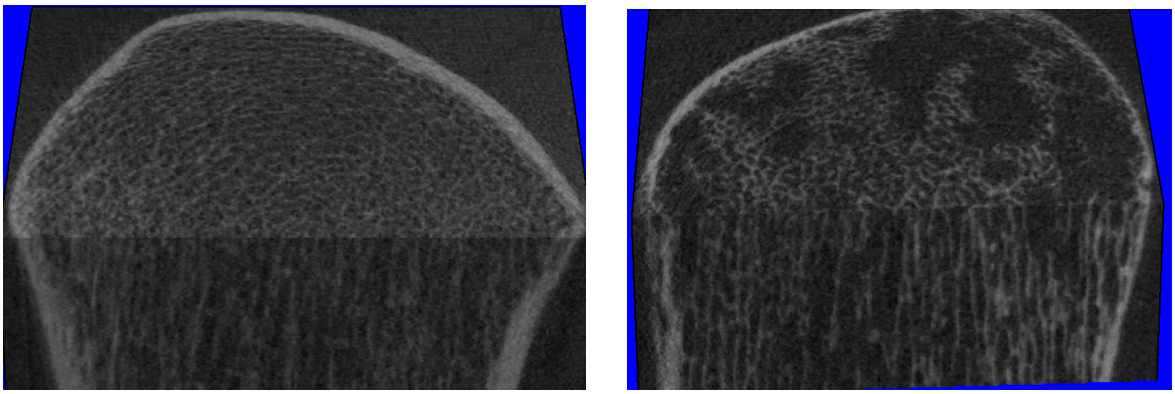
Background: Spinal cord injury (SCI) causes rapid bone loss due to a reduction in bone formation at the basic cellular unit (BMU) level and increased rate of bone remodelling at the surface level, changes that result in microstructural deterioration and increased fracture risk. There is lack of information concerning the effects of paralysis on bone microstructure. We hypothesised that SCI individuals have i) a severe trabecular bone microstructural deterioration ii) higher cortical porosity in comparison to controls.

Methods: We studied 31men with chronic complete SCI (age 43.5±14.2 yrs, duration of paralysis of 1.7-22 yrs), and 90 age and sex-matched healthy ambulatory controls, recruited at Austin Health, University of Melbourne. Images of the non-dominant distal tibia were obtained using high-resolution quantitative computed tomography (HR-pQCT, Scanco, 82 micron isotropic voxel size). Manufacturer's and StrAx1.0 (StraxCorp, Melbourne, Australia) software were used to quantify trabecular and cortical compartments indices.

Results: Compared with controls, SCI cases had 2.3, 1.8, 1.7 and 2.5 SD higher porosity in the total cortex, compact cortex, inner and outer transitional zones and 1.7SD lower matrix mineralisation density. Total and cortical vBMD were reduced by 2.4 and 1.7 SD, respectively (all $p<0.01$).

Trabecular bone volume fraction was 2.4SD lower in cases due to1.4 SD lower number of trabeculae and 6 SD higher separation. Trabecular bone surface and connectivity density were decreased by 0.9 and 1.4 SD, respectively (all $p<0.01$).

Conclusion: We infer that spinal paralysis produces profound and rapid loss of cortical and trabecular bone suggesting antiresorptive therapy should be commenced at the time of presentation.



HR-pQCT axial images of non- dominant distal tibia 27 years old men. Healthy participant (Left), patient with Spinal cord injury (right)- 2 years after injury. Thinner cortex , higher cortical porosity and fewer trabeculae and higher trabecular bone pattern inhomogeneity on the on Spinal cords injury patient.

Plenary Poster P15
Pharmacological effect of PTH against skeletal pain involves changes in neuronal gene transcription in ovariectomized rats

Tomoya Tanaka 1, Ryoko Takao-Kawabata 2, Tadahiro Iimura 1
1. Ehime University
2. Asahi Kasei Pharma Corporation

Clinical studies reported that teriparatide (TPTD), a human parathyroid hormone analog, significantly reduced back pain in osteoporotic patients. However, pharmacological action of TPTD on neuronal system remains elusive. This study investigated the pain-relieving effect of TPTD on primary sensory neurons in ovariectomized (OVX) rats.

Female SD rats of 12-weeks old were subjected to OVX or sham surgery. Four weeks after surgery, saline or TPTD at a dose of 30 μg/kg body weight were subcutaneously injected to the specimens on 3 times weekly for 4 weeks.

We first conducted two behavioral tests to observe the pain-relieving effect of TPTD on the OVX rats. The plantar test showed that the OVX rats gradually reduced the withdrawal latency over the time course of observation, indicating thermal hyperalgesia caused by OVX. The TPTD-treated OVX group significantly recovered the reduced latency on the initial administration and later days, compared to the vehicle-treated OVX group. The von Frey test also showed that the withdrawal threshold in the TPTD-treated OVX group was significantly higher than that in the vehicle-treated OVX group at 4 weeks after the initial administration.

To investigate the molecular basis, we next conducted RNA sequencing of the dorsal root ganglia (DRG) collected from the rats in these experimental groups. This analysis demonstrated that changes in transcriptomal profile of pain-related genes were involved in the pharmacological effect of TPTD.

We further assessed whether primary sensory neurons were direct targets of TPTD. Immunofluorescence demonstrated that most neurons in DRG expressed substantial levels of PTH1R. TPTD treatment dose-dependently activated a PTH receptor pathway in cultured neuronal cells obtained from DRG, suggesting direct effect of TPTD on primary sensory neurons. Collectively, our findings suggest that TPTD targets neuronal cells as well as bone cells to achieve its pharmacological action.

Plenary Poster P16
Compounds Extracted from Dragon's Blood Resin Inhibit RANKL-Induced Osteoclast Formation and Function and Suppress Estrogen Deficiency-Induced Osteoporosis in Mice

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Dragon's Blood resin is an ancient herbal remedy used for a range of medicinal purposes. It contains many active compounds that have demonstrated antioxidant and anti-inflammatory activity; however, their effects on osteoclast formation and osteoporosis have not been previously determined. Here we have investigated the in vitro and in vivo effects of compounds isolated from Dragon's Blood resin on osteoclast differentiation and osteoclast-mediated bone loss.

Six compounds (Loureirin [Lr] A, LrB, LrC, LrD, Cochinchinenin[Cc] A and CcC) extracted from Dragon's Blood resin were tested in mouse bone marrow RANKL and M-CSF-induced osteoclastogenesis assays. LrB was found to have the strongest inhibitory effect on osteoclast formation among these six compounds with similar molecular structures. LrB dose-dependently inhibited RANKL-induced osteoclast formation and bone-resorption function, and also suppressed actin ring formation and ROS production. The expression of osteoclastic genes including Acp 5 (TRAcP), Atp6v0d2 (V-ATPase-d2), Ctsk, Mmp9 and Ctr (Calcitonin Receptor), and some associated proteins products including V-ATPase-d2 and CTSK, were also reduced. LrB significantly abrogated p38 phosphorylation in the p38/MAPK signaling pathway, and also blocked IkB-α degradation in the NF-κB signaling pathway; but had little effect on ERK and JNK phosphorylation in the ERK/MAPK and JNK/MAPK signaling pathways. Furthermore, LrB was able to inhibit the expression of NFATc1 protein and RANKL-stimulated calcium oscillation. In vivo assessment of LrB efficacy in an ovariectomised (OVX) mouse model revealed that LrB treatment protected against OVX-induced bone loss, with treated mice demonstrating increased bone volume and trabecular number.

These findings suggest that Dragon's Blood resin containing LrB might be a promising agent to treat osteoporosis.

Plenary Poster P17
Role of individual components of sarcopenia in fracture risk prediction in elderly women and men

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The relationship between sarcopenia and fracture is controversial. We aimed to assess the contribution of individual components of sarcopenia (muscle mass, strength and function) to fracture risk prediction in elderly over and above BMD and clinical risk predictors.

The study involved 683 women and 330 men aged 60+ years from the ongoing Dubbo Osteoporosis Epidemiology Study, who had a total body BMD scan done. The incidence of fracture was ascertained by X-ray report between 2000 and 2017. Clinical data, lean muscle mass (MM), BMD, quadriceps strength (QS), gait speed (GS), sit-to-stand (STS), functional reach (FR) and get-up-go (GUG) were measured biannually. The worst quintile of the test result was used to operationally define poor performance. Multivariable Cox proportional hazards models including age, height, weight, prior fracture, falls and co-morbidities were used to quantify the association between individual components of sarcopenia and fracture risk.

There were 201 incident fractures over 6,487 person-years of follow-up in women, and 56 fractures over 3,003 person-years in men, yielding incidence rates of 31/1,000 person-years (95% CI: 27-36), and 19/1,000 person-years (14-24) in women and men, respectively. In women, none of the sarcopenia components independently contributed to fracture risk in multivariable analysis, possibly due to effect of BMD which contributed more to fracture risk in women than men. By contrast, in men, the worst quintile of STS, GUG and GS was associated with a 2-3-fold increased fracture risk over and above the other risk factors [Hazard ratios 2.67 (95% CI; 1.34–5.33), 3.15 (1.45–6.83), and 3.33 (1.63–6.80), respectively]. FR, MM and QS did not contribute to fracture risk.

In conclusion, in men but not women, impaired functional muscle performance tests, including sit-to-stand, get-up-go and gait speed were associated with increased fracture risk, suggesting potential targets for therapeutic intervention.

Plenary Poster P18

Tibia cartilage thickness and subchondral bone microarchitecture: varus- and valgus-aligned osteoarthritic knees vs. controls

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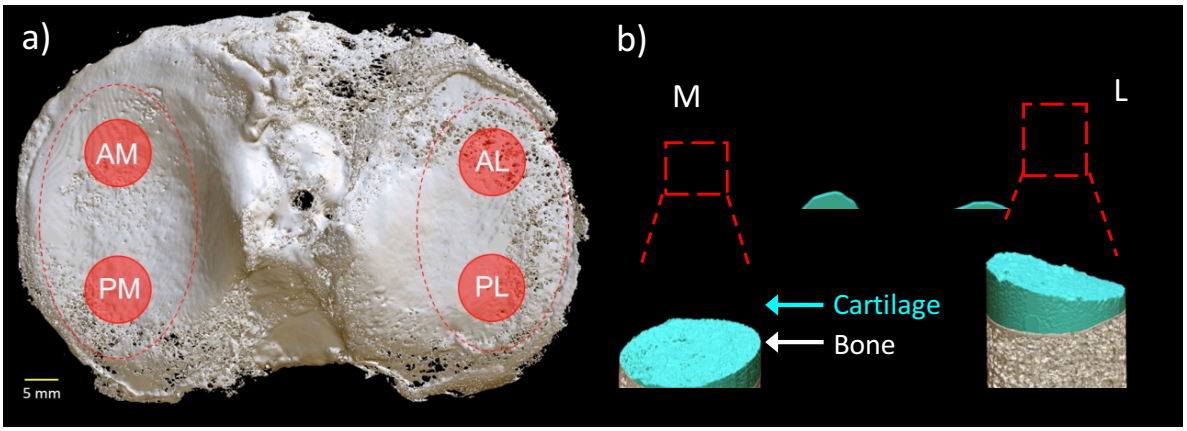
Introduction: In knee osteoarthritis (OA), subregional changes in proximal tibia subchondral trabecular bone (STB) microarchitecture and cartilage may reflect joint loading. However, reports are conflicting. Moreover, the effect of joint alignment remains unexplored. This early analysis in an ongoing study quantifies tibia cartilage and STB of end-stage knee-OA patients with varus- or valgus-aligned joints, compared to control (non-OA) knees.

Methods: Tibial plateaus from 24 knee-OA patients (69±7years, 89±16kg; varus-aligned (n=17), valgus-aligned (n=7); knee arthroplasty) and 15 cadavers (62±13years, 83±16kg; controls) were micro-CT scanned (17µm/pixel). OA joint alignment was classified radiographically. Cartilage thickness (Cg.Th), STB bone volume fraction (BV/TV) and corresponding medial-to-lateral (M:L) ratios were explored in four subregions (anteromedial (AM), anterolateral (AL), posteromedial (PM) and posterolateral (PL) condyles). Varus-OA and valgus-OA groups were compared to controls in these parameters (Bonferroni-adjusted Mann-Whitney U-tests).

Results: Compared to controls, varus-OA exhibited significantly lower Cg.Th in AM ROI (-62%, $p<0.05$), but higher laterally (up to +66%); in valgus-OA it was higher medially (+56% in PM ROI, $p<0.05$). Whereas in controls the Cg.Th M:L ratios were close to unity (range: 0.8-1.1), in varus-OA they were all below (0.2-0.6, $p<0.05$) and in valgus-OA similar to or higher than in controls (up to +40%).

Compared to controls, BV/TV in varus-OA was significantly higher (up to +49%) medially (AM, PM), whereas in valgus-OA it was higher laterally (up to +76%, AL, $p<0.05$). In varus-OA, all M:L BV/TV ratios ranged between 1.6-2.4, whereas in controls between 0.9-2.1. In valgus-OA, they were lower (up to -47%, AM:AL and PM:AL, $p<0.05$) than in controls and closer to unity (0.8-1.1).

Conclusions: OA and non-OA tibias differ significantly in Cg.Th and STB microarchitecture depending on joint alignment, suggesting that joint structural changes in OA reflect medial-to-lateral load distribution differences upon the tibial plateau. This may contribute to improve our understanding of the disease.



Micro-CT 3D rendering; (a) superior and (b) coronal view of tibial plateau, showing positions of the 4 ROIs examined.

Plenary Poster P19
Irradiation enriches for engraftment of hematopoietic progenitors but not osteoprogenitors in a mouse model of Osteogenesis Imperfecta

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Background: Osteogenesis imperfecta (OI) is a genetic bone fragility disorder. To treat OI, preclinical and clinical trials have aimed to use bone marrow transplantation (BMT) to engraft donor cells able to produce normal collagen. We speculated that marrow ablation via irradiation could enhance progenitor engraftment following BMT in a mouse model of dominant OI (Col1a2G610C).

Methods: Wild type (WT) and Col1a2G610C (OI) mice were exposed to 0 or 600 cGy of radiation and received whole marrow transplants by tail vein injection. Unlike prior studies, OI donor cells were used as an additional control. Dual-emission x-ray absorptiometry (DEXA), microCT, and biomechanical testing were used to measure BV, BMD, and strength. In a parallel cell tracking study, donor marrow was taken from mice constitutively expressing tdTomato. The osteopoietic versus hematopoietic fate of donor cells was examined using fluorescent stains for alkaline phosphatase (AP) and tartrate-resistant acid phosphatase (TRAP) activity.

Results: At week 3, analysis of BV and BMD showed no difference between BMT with and without irradiation versus untreated controls for both genotypes. Notably, prior clinical reports of engraftment autonomous improvements in bone quality were not seen with either wild type or OI donor cells. These metrics did show significant decreases in Col1a2G610C bone compared to wild type controls. In lineage tracking studies, irradiation considerably enhanced engraftment of tdTomato+ cells. However, tdTomato+ cells predominantly expressed TRAP and not AP, indicating engrafted donor cells were chiefly from the hematopoietic lineages.

Conclusion: This study represents an optimal approach to testing cell-based therapies with cell tracking and functional outcomes. Transplantation of mutant cells enables separation of the benefits from engraftment versus those associated with an acute inflammatory response produced by cell injection. Nevertheless, these data highlight that irradiation does not ablate the osteopoietic compartment and is thus unsuitable for enhancing bone cell therapies.

Plenary Poster P20
Correlative Imaging of Structural and Molecular Transport Compartmentalisation in an In Vivo Osteoarthritis Model

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Osteoarthritis is the most prevalent cause disability in aging adults, yet its underlying mechanisms remain poorly understood. Complex interactions between tissues of the entire joint underpin multifactorial processes of structure and function degeneration. Multiscale mechanobiology and the interfacing circulatory system facilitate molecular transport between musculoskeletal compartments. Understanding age and disease-related transport disruptions may have important implications for deciphering osteoarthritis aetiology and pathogenesis. Multimodal imaging facilitates exploration of such interactions across lengths-scales. This study probes transport through joints dependent on molecular-size-selectivity and functional barrier properties of boundary-membranes.

Dunkin-Hartley guinea pigs (DHGP), an animal model for spontaneous, age-related knee osteoarthritis, exhibit pathology comparable to humans; extending to the molecular level1-4. A mixed-bolus of neutral-charged fluorescent-tagged dextrans, 70kDa (red-tracer) and 10kDa (green-tracer), was injected via the heart into two anaesthetized cohorts of 8-10-month and 17-19-month animals. Young and old animals correspond to middle-aged5 and aged humans4, respectively. After five minutes' circulation, animals were euthanized, and knees resected. Left knees were cryofixed, followed by serial-episcopic blockface fluorescence imaging (BioInVision CryoViz, USA). Contralateral specimens were imaged using confocal fluorescence microscopy (Leica TCS SP5 II, Germany) following paraformaldehyde-fixation and polymethylmethacrylate-embedding.

Episcopic imaging showed size-based tracer separation between compartments. Younger joints exhibited significantly greater green-tracer concentrations. Tracer was not observed in boundary tissues i.e. growth plate, periosteum and cartilage.

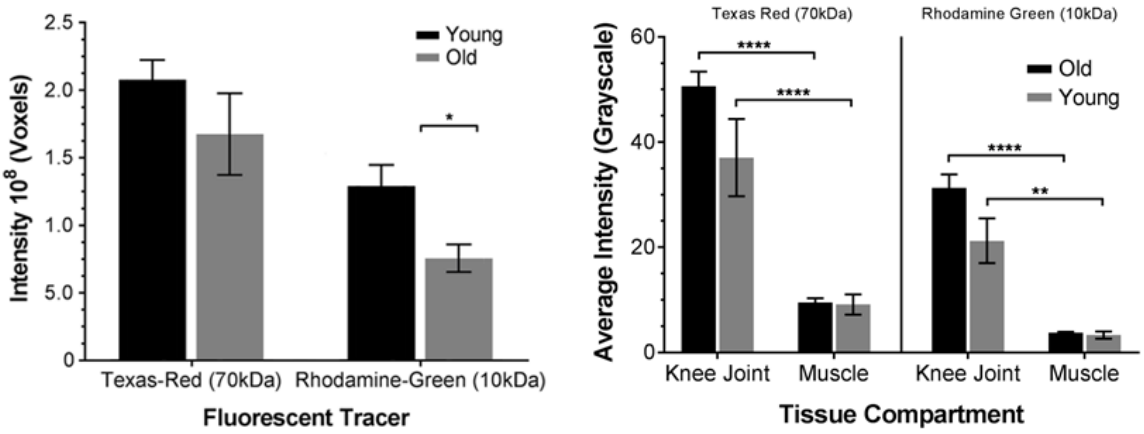

Figure 1. A. Voxel quantification of red and green-tracers demonstrate that older animals exhibit low tracer concentrations while younger animals exhibit significantly higher concentrations. B. Average intensity of red and green-tracers demonstrate significant differences in tracer attributable to tissue type as well as animal age. Muscle exhibited significantly less red and green-tracer than did tissues of the knee joint for both younger ($P<0.0001$ and $P<0.0001$, respectively) and older groups ($P<0.0001$ and $P< 0.01$, respectively).

Confocal microscopy enabled high-resolution structural and functional imaging i.e. trabecular surface osteoclastic activity. It confirmed the presence of red-tracer in marrow and revealed small, round red-tracer aggregations distributed in close association to marrow vasculature (Fig 2B). It also confirmed macromolecular transport across the cartilage surface and subchondral plate, with strongest signal in cartilage-traversing channels. (Fig 2C,D).

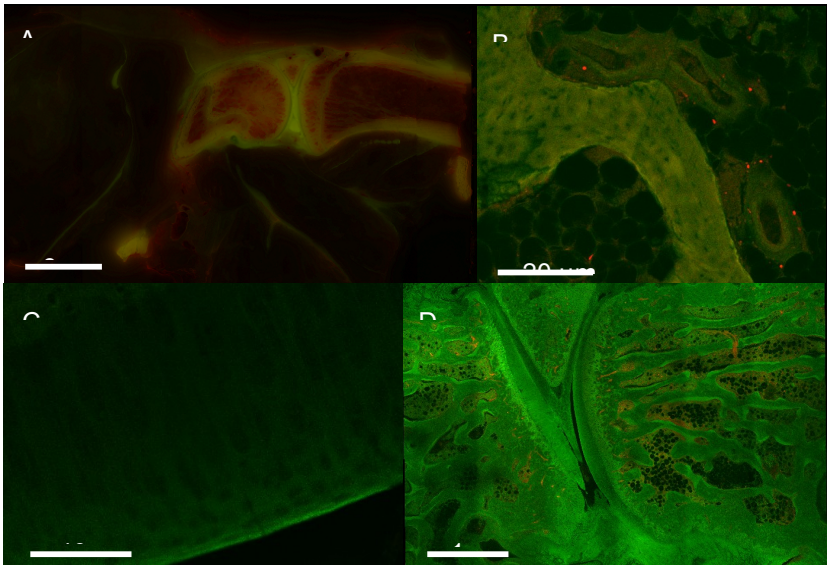

Figure 2. A. Episcopic blockface image showing muscle tissue showed significantly less tracer than other tissues (*$p < 0.01$, Fig 1B, arrowheads), with red and green fluorescence limited to bounding fasciae. B,C,D. Confocal images illustrating round aggregations of red-tracer, transport channels across cartilage and tracer delineation of tissues, respectively.

Implicated is the role of molecular communication as a potential factor in osteoarthritis pathophysiology. A greater understanding may aid to slow joint degradation and modulate joint tissue health by harnessing spatiotemporal transport

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Poster Presentations

Poster P21
Bisphosphonate-related osteonecrosis of the jaw-like lesion is prevalently induced in a chemotherapeutic dose-dependent manner in mice

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Aims: Cyclophosphamide is used to treat multiple myeloma. Intravenous bisphosphonates are used to reduce skeletal-related events such as pathological vertebral fractures and bone pain. The aim of this study was to investigate the effects of cyclophosphamide (chemotherapeutic drug) monotherapy and cyclophosphamide/bisphosphonate combination therapy on tooth extraction socket healing in mice.

Methods: Low-dose cyclophosphamide (CY) (50 mg/kg; CY-L), moderate-dose CY (100 mg/kg; CY-M), high-dose CY (150 mg/kg; CY-H), and bisphosphonate [Zometa (ZA): 0.05 mg/kg] were administered for 7 weeks. Each dose of CY and ZA in combination was also administered for 7 weeks. Both maxillary first molars were extracted at 3 weeks post-drug administration. Euthanasia was performed at 4 weeks after tooth extraction. Gross wound healing, microCT analysis, histomorphometry, and immunohistochemistry were used to qualitatively evaluate osseous and soft tissue wound healing of tooth extraction sockets.

Results: CY monotherapy rarely induced open wounds, although delayed osseous wound healing occurred in a CY dose-dependent manner. Interestingly, ZA monotherapy did not induce BRONJ-like lesions in mice. In contrast, CY/ZA combination therapy prevalently induced BRONJ-like lesions with impaired osseous healing and compromised soft tissue healing in a CY dose-dependent manner. An anti-angiogenesis was noted regardless of CY dose and ZA administration, even though only CY-M/ZA and CY-H/ZA combination therapies induced BRONJ-like lesions.

Discussion: Our findings suggest that high-dose CY could become a risk factor for the development of BRONJ following tooth extraction only when CY is used together with ZA. In addition to anti-angiogenesis, other factors may contribute to the pathoetiology of BORNJ. Caution should be taken when BRONJ model is created and evaluated using rodents.

Poster P22
Effect of mechanical loading on bone around threaded implants in rat maxillae

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Aim: Dental implant treatment has been widely applied. However, the effects of mechanical loading via implants on bone around implants are not clear. The aim of this study was to investigate the effects of mechanical loading on bone quality based on bone cells and the orientation of collagen fibers in rat maxillae.

Methods: Female wistar rats were used. Threaded implants were placed at 3 weeks after tooth extraction of both maxillary first molars. Repetitive mechanical load was applied via random selected site of implants for 5 weeks (3Hz, 1800 cycles, twice a week). No load was applied on the remaining site (n=7/group). Micro computed tomography, histomorphometry, and immunohistochemical analyses were performed. Preferential alignment of collagen fibers was also analyzed using birefringence measurement system. Moreover, quantitative polymerase chain reaction was conducted using bone samples at 30 minutes after the onset of mechanical load.

Results: Bone mass around implants were the same, irrespective of mechanical loads. In cancellous bone, both numbers of osteocyte and osteoblast were significantly increased inside and outside of the implant threads. Osteoclasts increased only outside of the threads. Mechanical load enhanced several bone cell-related genes. Mechanical load significantly increased Semaphorin3A, but not Sclerostin and Periostin. Moreover, mechanical load induced the preferential alignment of collagen fibers inside and outside the threads.

Conclusion: Mechanical repetitive load via implants influenced bone cells and collagen alignment both inside and outside of the implant threads. These findings suggest that mechanical load adapts bone quality around implants. Semaphorin3A may contribute to controlling bone quality around implants under loaded conditions.

Poster P23
Marrow Adipose Tissue drives Bone and Red Marrow Volume Decline in ageing Men

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Background: Negative correlation between marrow adipose tissue (MAT) and bone volume (BV/TV) is known. However, the question remains whether due to highly lipotoxic properties, MAT plays an active role in bone loss, or simply marrow fills the voids created by bone resorption. We hypothesise that, unlike red marrow (RM), MAT drives bone resorption and hence is negatively associated with both bone and red marrow volumes.

Materials and methods: Cross-sectional study in healthy men (n=96; mean age±SD=67.5±5.5) imaged by single energy computed tomography (CT) and DXA at hip. Using CT images BV/TV, MAT and RM within left and right hip marrows [neck and trochanter] were quantified. Image analyses were carried out using Slice-O-Matic® software.

Results: All femoral regions showed a strong negative correlation between MAT% and BV/TV (R=-0.836 to -0.972, $p<0.001$). There was also a consistent negative association between MAT vs. BMD of femoral neck and total hip (R=-0.274 to -0.370, p=0.115 to 0.030). Unlike MAT, RM did not show any association with age (R=-0.135 to 0.050, p=0.194 to 0.780) or BV/TV (R=-0.13 to 0.004, p=0.087 to 0.966). With increasing MAT, RM volume slightly decreased in all ROIs, but the associations reached significance only in right trochanter ROI (r=-0.221, p=0.033). MAT as a fraction of total marrow [MAT/(MAT+red marrow)] was also negatively associated with bone marrow in all ROIs (r=-0.811 to -0.874; $p<0.001$).

Conclusion: With increasing MAT% both BV/TV% and RM% regressed. Evidence suggests that age-associated fat relocation into bone marrow and its metabolites such as palmitic acid and inflammatory cytokines can create a severe lipotoxic and anti-osteoblastogenic and anti-hematopoietic environment. Based on the above we conclude that MAT plays an important role in the bone loss observed with aging in older men.

Poster P24
Distal Radius Bone Microarchitecture: what happens between age 25 and old age?

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Purpose: The aim of this study was to describe differences in bone geometry, volumetric bone mineral density (vBMD) and microarchitecture parameters at the distal radius between older and young adults.

Methods: Bone geometry, trabecular and cortical parameters and vBMD at distal radius were collected using high-resolution peripheral computed tomography (HRpQCT) and analysed using StrAx in 201 participants from the prospective Tasmanian Older Adult Cohort study (mean age 72.2 years, range 61.9-89.4 years, female 47%) and 196 participants from the T-bone study (mean age 25.5 years, range 24.1-27.6 years, female 38%). Unpaired t-tests were used to compare means.

Results: Older adults had larger cross-sectional area of the transitional zone (outer 30.96mm2 vs. 28.38mm2, inner 36.34mm2 vs. 32.93mm2) and thicker transitional zone bone area (outer 0.57mm vs. 0.54mm, inner 0.71mm vs. 0.65mm) compared to young adults. In addition, the prevalence of cortical porosity (54% vs. 49%) and porosity of the transitional zone (outer 42% vs. 37%, inner 82% vs. 81%) were higher in older adults than in young adults. vBMD of total bone area (370.73 mg hydroxyapatite (HA)/cm3 vs. 424.57 mg HA/cm3), vBMD of transitional zone (outer 899.29 mg HA/cm3 vs. 959.52 mg HA/cm3, inner 385.47 mg HA/cm3 vs. 408.34 mg HA/cm3), cortical and trabecular vBMD (734.51 mg HA/cm3 vs. 801.04 mg HA/cm3, 144.19 mg HA/cm3 vs. 178.02 mg HA/cm3) were all significantly lower in older adults. There were no significant differences between older and young adults in total, cortical, and trabecular cross-sectional area or cortical and trabecular thickness.

Conclusion: Compared to young adults at the time of peak bone mass, older adults have an increase in transitional zone bone size, decreased vBMD measures and increased prevalence of porosity, with the most significant differences in the compact cortex.

Poster P25
Vitamin D levels in adolescence is an independent predictor of bone mineral density and microarchitecture in early adulthood

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Menzies Institute for Medical Research

This prospective study aimed to describe the association of serum vitamin D levels at different stages of growth with bone measures in adolescence and early adulthood.

Four hundred and fifteen participants were followed from age 8 to 16 and 201 of them followed to age 25. Areal bone mineral density (BMD) at the spine, hip and total body (by DXA) was measured at age of 16 and 25, and trabecular and cortical bone measures at radius and tibia measured at age 25 (by High Resolution Peripheral Quantitative Computerised Tomography). Serum 25-hydroxyvitamin D (25OHD) levels were measured at age 8, 16 and 25. Multivariable linear regression was used to analyse the association of 25OHD levels at age 8, 16 and 25 with bone measures at age 16 and 25.

There were weak to moderate correlations between 25OHD levels at each age (correlation coefficients=0.32 to 0.45). Vitamin D at age 8 had no significant association with any bone measures at age 16 or 25 after adjustment for age, sex, body size and season. Serum 25OHD levels at age 16 were significantly associated with higher BMD at all sites at age 16 and 25 except for spine BMD at age 25. They were also associated with decreased radial porosity and better trabecular microarchitecture with higher density, increased trabecular number and reduced separation at radius and tibia at age 25. 25OHD at age 25 was only significantly associated with hip BMD and tibial trabecular number at age 25.

Vitamin D levels in adolescence, to a limited extent at age 25 but not in earlier childhood has beneficial associations with BMD and bone microarchitecture in early adulthood. Optimising vitamin D particularly during adolescence should be a priority.

Poster P26
Effect of local PTH administration on maxillary bone around implants in osteoporotic rats.

Inaba Nao

Objectives: Intermittent administration of parathyroid hormone (PTH) has been demonstrated to promote osseous healing after tooth extraction. However, the effects of local administration of PTH on bone around implants are not clear. The aim of this study was to investigate the effect of systemic and local administration of PTH on maxillary bone around implants in osteoporotic rats.

Materials and Methods: Female Wistar rats (8 weeks) were used. All rats were bilaterally ovariectomized to induce osteoporosis. Both maxillary first molars were extracted at 8 weeks after ovariectomy. Implants were placed at 3weeks post-extraction. Intermittent PTH administration was systemically and locally performed just after the implant placement for 2 weeks, and then euthanized. Saline was used as control (VC). Maxillae and long bones were dissected. cDNA was created using maxillary bone around implants. Micro computed tomography and quantitative polymerase chain reaction were performed.

Results: Both local and systemic PTH injection for 2 weeks significantly increased long bone volume fraction (BVF), trabecular thickness (Tb.Th), trabecular number (Tb.N) and bone mineral density (BMD), and decreased trabecular separation (Tb.Sp) when compared with VC. Moreover, PTH therapy significantly increased BVF, Tb.Th, Tb.N and BMD, and decreased Tb.Sp in bone around implants, regardless of administration routes. However, relative expressions of bone-related genes were significantly different between systemic and local PTH therapy.

Conclusion: Local and systemic PTH administration has the same effect on long bone structures. It has been demonstrated that PTH therapy is effective to improve osseous wound around implants, regardless of administration routes. Different gene expressions in bone around implants between systemic and local PTH administration may contribute to the development of a novel approach of PTH therapy in implant dentistry.

Poster P27
Aortic calcification is associated with accelerated five-year decline in muscular strength in older Australian women

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2. Edith Cowan University
3. Institute for Aging Research, Beth Israel Deaconess Medical Center and Harvard M

Objective: Determine the association between AAC and neuromuscular function over five years.

Participents: Ambulant women over 70 years old residing in Perth, Western Australia who participated in a randomised controlled trial of calcium supplementation against placebo on fracture outcomes. One thousand and forty-six women [mean age=74.9 ±2.6 years; BMI=27.1 ±4.4 kg/m2] were included.

Measurements: Lateral spine images captured during bone density testing were scored for AAC [AAC24; 0-24] at baseline. Severe AAC [AACsev] was defined using established cut points [AAC24>5]. At baseline and follow-up, isometric grip strength was assessed using a dynamometer. Mobility was assessed by the Timed-Up-and-Go (TUG) test. Using pre-defined criteria, muscle weakness was considered as grip strength <22kg and poor mobility defined as TUG>10.2s. A subset of women had appendicular lean mass [ALM] determined by dual-energy x-ray absorptiometry at baseline and follow-up [n=261].

Results: AACsev was evident in 193 [18.5%] women. Average decline in grip strength after five years was greater in those with AACsev than those without (3.6 ±3.7 vs. 2.9 ±4.2kg; p=0.034). This remained significant after adjustment for age, treatment allocation, diabetes, smoking history, renal function, medical record derived prevalent vascular disease, BMI and physical activity [β=-0.184; 95% confidence interval: -0.361, -0.008; p=0.040]. AACsev was not associated with five-year changes in TUG or ALM in univariable or multivariable analyses (all p>0.05).

Conclusion: In older women, severe aortic calcification was associated with greater five-year decline in muscle strength, but not TUG or ALM. These findings support the concept that vascular disease may have an effect on the loss of muscular strength.

Keywords: aortic calcification; physical function; grip strength; mobility; older women.

Poster P28
Effects of gravity change on medaka fish hard tissues

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Background and purpose: Life on earth is constantly affected by gravity, with bone tissues also known to be sensitive to its influence. On the other hand, the effects of gravity on teeth and periodontal tissue are largely unknown. Since the direction of tooth growth is correlated with the direction of gravity, we considered that gravitational force may be involved in generation and maintenance of teeth, as well as periodontal tissues.

Methods and Results: We utilized a biological gravity experimental device developed in research studies conducted at the International Space Station. TRAP promoter-EGFP transgenic Japanese rice fish (medaka) with fluorescent protein labelling of osteoclasts were reared under a gravitational force 5 times greater than normal (5 G). We analyzed the influence of gravity on the development of pharyngeal dental bones and other tissues of medaka. In pharyngeal teeth of fish reared under 5 G for a period of 6 months, the EGFP-positive area was reduced by approximately 85% and the fluorescent intensity of alizarin red-positive sites was decreased by about 40%, indicating that both the number of osteoclasts and amount of calcium deposition were decreased. Furthermore, entire sequence analysis of pharyngeal dental bone tissues of medaka reared under 5 G for 1 week showed that the expression level of the AP-1 gene group, related to bone metabolism, was decreased. These results suggested that bone turnover of osteoclasts and osteoblasts for maintaining pharyngeal tooth bone tissues is reduced by hypergravity. In addition to pharyngeal tooth bones, micro-CT analysis revealed abnormally formed otoliths, which function to control equilibrium sensation and vertebral curvature towards the dorsal side.

Discussion: Our findings indicate that gravity regulates gene expression, bone turnover by osteoblasts and osteoclasts of teeth and periodontal tissues, and homeostasis of otoliths and vertebrae.

P30
Denosumab failure associated with escape from suppression of bone resorption

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Western Health

We present the case of a 62-year old woman with scleroderma who did not respond to denosumab secondary to premature escape from suppression of bone resorption.

Our patient was initially diagnosed with osteoporosis 15 years previously based on bone densitometry, with lumbar spine and femoral neck T-scores of -4.05 and -3.69 respectively. There have been no previous fragility fractures and she has not been exposed to glucocorticoid therapy. Initial treatment with oral bisphosphonates was changed to denosumab due to patient choice and intermittent compliance. Over the subsequent 5 years, she received denosumab 60mg via subcutaneous injections every 6 months. Although she did not sustain any new fractures, there was no improvement in her bone densitometry. Significantly, whilst serum C-terminal telopeptide of type 1 collagen (CTX) and procollagen type 1 N propeptide (P1NP) demonstrated initial suppression at 3 months post denosumab dose, these were elevated prior to the next dose at 6 months, suggesting escape from the suppressive effects of denosumab.

Studies of denosumab show sustained suppression of bone turnover if given every 6 months, with escape occurring approximately 2 to 3 months after cessation of denosumab. Whilst escape from denosumab has been previously reported in malignancy, there has only been one reported case in the setting of denosumab for osteoporosis. Although this is the second case report of escape from denosumab in osteoporosis and the first in a patient with scleroderma, this phenomenon is likely to be under-reported in patients who do not respond to denosumab. We suggest that clinicians consider the possibility of escape in patients with poor response to denosumab who are compliant, and suggest the measurement of bone turnover markers in these patients.

P31
What is the natural history of Camurati-Englemann disease?

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Aims: Camurati-Englemann disease [CED] is a rare craniotubular bone dysplasia characterised by osteosclerosis of the long bones and skull that results from dominantly-inherited mutations in TGFB1. Most reports of CED emphasise severe cases of childhood onset, with bone pain, muscle weakness, anorexia and gait disturbance, but there may be marked phenotypic variability. Because of its rarity, there is thus uncertainty as to the full clinical spectrum of CED, and its natural history. We have had the opportunity to assess these aspects in a large family with CED.

Methods: We studied nine adults and one child [age 6-68], who all carried the familial TGFB1 mutation [R218H]. We detailed individual histories of symptoms, and examined both skeletal radiographs and 99Tc-MBP bone scintiscans. Two nuclear medicine specialists blindly ranked the bone scintiscans according to disease activity.

Results: Three subjects had severe symptomatic disease: a 6 year old with pain and gait disturbance, and two young adults who presented with cranial nerve palsies from skull involvement. The remaining 7 were largely asymptomatic, but several had experienced limb pain in their late teens, that had resolved without specific treatment. Current disease activity, assessed by scintigraphy, was inversely related to age. In several subjects there was a striking disparity between their extensive radiographic involvement and minimal disease activity on the scan, suggesting that the active disease process could abate spontaneously. We documented this in one of the severely affected subjects who had two scintiscans 15 years apart; disease activity was clearly reduced on the later scan.

Conclusion: Although CED can undoubtedly cause severe symptoms, particularly if of onset in childhood or there is extensive skull involvement, the data from this family suggests that minimally symptomatic disease may be more common than previously recognised, and that disease activity tends to abate spontaneously with age.

P32

Dietary acid load and risk of diabetes mellitus among postmenopausal Women in Malaysia

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Dietary acid load has been found to be associated with chronic diseases especially cardiovascular disease, diabetes, cancer and osteoporosis. Most of the above studies were conducted among Caucasian with limited information available among postmenopausal Asians population. The purpose of this cross sectional study was to evaluate the prospective relationship between dietary acid load and risk of diabetes among postmenopausal women in Malaysia. A total of 217 participants aged 51-85 years, were recruited from 8 out of 15 affiliates of the National Council of Senior Citizens Organizations Malaysia (NACSCOM). Sociodemographic, lifestyle factors, medical history, anthropometric data and biochemical samples were collected during visitation. Dietary acid load was estimated as potential renal acid load (PRAL) from nutrient intake assessed by a validated semi-quantitative food frequency questionnaire (FFQ). Average PRAL score was 13.44 ± 19.08. Multivariate linear regression analysis revealed that PRAL (standardized regression coefficient = 0.139, p = 0.039, 95% CI 0.000, 0.013) was positively associated with blood glucose level after adjusted for covariates (age, duration of postmenopause, physical activity level, years of education). Using fasting plasma glucose as the surrogate measure for risk of diabetes, our findings suggest that a high dietary acid load PRAL score was associated with an increased risk of diabetes mellitus in postmenopausal women. Further studies including cohort and interventional studies modifying acid-base dietary intake are essential to delineate the possible role of low dietary acid load for the prevention of diabetes mellitus.

P33

Associations between serum sodium concentration and bone health measures in individuals who use antiepileptic drugs

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Aim: Hyponatraemia (serum [Na+] <135mmol/L) is associated with increased risk of osteoporosis and fractures. Antiepileptic drugs (AEDs) are also independently associated with increased risk of osteoporosis and fractures, and can induce hyponatraemia. The aim of the study was to evaluate associations between serum sodium concentration and bone health measures in AED users, which might help elucidate a possible mechanism by which AEDs relate to bone health.

Methods: This cross-sectional study included patients, aged 18-75 years, who had been treated with AEDs for more than one year. These patients all had bone mineral density (BMD) scans done, with serum sodium concentrations recorded for the ten-year-period prior to their index BMD scans. Clinical data, including fracture history, AED use and duration, were obtained by questionnaire.

Results: Seventy-one patients were recruited. Total hip BMD adjusted for age, height and weight was negatively associated with the number of hyponatraemic events recorded (Rho=-0.245, p=0.04). Serum sodium concentrations were not associated with specific AED use. Reported history of fracture was significantly greater with enzyme-inducing AED (EIAED) and non-enzyme-inducing AED (NEIAED) polytherapy compared to patients using EIAED or NEIAED monotherapy (p=0.04). Those who reported a history of fracture also reported longer duration of NEIAED use than those

who did not report a history of fracture (p=0.01). Duration of oxcarbazepine use was marginally negatively correlated with total hip BMD (Rho= -0.949, p=0.05).

Conclusion: Although serum sodium concentration was associated with impaired bone health, it did not appear to provide an explanatory mechanism for poor bone health associated with use of specific AEDs. Further prospective studies with a larger sample size are required to investigate (i) the association between serum sodium concentration and bone health in AED users and (ii) the effect of its reversibility with electrolyte restoration, to improve preventative management of bone health in AED users.

P34

Rates and predictors of bone mineral density monitoring after commencement of endocrine therapy for breast cancer: 5-year experience from a tertiary referral breast cancer surgery service

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Background: Preservation of bone health is a priority in women on endocrine therapy for hormone-sensitive breast cancer. The use of aromatase inhibitors (AI) have been shown to accelerate bone mineral density loss, while in contrast, tamoxifen has bone-protective effects. This study explores the rates and factors predictive of bone mineral density monitoring among women commenced on endocrine therapy for breast cancer at our tertiary referral breast service.

Methods: Medical records of all women diagnosed with non-metastatic breast cancer at our breast surgery service between August 2007 and June 2012 were examined. All women commenced on either an AI (anastrazole or letrozole) or tamoxifen and underwent a baseline dual-energy x-ray absorptiometry (DEXA) scan within 6 months of starting endocrine therapy were included. The rate of follow-up DEXA completion was analysed and multivariable regression analysis was used to identify independent risk factors.

Results: Among 432 new breast cancer patients diagnosed at our service, 224 (51.9%) fulfilled the inclusion criteria. The median age was 61 (IQR 56, 70), and 151 (67.4%) women were taking an AI. 71.0% of the sample had follow-up DEXA scans (AI 82.8%; tamoxifen 47.2%, p<0.0001). The follow-up DEXA rate was 66.1% among women with a normal baseline DEXA (AI 78.8%; tamoxifen 37.1%, p<0.0001), while the rate among patients identified as osteopaenic or osteoporotic on baseline DEXA was 76.9% (AI 87.3%; tamoxifen 56.8%, p=0.001). Multivariable analyses identified aromatase inhibitor use and baseline DEXA as the only factors showing independent correlation with follow-up DEXA.

Conclusion: We report high rates of bone mineral density monitoring among women on endocrine therapy at our breast service, particularly with aromatase inhibitor use and with poor bone health on initiation of treatment. Multivariable analysis confirmed aromatase inhibitor use and baseline bone health as significant factors associated with monitoring.

P35

Colecalciferol initiation post-minimal trauma fracture

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Aim: To determine the proportion of patients admitted to a major tertiary teaching hospital in Australia aged 50 years and older with a confirmed neck of femur or vertebral minimal trauma fracture, who are commenced on colecalciferol supplementation by discharge, and to report the percentage of patients with serum colecalciferol levels measured.

Methods: A sub-analysis of a retrospective audit of electronic medical files for patients admitted with a minimal trauma fracture of the hip or vertebra between 1st January 2016 and 30th June 2016 was conducted.

Results: A total of 406 patients were screened and in this sub-analysis, 64 patients were eligible for inclusion, within these 64 patients, 38 were not on any vitamin or mineral supplementation at admission. Of these, 26 patients (68.4%) had their serum colecalciferol levels measured, and 21 patients (55.2%) overall were initiated on colecalciferol.

Conclusion: Over half of patients with a minimal trauma fracture were commenced on colecalciferol therapy, but a noteworthy proportion of patients remain untreated. Patients with colecalciferol levels are more likely to be initiated on therapy compared to those of whom levels were not taken during admission. This is a missed opportunity for intervention that may place patients at a higher risk of subsequent fracture; therefore effective strategies should be implemented to address this treatment gap in the future.

P36

Repurposing a fracture risk calculator as a screening tool for women at risk for sarcopenia

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Background: The identification of sarcopenia requires densitometry or bioelectric impedance analysis to measure muscle mass, dynamometry for muscle strength, or assessment of gait speed. A simple, feasible tool is needed for identifying individuals at risk for sarcopenia. As there are some shared characteristics between individuals with sarcopenia and osteoporosis, we aimed to test the performance of FRAX for discriminating individuals at risk for sarcopenia.

Methods: In this longitudinal component of the Geelong Osteoporosis Study, baseline FRAX(Australia) scores were determined for 310 women, and sarcopenia assessed 15yr later using DXA-derived low relative appendicular lean mass (Lunar; rALM<5.5kg/m2) and poor handgrip strength (Jamar; HGS<20kg). We tested FRAX scores for hip fracture and major osteoporotic fracture (MOF), with and without BMD. The area under the Receiver Operator Characteristic (AUROC) curve quantified the discriminate performance of FRAX scores for predicting sarcopenia.
Results: At baseline, median(IQR) values were age 54(48-61)yr, HIP-FRAX(noBMD) 0.20(0.10-0.80), MOF-FRAX(noBMD) 1.70(1.0-3.63), HIP-FRAX(+BMD) 0.10(0.00-0.43), MOF-FRAX(+BMD) 1.60(1.00-3.33). At follow-up, sarcopenia was identified for 14 (4.5%) of participants.

(i)FRAX-without-BMD: AUROC was 0.859 for Hip-FRAX, and 0.839 for MOF-FRAX. Optimal thresholds were 1.0 for Hip-FRAX (sensitivity 78.6%, specificity 74.3%) and 4.2 for MOF-FRAX (sensitivity 71.4%, specificity 72.3%).

(ii)FRAX-with-BMD: AUROC 0.808 for Hip-FRAX, and 0.802 for MOF-FRAX. Optimal thresholds were 0.6 for Hip-FRAX (sensitivity 64.3%, specificity 77.0%) and 3.9 for MOF-FRAX (sensitivity 64.3%, specificity 73.0%).

Conclusion: FRAX scores calculated without BMD predicted the risk for sarcopenia with reasonable sensitivity and specificity. Given that the clinical risk factors are identified without the need for equipment, and that the on-line calculator is widely accessible, there is potential for this assessment modality of FRAX to be used for screening individuals at risk of sarcopenia. Our results suggest that individuals with a Hip-FRAX score above 1.0 (8th decile) are at risk for sarcopenia, and may require further investigation and management.

P37

Associations between Circulating Osteoprogenitor (COP) cells, Parathyroid Hormone, Vitamin D and function in older adults

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Introduction: Circulating osteoprogenitor (COP) cells have been linked to bone formation and osteogenesis during the initial stages of fracture healing. Recent studies have shown a strong association between low percentages of COP cells (%COP) and frailty and disability. Additionally, increased levels of COP cells have been associated to low bone mineral density. However, the association between serum levels of Parathyroid hormone (PTH) and vitamin D remain unknown. In this study we aimed to identify the potential associations between COP cells, parathyroid hormone, vitamin D and their clinical significance in older adults.

Methods: A random sample of community-dwelling individuals aged 65 and older enrolled in the Nepean Osteoporosis and Frailty (NOF) Study (mean age 82.8; N = 77; 70% female). COP cells were identified by flow cytometry using selective gating of CD45/OCN+ cells and by Mean Fluorescence Intensity (MFI). Logistic regression models estimated the relationship between the percentage of COP cells and prevalent disability and frailty.

Results: There was a positive correlation between PTH levels and COP cells ($p=0.003$). A trend towards a negative correlation for vitamin D and COP cells was also evident ($p=0.060$). No correlations were found between COP cells and bone mineral density at the heel.

Conclusions: COP cells were positively correlated with PTH levels in older adults. This may suggest involvement of COP cells in bone turnover in particular the formation and mineralisation of bone. Whether pathological states such as hyperparathyroidism and vitamin D deficiency are also associated with significant changes in %COP remains to be determined.

P38
Vertebral Fracture Identification - A Different Approach

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Aim: Vertebral fractures often go undiagnosed and untreated. Diagnoses with imaging also are fraught with difficulty because of the lack of uniformity in description of vertebral fractures.

Methods: As part of our role in CDHB Fracture Liaison Service (FLS) all images are available electronically through a shared depository that is imported into HCAS Healthcare analytic systems, HPE. Through this we were able to access vertebral fractures as diagnosed as on the x-ray report. With the HCAS system we have then used a clinical health ontology – SNOMED CT (https://www.snomed.org/). Snomed clinical terms is the most comprehensive and precise clinical health terminology product that enables us to search in code of terms and small sentences within clinical notes and radiological reports. This incorporates words such as anterior, wedge, fracture, vertebra, closed, thoracic, lumbar, collapse or a combination. We have used this software to extract those individuals who are clearly identified as having thoracic and vertebral fractures. These are reviewed visually online.

Results: The results so far have been in over 700 patients identified; approximately 30% were already on treatment and had had appropriate investigations. Another 30% had not been identified and were thus followed up by FLS. A further approximately 30% were either miss classified, had other additional clinical factors. The initial 700 patients provided with information that was robust enough for us to embark upon this in a prospective manner. From August 2017 we have identified over a 100 patients monthly of which 10 – 15 % have been previously undiagnosed and untreated.

Conclusion: This approach in using digital images and clinical notes search in our health system has streamlined the approach to diagnosing and treating those people with vertebral fractures without the use of significant additional resources.

P39

Associations between Bipolar Disorder and bone: A systematic review

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Background: Poor bone health is a likely physical comorbidity of bipolar disorder (BD), a severe, episodic mental illness, affecting approximately 2.4% of the population. Data suggests that pharmacological treatments used to manage BD may also negatively influence bone. This systematic review examines the literature regarding associations between BD and bone health.

Methods: We conducted an e-search of PubMed, PsychINFO and CINAHL to identify studies that investigated associations between BD and bone in adults aged ≥18. Two reviewers determined eligibility according to pre-determined criteria, and methodological quality was assessed using a previously published methodological scoring system. Due to heterogeneity a best-evidence synthesis was performed.

Results: Our search yielded 1,409 articles, of which three (all cohorts) met predetermined criteria. The studies, from Taiwan and the United States, analysed administrative data albeit spanning different years and comprised a total of 344,497 participants. No studies investigating bone quantity or quality were identified. Those with BD, compared to those without, had an increased risk of fracture (range 20%-80%): in those with BD, fracture-free survival time was 20-30% shorter amongst anticonvulsant users, and survival time without fracture decreased substantially with advancing age, and for women (10-30% shorter than men). For hip fracture alone, the use of anticonvulsants (any type, and enzyme-inducing) shortened the time to fracture ~2.5-fold, as did the recency of use. Fracture incidence per 1,000 person years (py) was 21.4 and 10.8 in those with and without BD, respectively. In those with BD, fracture incidence per 1,000 py was greater amongst anticonvulsant users compared to non-users (35.8 vs 21.8, respectively).

Conclusion: Increased fracture was observed in individuals with BD, independent of older age, female sex, comorbidities and medication use. Anticonvulsant use compounded this effect, and survival time was further shortened with recency of use. These findings herald further studies in this area of enquiry.

P40

Factors associated with the uptake of bone mineral density scans following fracture

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Aim: Current literature suggests that the uptake of bone mineral density (BMD) assessment following fracture is suboptimal. We aimed to identify factors associated with the uptake of BMD assessment during the year following fracture.

Method: The Geelong Bone Density Service records were searched for 1,192 participants enrolled in PROFRAC, a study examining risk factors and outcomes associated with fracture. Incident fractures were identified from radiological reports from the University Hospital Geelong. Exclusions were for fractures of the toe (n=39), finger (n=95) or death during the study period (n=22). Cause of fracture, education level, general health prior to fracture, physical activity, fracture history and the presence of osteoporosis were documented by self-report. Odds ratio (OR) and confidence intervals (CI) were determined using logistic regression.

Results: Among the final sample of 1,036 [43.9% male, median age 59.6 (IQR 41.6, 73.8) years], 166 (16.0%) participants had a BMD assessment in the year post-fracture, of whom 102 (61.5%) had suffered a major osteoporotic fracture. The odds of having a BMD assessment increased with each year of age (OR 1.02, 95%CI 1.01-1.03), female gender (OR 2.27, 1.46-3.55), past diagnosis of osteoporosis (OR 1.86, 1.20-2.89) history of BMD scans (OR 2.48, 1.66-3.69) and presence of a major osteoporotic fracture (OR 1.52, 1.03-2.23). Cause of fracture, education level, physical activity, fracture history or general health prior to fracture were not associated with BMD assessment in the year post-fracture.

Among those 417 participants clinically indicated for BMD assessment (aged 50 years or older with a low trauma fracture), only 100 (24.0%) had their BMD assessed.

Conclusion: These data indicate that uptake of BMD scans following fracture is low. Men and those without a known history of osteoporosis or BMD scans are less likely to have a BMD scan post fracture and should be targeted for BMD referral.

P41
Cardiovascular Disease and bone health in Gambian men and women

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Eighty percent of global cardiovascular disease (CVD) mortality occurs in low-middle-income countries. Evidence shows an increased rate of fracture following major cardiovascular events and elevated blood pressure. This study aimed to investigate the associations between cardiac workload and bone outcomes in adult Gambians.

The Gambian Bone and Muscle Ageing Study recruited 249 women and 239 men ([mean±SD] 61.1±12.5 and 60.8±12.3years). Blood pressure and heart rate were measured with the OMRON705IT. Rate pressure product (RPP), an indicator of cardiac workload, was calculated as heart rate*systolic blood pressure (bpm*mmHg). DXA was used to measure aBMD and bone mineral content (BMC) at the whole-body, total-hip, femoral-neck, lumbar-spine and radius. Linear regressions explored the relationship between RPP and bone outcomes, adjusting for age, weight, height; including a sex-RPP interaction. Results are expressed as β-coefficients[95% CI] of percentage unit change in bone outcomes and sex-RPP interaction p-values (p-int).

BMI was greater in women (22±4) than men (21±3), p=0.001; blood pressure was similar in men and women. With greater age, RPP was higher in women than men (p<0.0001). In unadjusted analyses, RPP was negatively associated with all bone outcomes in women but not in men. Following adjustments, every 1% increase in RPP was negatively associated in women with: whole-body aBMD (-0.08%[-0.12,-0.03],p-int=0.007), whole-body BMC (-0.12%[-0.19,-0.05],p-

int=0.02), femoral-neck BMC (0.19%[-0.31,-0.07],p-int 0.05), radius aBMD (-0.2%[-0.28,-0.11],p-int<0.0001) and radius BMC (-0.19%[-0.28,-0.10],p-int=0.008). No such associations were found in men.

The prevalence of non-communicable chronic diseases of older age is increasing as The Gambia transitions. Timely osteoporosis and CVD prevention will help to reduce fracture risk.

P42

A BMP signaling-target gene Atoh8 increases bone volume in adult mice

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Although bone morphogenetic protein (BMP) is the major driver of osteoblastic bone formation, it is unclear whether it directly controls bone resorption. Here, we purified Atonal Homolog 8 (Atoh8) gene to be up-regulated upon BMP-2 stimulation in mouse bone marrow stroma cells ST-2 by microarray assay. In MC3T3-E1 osteoblasts, induction of Atoh8 was confirmed by qRT-PCR to be increased from 1 h of BMP-2 stimulation, and subsequently elevated for over 48 h. The direct binding of BMP-activated Smad1 to the promoter region of Atoh8 gene was confirmed by chromatin immunoprecipitation and the luciferase reporter assay. Our conventional Atoh8 knockout (KO) mice line were viable and fertile. The skeletal preparation of the newborn KO mice showed mild retardation in bone formation of carvariae, claviculae and vertebral bones, although the overall body size was normal. At 8 weeks of age, KO mice were healthy whereas the body weight and the size were mildly reduced. The μCT and bone histomorphometry analyses of bones of KO mice revealed that bone volume was significantly decreased while bone formation was not altered, however, the osteoclast number was increased. Although the cell-autonomous osteoclast differentiation in KO primary bone marrow cell or spleen cell was normal, co-culture with KO bone stroma cells enhanced the osteoclast differentiation of bone marrow cells. The Rankl /Opg expression ratio in KO osteoblasts were elevated. These results suggested that Atoh8 suppressed osteoclast formation by suppressing expression ratio of Rankl/Opg in osteoblast to prevent bone loss. In addition to the promotive role in bone formation, BMP signaling may suppress bone resorption by inducing Atoh8, which molecule may be a novel target to treat bone loss.

P43

Prior Fracture is Associated with Lower Bone Material Strength Index in Men

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Purpose: BMD does not fully explain fracture risk, as the largest absolute number of fragility fractures occur in people without severe deficits in BMD. Impact microindentation (IMI) is an emerging technique, which measures bone material strength index (BMSi) in vivo. However, it is unclear how well the technique identifies bone fracture propensity. The aim of this study was to evaluate the association between BMSi and prior fracture in men.

Methods: BMSi was measured using the OsteoProbe for the first 266 men (ages 33-92yr) assessed in the current phase of the Geelong Osteoporosis Study. Prior fracture was defined as a moderate/low trauma fracture in adult life (≥20yr), and was self-reported and confirmed radiologically where possible. Multiple regression models were used to determine whether any differences in BMSi values between those with or without prior fracture were independent of other risk factors for fracture including BMD, age, weight, height, body mass index (BMI), parental hip fracture, alcohol consumption and smoking.

Results: There were 26(9.9%) men with prior fracture (5 rib, 4 ankle, 3 tibia, 3 humerus, 2 hip, 2 wrist, 2 clavicle, 3 foot, 1 vertebral, 1 elbow). Mean (±SD) for BMD, age, weight, height and BMI were 0.967±0.122g/cm2, 63.7±12.5yr, 81.3±10.9kg, 174.3±6.8cm and 26.7± 3.1kg/m2 respectively.

Mean BMSi for men with and without prior fracture was 80.4 (78.2-82.6) vs 83.1(82.4-83.9; p=0.04). The lower mean BMSi for men with prior fracture persisted after adjustment for age and BMI. Adjusted mean BMSi values were 80.5(78.4-82.7), 83.2 (82.4-83.9) for men with and without prior fracture (p=0.04), respectively. Excluding foot fractures did not alter the findings. No other cofounders were identified.

Conclusions: Mean BMSi values were significantly lower in men with a prior fracture. These data support the notion that IMI using the OsteoProbe may be a useful technique for identifying men with bone fragility.

P44
FRAX as a screening tool for frailty

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Aims: Frailty is characterised by age-related decline in physical, psychological and social functioning. Many of the features of frailty overlap with underlying risk factors for fragility fractures. This study aimed to investigate whether FRAX® could be used as a screening tool to identify frail individuals.

Methods: This longitudinal study included 303 women aged 60-90 years enrolled in the population based Geelong Osteoporosis Study. FRAX (Australia) 10- year probabilities of major osteoporotic fracture (MOF) and hip fracture were calculated with and without BMD at baseline. Frailty was determined at the 15-year follow-up using a modified Fried frailty phenotype index, a five-item tool that groups individuals into frail (3 ≥ items), pre-frail (1-2 items) and robust (zero items). For this analysis, frailty was dichotomised, defining it as having three or more items and non-frail defined as having less than three items (pre-frail and robust pooled). We used Receiver Operating Characteristic (ROC) curves and diagnostic characteristics tests to investigate the discriminatory ability of the FRAX tool to predict frailty.

Results: Of 303 women (mean age of 70.9±7.3 years), 51 (16.8%) were considered frail at follow-up. In descending order, the areas under the ROC curve were MOF-FRAXnoBMD 0.730, hip-FRAXnoBMD 0.712, MOF-FRAXBMD 0.725, hip-FRAXBMD 0.700 and BMD alone 0.627 (all p-values < 0.001). The optimal threshold for identifying frailty was 2.6% 10-year fracture probability for MOF-FRAXnoBMD (sensitivity 70.6%, specificity 66.2%).

Conclusion: MOF-FRAXnoBMD was identified as the best model for predicting frailty displaying acceptable sensitivity and specificity for a screening tool. FRAX is an internationally used tool, easy to administer and utilising information routinely collected in clinical settings. These data suggest that FRAX scores might be useful in the clinical setting for screening older women at risk of becoming frail, particularly where access to densitometry measures are not available.

P45
Computed effects of PTH treatments in Osteoporosis by using a new mechanistic PK/PD model

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Osteoporosis is a common age- and/or sex- related bone disease which ultimately leads to bone fractures. The only available and approved anabolic treatment is PTH(1-34). This drug exhibits a dual action, i.e. anabolically or catabolically depending on the administration type, i.e. given continuously versus intermittently. Only a few computational pharmacokinetic-pharmacodynamic (PK/PD) models tried to simulate the actions of PTH(1-34) on bone modeling and remodeling. However, to the authors' knowledge, no comprehensive model has been developed to simulate the dual action of PTH(1-34) on human bone. Here, we developed and tested a mechanistic PK/PD model of the action of PTH(1-34) on human bone remodeling that includes mechanobiological feedback. This model simulates changes of bone cells in a volume of interest due to different catabolic or anabolic stimuli. We simulate changes of bone matrix fraction (fbm) in postmenopausal osteoporosis together with subcutaneous daily-injections of PTH (1-34) for a period of 2 years. Once calibrated on clinical data of BMD changes, the model can be used to predict site-specific bone response due to different PTH(1-34) doses. Our simulations show that change of fbm versus dose response is non-linear and that change of fbm is bone-site specific. At lumbar spine a 20 μg dose of PTH(1-34) leads to 8.4% while the femoral neck only a 3.0% change is observed. These simulation results are in good agreement with experimental data reported in Leder et al. 2014 (JCEM, vol. 99(5), pgs. 1694–1700): 9.4 ± 1.13% at lumbar spine and 2.82 ± 0.72% at femoral neck. To conclude, our model is able to closely mimic bone physiology and can be used to predict site-specific changes in bone due to different PTH dosing, different mechanical loading configurations (i.e., physical activity) or to combined cases in order to test the synergistic action between PTH treatment and mechanical loading.

P46

The effect of a high fat high fructose diet on the osseointegration of titanium implants in a rodent model

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The aims of this study were to analyse the effects of a diet reported to induce insulin resistance and impaired glucose tolerance on bone microarchitecture and the osseointegration of titanium implants in a female rodent model.

Twenty female Sprague Dawley rats were randomly assigned to two groups: 1) Normal Diet (ND), and 2) High Fat High Fructose (HFHF) diet – 60% fat and 20% w/v fructose enriched water. Titanium implants 4.1mm length x 1mm diameter with a sandblasted and acid etched surface were placed in the right tibial metaphysis at week 1 in the ND group and at week 6 in HFHF group and allowed to heal for an 8 week period. Metabolic changes were measured by insulin and oral glucose tolerance tests. Three dimensional bone morphometric structures, relative bone volume (BV/TV), relative bone surface (BS/TV), trabecular thickness (Tb.Th), trabecular separation (Tb. Sp), trabecular number (Tb.N) in both the trabecular and cortical bone including the bone-to-implant contact (BIC) of the peri-implant bone formed around the implant were analysed using micro computed tomography.

Glucose and insulin tolerance was not significantly impaired at any time point in the study for the HFHF group. Analysis of tibial trabecular bone revealed a significant decrease in the BV/TV ($p<0.05$), and the Tb.N ($p<0.05$) in the HFHF group. Analysis of cortical bone in the tibial diaphysis revealed no significant differences in any of the parameters between the groups. Analysis of the peri-implant bone formed around the implant revealed a significant decrease in bone-to-implant contact ($p<0.01$) in the HFHF group.

This data indicated that despite the absence of impaired insulin and glucose tolerance, there was a significant negative impact on trabecular but not cortical bone microarchitecture and significantly reduced osseointegration.

P47

Handgrip strength and muscle quality in Australian Women: Geelong Osteoporosis Study (GOS)

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Deakin University

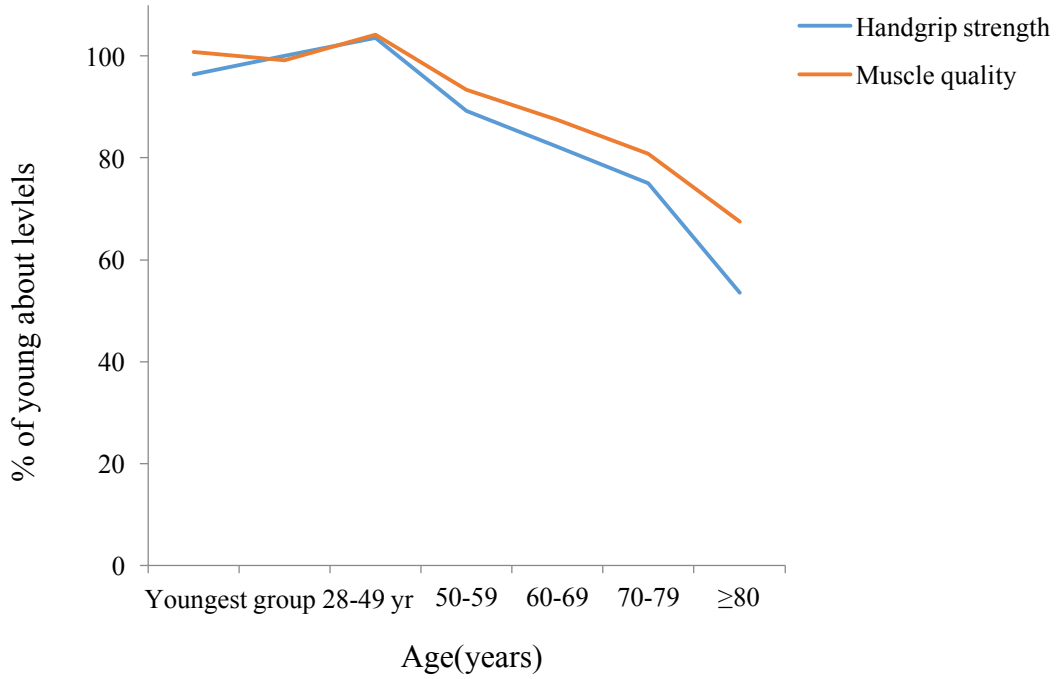
Background: Low handgrip strength (HGS) is a measure of poor skeletal muscle performance and a marker of ill-health and frailty. Muscle quality (MQ) is a measure of muscle strength relative to muscle mass. We aimed to develop normative data for both HGS and MQ and determine the relationship with age and BMI for Australian women.

Methods: This cross-sectional analysis included data for 792 women (median age 57yr; IQR 43-70) assessed at the 15-year follow-up of the Geelong Osteoporosis Study (2011-2014). HGS was measured twice with a dynamometer (Jamar) for each hand and the mean of the maximums was used. Mean DXA-derived lean mass (Lunar) for the arms was used to calculate MQ as HGS/lean mass (kg/kg). BMI was calculated using measured weight and height and categorised as normal (BMI<25.0kg/m2), overweight (BMI 25.0-29.9kg/m2) and obese (BMI>30.0kg/m2). Intergroup differences were tested using ANOVA.

Results: HGS ranged from 2.0 to 43.5 kg and MQ from 1.1 to 18.6. Mean values (SD) for normal BMI, overweight and obese groups for HGS were: 25(6.8), 24(6.9), 24(6.9) kg, $p = 0.14$; and for MQ: 12.0(2.7), 11.0(2.6), 9.9(2.6), $p < 0.001$, respectively. Our data show that mean HGS and MQ remained stable until the fifth decade, then declined steadily with increasing age; therefore, we used data for women (n=283) aged 28-49 years as the young adult reference group, with mean(SD) values for HGS = 28(6.2) and MQ = 12(2.5). Age-related values for HGS and MQ in relation to the young adult reference group are shown in the Figure.

Conclusion: Mean HGS and MQ declined with advancing age in older women. Our data suggest that while mean HGS appeared to be independent of BMI, MQ decreased in the face of increasing adiposity. This pattern is likely driven by age-related differences in both body composition and muscle strength.

Figure HGS, MQ and age in relation to the young adult reference group.



P48

Degraded microarchitecture in Australian men and women as assessed by Trabecular Bone Score

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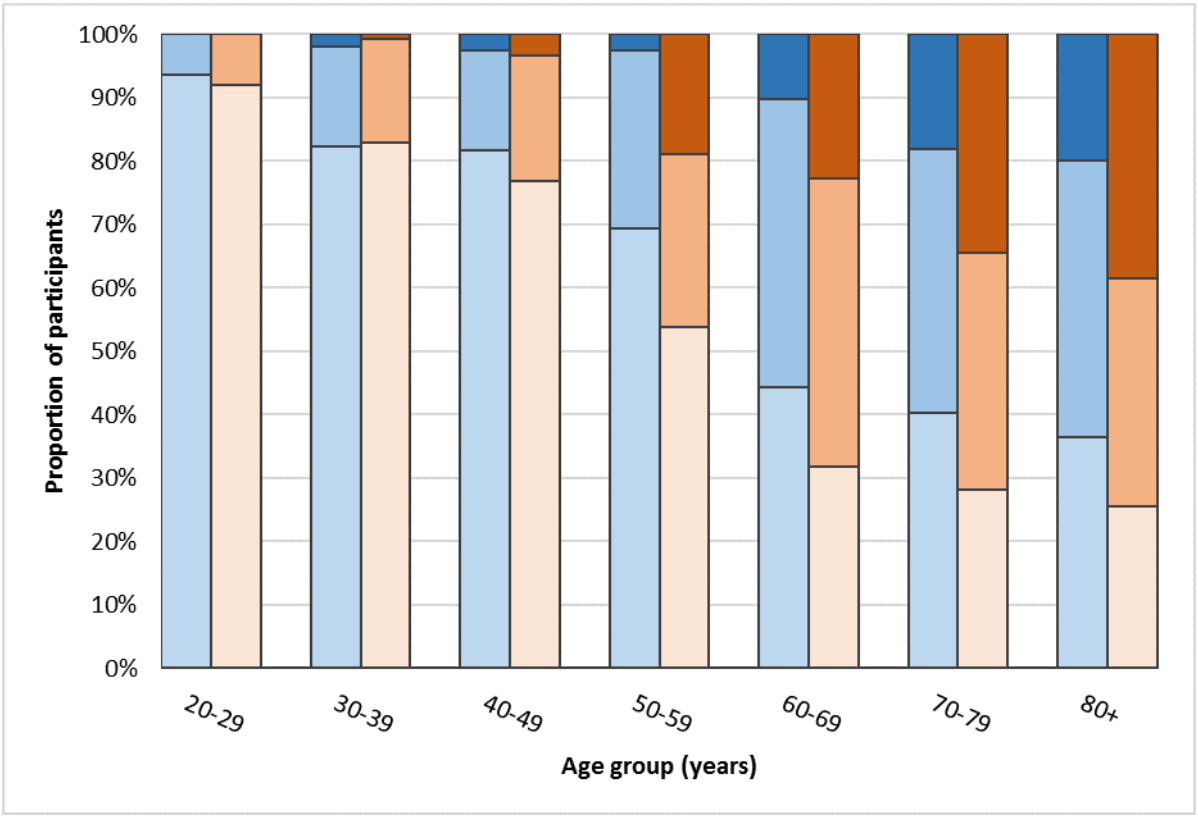
Purpose: Trabecular bone score (TBS) is a novel tool for indirectly assessing trabecular microarchitecture at the lumbar spine, providing information complementary to areal BMD. The aim of this study was to determine the proportions of Australian men and women with degraded microarchitecture, using TBS.

Methods: This study included 894 men and 682 women (aged 24-98yr) enrolled in the Geelong Osteoporosis Study. TBS was determined by analysis of lumbar spine DXA scans (Lunar Prodigy) using TBS iNsight software (Version 2.2). T-scores were calculated for TBS using the young adult (aged 20-39) mean and standard deviation, and each participant was categorised as normal (T-score above -1.0), partially degraded (T-score between -2.5 and -1.0) or degraded (T-score less than -2.5).

Results: Microarchitecture was considered normal in 37.2 (54.5%), partially degraded in 197 (28.9%), and degraded in 113 (16.6%) women. Of all men, 531 (59.4%) had normal microarchitecture, 283 (31.7%) had partially degraded microarchitecture, and 80 (8.9%) had degraded microarchitecture. The proportion of participants classified as having degraded microarchitecture increased as age increases, as seen in Figure 1. Further, for almost every age group, the proportion of women classified as having degraded microarchitecture was larger than the proportion of men.

Conclusion: This study describes the distribution of TBS classifications by T-score across the age range for both men and women.

Figure 1 Proportion of participants in trabecular bone score categories by age group, for men and women. Data for men are presented in blue (left), with data for women presented in red (right). Dark shades represent degraded microarchitecture, medium shades represent partially degraded microarchitecture, and light shades represent normal microarchitecture.



P49

Clinical utility of routine parathyroid hormone measurement in women commenced on endocrine therapy for hormone sensitive breast cancer

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Introduction: Serum calcium and vitamin D measurement is recommended in the evaluation of bone health in women commenced on endocrine therapy for breast cancer. However, the clinical value of parathyroid hormone (PTH) measurement, particularly in the prediction of bone loss, is unclear.

Methods: All women over 50 years diagnosed with non-metastatic breast cancer and started on endocrine therapy (anastrazole, letrozole or tamoxifen) at our service between August 2007 to June 2012 were retrospectively identified. For inclusion, patients were required to have had a baseline dual-energy x-ray absorptiometry (DEXA) scan and blood test within six months of starting endocrine therapy, and at least one follow-up DEXA scan prior to ceasing or switching endocrine treatment.

Results: 139 patients (113 aromatase inhibitor [AI], 26 tamoxifen) were included. Median age was 65 years (IQR 56, 68) and median time between baseline and final DEXA was 3.5 years (IQR 3.0, 4.4). Fifty-five patients (39.6%) had osteopaenia, and twenty patients (14.4%) had osteoporosis. Tamoxifen use resulted in a significant increase in femoral neck (FN) T-score per year compared with AI use (0.078 versus -0.110, p=0.001). On linear regression analysis, PTH was negatively associated with increased baseline FN T-score (p=0.043), while no significant association was identified with vitamin D or calcium. In women taking AI, a non-significant trend towards improvement in the percentage change in lumbar spine BMD per year (0.964% versus -1.043%, p=0.087) and FN T-score per year (-0.867 versus -0.111, p=0.740) was observed with a PTH >5.90pmol/L compared to a PTH ≤5.90pmol/L. Following PTH measurement, four patients (2.9%) were diagnosed with primary hyperparathyroidism, with three undergoing parathyroidectomy.

Conclusion: Serum PTH should be routinely measured on initiation of endocrine therapy, as it may provide information regarding baseline bone health and detect previously undiagnosed parathyroid disease, but is of no predictive value in determining future bone loss.

P50

Human cartilage influences the crystallization of monosodium urate (MSU) and the inflammatory response to MSU crystals: understanding the link between osteoarthritis and gout

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Background: In people with gout, osteoarthritic joints are preferentially affected by monosodium urate (MSU) crystal deposition and gout flares.

Aim: To examine the effects of cartilage on MSU crystallization, and determine whether cartilage alters MSU crystal-induced inflammation.

Methods: Human cartilage homogenates were prepared from macroscopically-healthy and macroscopically-diseased knee samples obtained from patients undergoing knee arthroplasty. Crystallization assays were used to test the effects of cartilage homogenates or specific cartilage factors (purchased commercially) on MSU crystallization. Changes in urate solubility, nucleation (crystal appearance time), crystal growth and morphology, and total crystal formation were assessed over 24h. For inflammatory assays, MSU crystals grown with or without cartilage homogenates were added to THP-1 monocytes for 24h and cytokine release was measured by ELISA.

Results: Addition of 5% and 10% healthy homogenized cartilage increased the total weight of MSU crystals formed, and resulted in formation of shorter MSU crystals, compared to control (no cartilage). In morphological analysis, MSU crystal bows were observed in the presence and absence of cartilage; however bows formed in the presence of homogenized cartilage were significantly shorter than control bows (Figure); this effect was augmented when homogenized cartilage was further degraded by collagenase. There were no differences between healthy cartilage and diseased cartilage homogenates in all crystallization assessments. Type II collagen, but not other specific cartilage factors, also led to the formation of shorter MSU crystals. No differences in urate solubility or nucleation times were observed. In THP-1 cell

assays, MSU crystals grown in the presence of healthy homogenized cartilage significantly increased release of IL-8 (but not IL-1 or TNF- compared to control crystals.

Conclusions: Human cartilage increases MSU crystal formation, influences crystal size, and promotes the release of some pro-inflammatory cytokines in response to MSU crystals. These processes may contribute to the preferential clinical presentation of gout in osteoarthritic joints.

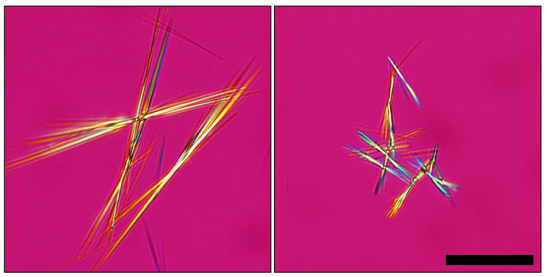

Figure: MSU crystal bow-like structures formed in the absence (left) or presence (right) of 10% healthy cartilage homogenate after 24 hours, viewed using polarizing light microscopy. Scale bar represents 30μm.

Disclosure: This study was funded by an investigator-initiated grant from AstraZeneca and Ironwood Pharmaceuticals, who had no role in the conduct and reporting of the study.

P51

How Well Does FRAX (Aus) Adjusted for Trabecular Bone Score Predict Incident Fractures? Data from the Geelong Osteoporosis Study

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Aims: The aim of this study was to determine how well (i) FRAX(Aus) with BMD (FRAXBMD) and (ii) FRAX(Aus) with BMD, adjusted for trabecular bone score (FRAXTBS), predicted both hip and major osteoporotic fractures (MOFs) in Australian men.

Methods: Men (n=591, aged 69.2±12.2yr) enrolled in the Geelong Osteoporosis Study were included. Clinical risk factors including femoral neck BMD were assessed at baseline (2001-2006). Absolute fracture risk was estimated using FRAXBMD and FRAXTBS. Incident fractures were verified radiologically over a 10-year follow-up period. The predicted number of fractures was calculated by multiplying FRAX scores by the proportion of time followed-up. The difference between observed and predicted number of fractures was assessed using a Chi-squared test. Sensitivity, specificity and AUC were calculated.

Results: FRAXBMD predicted hip fractures well (13 observed vs 9 predicted, p=0.389), but MOFs were underestimated (47 observed vs 22 predicted, p=0.002). FRAXTBS also predicted hip fractures well (13 observed vs 9 predicted, p=0.389), however the number of MOFs was underestimated (47 observed vs 25 predicted, p=0.007). Both FRAXBMD and FRAXTBS had similar AUCs for both hip and MOFs (Figure). Sensitivity was higher for FRAXTBS compared to FRAXBMD for both hip (84.6 vs 61.5) and MOFs (4.3 vs 2.1), with a slight reduction in specificity (hip: 79.9 vs 83.9; MOF: 98.9 vs 99.3). For men with hip fracture, five were considered low risk (<3%) and eight were considered high risk (≥3%) by FRAXBMD. For FRAXTBS, only two were considered low risk and the other 11 were considered high risk (p=0.185). For men with MOFs, only one man was considered high risk (≥20%) using FRAXBMD and only two by FRAXTBS (p=0.557).

Conclusions: FRAXBMD underestimated the number of MOFs. Adjusting the score for TBS improved sensitivity of predictions.

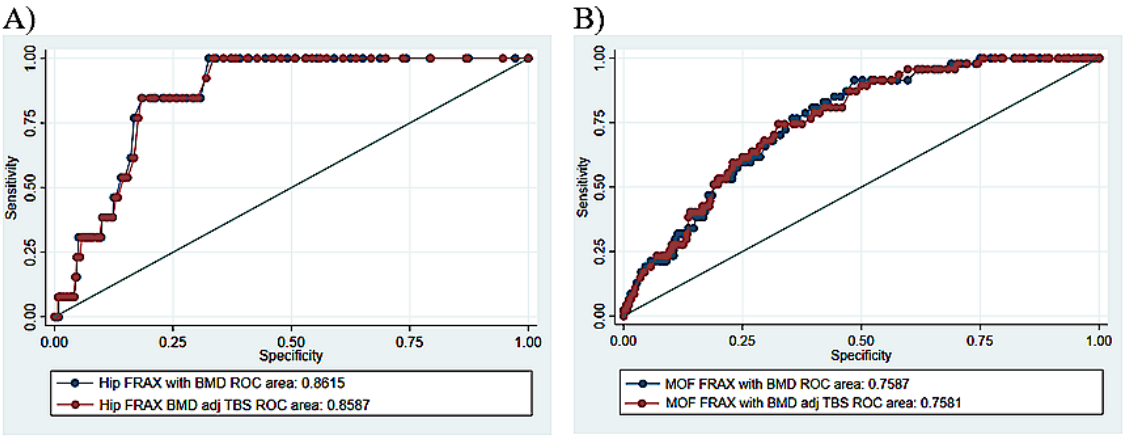

Figure: ROC curve showing the algorithms' (FRAX with BMD and FRAX with BMD, adjusted for TBS) ability to discriminate A) hip and B) major osteoporotic fractures.

P52

Association of adiposity measures in childhood and adulthood with knee cartilage thickness, volume and bone area in young adults

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3. Institute of Bone and Joint Research

Objective: To describe the associations of childhood and adulthood adiposity measures with knee cartilage thickness, volume and bone area in young adults.

Methods: Childhood and adulthood adiposity measures (weight, height, waist circumference and hip circumference) of 186 participants were collected in 1985 (aged 7-15 years) and during 2004-2006 (aged 26-36 years). Knee magnetic resonance imaging was conducted during 2008-2010 (aged 31-41 years), and cartilage thickness, volume and bone area were measured using a quantitative approach (Chondrometrics, Germany). Linear regressions were used to examine the above associations.

Results The prevalence of overweight was 7.6% in childhood and 42.1% in adulthood. Childhood weight was negatively associated with adult patellar bone area (β=-6.24, 95% confidence interval (CI) -10.25 to -2.22 mm2/kg), while adult weight was positively associated with bone area in medial femorotibial compartment (MFTC) (β=3.09, 1.44 to 4.74 mm2/kg) and lateral femorotibial compartment (LFTC) (β=1.85, 0.19 to 3.51 mm2/kg). Adult waist-hip ratio (WHR) was negatively associated with cartilage thickness (MFTC: β=-0.011, -0.022 to 0.000; LFTC: β=-0.013, -0.025 to -0.002 mm/0.01 unit), volume (Patella: β=-21.85, -37.77 to -5.93; LFTC: β=-23.87, -42.18 to -5.57 mm3/0.01 unit) and bone area (Patella: β=-4.69, -8.26 to -1.12; LFTC: β=-4.76, -9.47 to -0.06 mm2/0.01 unit). The change in WHR z-scores from childhood to adulthood was negatively associated with cartilage thickness (MFTC: β=-0.056, -0.109 to -0.003 mm), volume (patella: -83.10, -162.97 to -3.23; MFTC: -83.04, -158.07 to -8.00; LFTC: -100.55, -190.28 to -10.81 mm3) and bone area (patella: -21.35, -39.00 to -3.69; LFTC: -24.47, -47.36 to -1.58 mm2).

Conclusions: Childhood weight was negatively but adult weight was positively associated with adult bone area. Adult WHR and the change in WHR from childhood to adulthood were negatively associated with cartilage thickness, volume and bone area. These suggest early life adiposity measures may affect knee structures in young adults.

P53

Association of body composition, physical activity and physical fitness with knee cartilage thickness and subchondral bone area in young adults

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Objective: To describe the associations of body composition, physical activity and physical fitness with knee cartilage thickness and subchondral bone area in young adults.

Methods: Participants (n=186, aged 31-40 years, 48.4% females) were selected from the Childhood Determinants of Adult Health Study. Body composition, physical activity and physical fitness were measured during 2004-2006. Cartilage thickness and subchondral bone area were measured quantitatively (Chondrometrics 3.0, Germany) from knee magnetic resonance imaging taken during 2008-2010. Linear regressions were used to describe associations.

Results: Lean mass, but not fat mass, was positively associated with knee cartilage thickness (β=6.50 μm/kg, 95% confidence interval (CI): 0.86 to 12.13) and subchondral bone area (β=13.66 mm2/kg, 95% CI: 5.73 to 21.59). Physical activity measures (including walking, moderate activity, vigorous activity and total activity) were not associated with knee cartilage thickness or subchondral bone area. Physical fitness measures were positively associated with knee cartilage thickness (long jump: β=2.36 μm/cm, 95% CI 0.68 to 4.04; hand grip strength: 7.65 μm/kg, 1.53 to 13.77; physical work capacity: 1.04 μm/watt, 0.27 to 1.81) and subchondral bone area (long jump: β=4.25 mm2/cm, 95% CI 1.01 to 7.50; hand grip strength: 19.89 mm2/kg, 8.23 to 31.55; leg strength: 3.32 mm2/kg. 1.25 to 5.40; physical work capacity: 3.00 mm2/watt, 1.54 to 4.45). These associations largely became non-significant after adjusting for lean mass.

Conclusion: Physical fitness measures were positively associated with knee cartilage thickness and subchondral bone area in young adults, and these associations were largely mediated by lean mass.

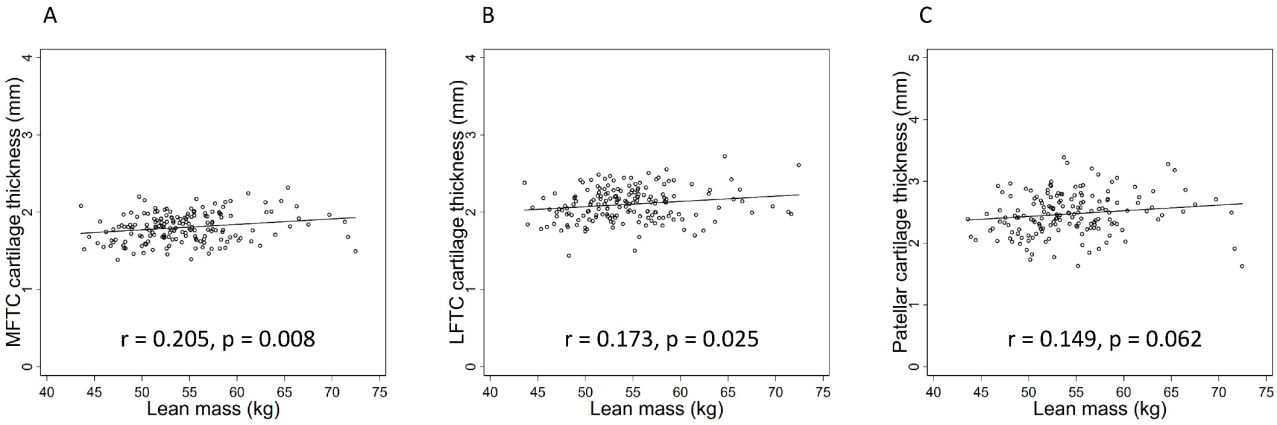

Figure. Scatter plots and linear regression lines for associations between lean mass and knee cartilage thickness (A: MFTC; B: LFTC; C: Patella).
Linear regression lines are from models adjusted for age, duration of follow-up, gender, knee injury and height.
MFTC, medial femorotibial compartment; LFTC, lateral femorotibial compartment.

P54

Bone-Specific physical activity associations with peripheral quantitative computed tomography derived measures of bone density and area

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The Bone-specific Physical Activity Questionnaire (BPAQ) is designed to provide a bone-relevant index of physical activity participation according to the mechanical loads borne across the lifespan. We have previously demonstrated positive associations between BPAQ scores and DXA-derived BMD and ultrasound-derived broadband ultrasound attenuation; however, it was unknown if relationships exist for peripheral quantitative computed tomography (pQCT) derived measures of bone density and area.

Aim: To examine relationships between historical bone-relevant physical activity and pQCT-derived parameters of bone density and area across the age span.

Methods: We recruited 532 healthy volunteers (277 males and 255 females) across a broad age-range (4-97 years). Peripheral quantitative computed tomography (XCT-3000, Stratec) was used to measure volumetric bone density and area of the non-dominant tibia at the 4%, 38%, and 66% sites and the non-dominant radius at 4% and 66% sites. Physical activity participation was recorded with the BPAQ to derive a past BPAQ (pBPAQ) score reflecting exercise loading history from birth. Pearson correlation analyses were used to examine relationships between pBPAQ scores and pQCT parameters adjusting for bone length to account for differences in body size.

Results: For the whole group, pBPAQ scores were associated with total density at 38% and 66% tibial sites and the 66% radial site ($r=0.145–0.261$, $p<0.05$) and total area at the 38% tibial site and 4% and 66% radial sites ($r=0.129–0.156$, $p<0.05$). For males, pBPAQ scores were associated with total density and area at all sites ($r=0.195–0.430$, $p<0.05$). For females, only total density at the 38% tibial site and total area at the 4% radial site were associated with pBPAQ scores ($r=0.179–0.185$, $p<0.05$).

Conclusion: Historical bone-relevant physical activity is associated with pQCT-derived parameters of tibial and radial bone density and area, particularly for men. Such results reinforce earlier findings of BPAQ associations with bone mass.

P55

Histone methylation involved in temporomandibular joint osteoarthritis

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2. National Center for Geriatrics and Gerontology

Temporomandibular joint osteoarthritis (T1MJOA) morbidity increases with age. Condylar articular cartilage degradation, which causes TMJOA, is suggested to be involved in articular chondrocyte metabolic imbalance as well as the whole body joints. Several studies implicated that cartilage homeostasis is related to epigenetic regulation such as DNA and histone chemically modification, however few studies have focused on condylar cartilage. In this study, we observed that chondrocyte H3K9 methylation level was decreased in degenerated condylar articular cartilage. Treatment with chaetocin, selective H3K9 methylation inhibitor, decreased the expression level of Sox9 and col Ⅱ , which are known as an early chondrogenic marker, in ATDC5 mouse chondroprogenitor cells. However, the expression level of colX and MMP-13, hypertrophic markers, was increased. Additionally, cell viability was reduced and caspase3/7 activity was promoted by chaetocin treatment. These results show that decrease of histone H3K9 methylation relate to condylar cartilage degradation, induction of late stage chondrocyte differentiation and negative regulation of chondrocyte homeostasis in ATDC5 cells. These results suggested that condylar articular cartilage degradation is involved in epigenetic regulation in TMJOA.

P56

Pain at multiple sites is associated with prevalent and incident fractures in older adults: a 5.1-year follow-up study

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This study aimed to examine the association between pain at multiple sites and fractures, and to explore whether this association is independent of falls risk and bone mineral density (BMD).

Data from a population-based study (mean age 63 years, 51% female) were utilized with measurements at baseline (n=1086), 2.6 (n=875) and 5.1 years (n=768). Presence/absence of pain at the neck, back, hands, shoulders, hips, knees and feet was assessed by questionnaire. Fractures were self-reported at each time-point. BMD was measured by Dual-energy X-ray absorptiometry. Baseline demographic, lifestyle, and falls risk assessment were obtained.

A total of 385 fractures were reported at three time-points and 86 incident fractures were observed over 5.1 years. Greater number of painful sites was associated with higher fracture prevalence at any site, and fractures occurring at the vertebral, non-vertebral and major sites (including the femur, radius, ulnar, vertebrae, rib and humerus) in univariate and multivariable analyses with adjustment for age, sex, body mass index, smoking history, physical activity, pain medication, BMD and falls risk. There was a dose-response relationship between number of painful sites and fracture prevalence at these sites (all P for trend <0.05). In addition, there was a dose-response relationship between number of painful sites and the risk of incident fractures occurring at any sites (relative risk (RR) 1.4, 95% CI 1.1-1.8) and major sites (RR 1.9, 95% CI 1.2-2.9) over 5.1 years after adjustment for confounders. No significant association between number of painful sites and prevalent and incident hip fractures was observed possibly due to the small sample size (7 prevalent and 3 incident hip fractures).

In conclusion, pain at multiple sites is associated with higher risk of prevalent and incident fractures, which is independent of falls risk and BMD, suggesting that widespread pain may be an independent marker of higher fracture risk.

P57

Factors secreted from MLO-Y4 osteocyte-like cells under inflammatory conditions inhibit C2C12 myoblast differentiation

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The presence of healthy muscle adjacent to fracture sites accelerates healing. It has been suggested that muscle progenitor cells that can differentiate into osteoblasts are recruited to the fractured bone. We hypothesised that in the inflammatory environment that develops at the early stages of fracture healing, osteocytes secret factors that inhibit differentiation of myoblasts into myotubes, thus allowing their subsequent trans-differentiation into osteoblasts.

MLO-Y4 osteocyte-like cells were cultured in 6-well plates and C2C12 myoblasts were seeded on inserts (ThinCert™). Inserts were placed over the MLO-Y4 cultures and IL1β was added at 10ng/mL. Four days later, C2C12 cells were fixed and stained with fluorescently-labelled myosin antibody and DAPI, and fusion index determined. Gene expression was

analysed using real-time PCR with TaqMan® assays. The results present values pooled from three independent biological repeats.

The differentiation into myotubes was similar when C2C12 cells were cultured alone, treated with IL1β, or co-cultured with MLO-Y4 cells (fusion index, mean±SEM: 27.9±2.7, 20.4±2.9 and 30.6±4.1, respectively). However, in the presence of both MLO-Y4 and IL1β the fusion index was much lower; 9.35±2.7, (P=0.02 vs. untreated, one-way ANOVA, Bonferroni's post-hoc). Comparison of gene expression in C2C12 cells treated with IL1β and cultured alone or in co-culture with MLO-Y4, found lower expression of muscle differentiation markers in co-cultured cells: myogenin (Myog) was 2.4-fold lower and myosin heavy polypeptides Myh1 and Myh8 were each 8.7-fold lower. When the experiments were repeated with TNFα (40ng/mL) instead of IL1β, the expression of Myog, Myh1 and Myh8 was13.7, 9.3 and 10.9-fold lower, respectively, in co-cultures.

We conclude that under inflammatory conditions, MLO-Y4 cells secrete factors that inhibit the differentiation of C2C12 myoblasts into myotubes. This inhibition may model an initial step in the process of recruitment of muscle progenitor cells that can further differentiate into osteoblasts and contribute to bone repair.

P58

Vegetable diversity, injurious falls and fracture risk in older women: a prospective cohort study

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Edith Cowan University
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Aim: Increasing vegetable intake is one of the cornerstones of dietary recommendations for better health. Benefits of increasing vegetable intake may include lower fracture risk. Despite vegetables containing a variety of nutrients and phytochemicals, the importance of vegetable diversity is less clear, especially in relation to injurious falling and fractures. This study examined the relationship between vegetable diversity and injurious falls and fractures leading to hospitalisation in a prospective cohort study of elderly Australian women (n=1429, ≥ 70 years).

Methods: Vegetable diversity was quantified by assessing the number of different vegetables consumed daily. Vegetable intake (75g serves per day) was estimated using a validated food frequency questionnaire at baseline (1998). Over 14.5 years, injurious falls (events=568, 39.7%) and fractures (events=404, 28.3%) were captured using linked health records.

Results: In multivariable-adjusted Cox regression models, women with greater vegetable diversity (per increase in one different vegetable per day) had lower relative hazards for injurious falls HR0.92; 95%CI(0.86-0.99) and fractures HR0.91; 95%CI(0.84-0.99). Overall, greater vegetable diversity (≥5 per day) was associated with lower hazards for injurious falls HR0.77; 95%CI(0.63-0.95) and fractures HR0.78; 95%CI(0.61-0.99) compared to lower diversity (≤3 per day). However, a significant interaction between daily vegetable diversity and vegetable intake was observed for injurious falls (pinteraction=0.03) and fractures (pinteraction<0.01). Specifically, the benefits of increased vegetable diversity were observed in those with the lowest vegetable intake (<2.2 serves per day), with a decrease in risk for an injurious fall HR0.83; 95%CI(0.71-0.98) or fracture HR0.74; 95%CI(0.62-0.89) for every different additional vegetable consumed.

Conclusion: Higher vegetable diversity was associated with lower injurious fall and fracture risks in older women with low vegetable intake. Public health recommendations focussing on increasing vegetable intake should consider promoting vegetable diversity to benefit those with the lowest vegetable intakes, especially in relation to injurious falling and fracture prevention.

P59

Relationship between Vitamin D status during different developmental stages and body composition in young adults

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2. University of Tasmania
3. Telethon Kids Institute
4. University of Notre Dame
5. Curtin University

Aim: Vitamin D plays a role in differentiation and metabolism of skeletal muscle cells, and may regulate the differentiation and metabolism of adipose tissue, but it is not clear if vitamin D status during growth influences body composition in early adulthood. We examined associations between vitamin D status in childhood, adolescence and young adulthood with body composition in young adults in a pregnancy cohort.

Methods: Participants were offspring of the Western Australian Pregnancy Cohort (Raine) Study. Serum 25-hydroxyvitamin D (25(OH)D) was measured at age 6, 14, 17 and 20, and body composition assessed using dual-energy x-ray absorptiometry (DXA) at age 20. We studied 821 participants (385 females) who had ≥3 serum 25(OH)D results and body composition data. Latent class growth analysis identified four vitamin D status trajectories: consistently lower (n=259), decreasing (n=125), increasing (n=138) and consistently higher (n=299). Analyses were adjusted for covariates including age, seasonality, TV watching, physical activity, energy and protein intake and in females, age at menarche and oral contraceptive use.

Results: In males, serum 25(OH)D at 17 and 20 years positively associated with lean body mass (LBM), and 25(OH)D at 20 years negatively correlated with fat body mass (FBM). In men in the consistently higher vitamin D trajectory, LBM at age 20 was 2.3-3.7 kg higher, and FBM 4.1-6.0 kg lower than in men with consistently lower or decreasing vitamin D trajectories (all $P < 0.05$). In females, serum 25(OH)D at 14, 17 and 20 years negatively associated with FBM. In women with increasing or consistently higher vitamin D trajectories, FBM at age 20 was 5.2-6.8 kg lower than in those with consistently lower vitamin D status (all $P < 0.05$).

Conclusion: Better vitamin D status during adolescence was associated with higher lean mass in males, and lower fat mass in both sexes at 20 years of age.

P60

Ten-year continued nonvertebral fracture reduction in postmenopausal osteoporosis with denosumab treatment

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Aim: Evaluate if reduction in nonvertebral fracture (NVFx) rate previously observed between years 4–7 compared with the first 3 years of denosumab treatment was maintained through 10 years in FREEDOM Extension (EXT) long-term group.

Methods: During FREEDOM, subjects were randomised to placebo or denosumab 60 mg Q6M for 3 years. Subjects who missed ≤1 dose could enrol in the 7-year EXT, all subjects were to receive open-label denosumab; up to 10 years (long-term) or up to 7 years (crossover). NVFx rate the first 3 years of denosumab was compared with
1) years 4–7 of denosumab in long-term and crossover groups separately and combined, and
2) years 4–10 of denosumab in the long-term group.

Results: 4,550 subjects enrolled in the EXT (N=2,343 long-term; N=2,207 crossover). NVFx rate (95% CI) in the long-term group was 1.98 per 100 subject-years (1.67–2.34) the first 3 years of denosumab and 1.54 (1.29–1.83) years 4–7 (Rate Ratio [RR] 0.79, p= 0.046). NVFx rate in the crossover group was 2.37 (1.97–2.84) the first 3 years of denosumab and 1.52 (1.24–1.87) years 4–7 (RR 0.65, p=0.002). NVFx rate in the combined group was 2.15 (1.90–2.43) the first 3 years and 1.53 (1.34–1.75) years 4–7 (RR 0.72, p<0.001). The long-term group NVFx rate was 1.44 (1.24–1.66) years 4–10 (RR 0.74 vs first 3 years, p=0.008). For 6,089 subjects exposed to denosumab in FREEDOM or EXT, the rate of bone safety events (ONJ or AFF) was 4.2 per 10,000 subject-years.
Conclusions: Compared with the first 3 years of denosumab treatment, longer duration of denosumab therapy was associated with a further decrease in NVFx rate through 10 years. Long-term reduction in bone remodelling is associated with cntinued increases in BMD and a favorable benefit/risk profile for bone.

Table. Comparison of nonvertebral fracture rates up tp 10 years of denosumab treatment

	First 3 years of denosumab treatment	Years 4-7 of denosumab treatment	Years 4–10 of denosumab treatment
Long-term subjects (N = 2,343)	140 fractures	126 fractures	184 fractures
Fracture rate (95% CI)	1.98 (1.67–2.34)	1.54 (1.29–1.83)	1.44 (1.24–1.66)
Rate Ratio (95% CI)	[Referent]	0.79 (0.62–1.00)	0.74 (0.60–0.93)
p-value		$p = 0.046$	$p = 0.008$
Cross-over subjects (N = 1,731)	123 Fractures	91 Fractures	
Fracture Rate (95% CI)	2.37 (1.97–2.84)	1.52 (1.24–1.87)	
Rate Ratio (95% CI)	[Referent]	0.65 (0.50–0.86)	
p-value		$p = 0.002$	
Long-term and cross-over subjects combined (N = 4,074)	263 Fractures	217 Fractures	
Fracture rate (95% CI)	2.15 (1.90–2.43)	1.53 (1.34–1.75)	
Rate Ratio (95% CI)	[Referent]	0.72 (0.61–0.86)	
p-value		$p < 0.001$	

P61

Denosumab reduced bone remodelling, eroded surface and erosion depth in cortical bone of iliac crest biopsies from postmenopausal women in the FREEDOM

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Aim: Report the effects of denosumab versus placebo on cortical bone histomorphometry from iliac crest bone biopsies obtained during FREEDOM trial.

Method: A total of 112 biopsies were evaluable for cortical bone histomorphometry, including 67 obtained at month 24 (37 placebo, 30 denosumab) and 45 at month 36 (25 placebo, 20 denosumab). Both cortices were analysed, if available. Endocortical (Ec) ES/BS, osteoclast surface (Oc.S/BS) and erosion depth (E.De) were assessed. Cortical porosity and Ec wall thickness (W.Th) were measured. Dynamic remodelling parameters were assessed for endocortical, periosteal and intracortical envelopes.

Results: The Ec structural variables, Oc.S/BS, ES/BS and mean and maximal E.De, were significantly lower in the denosumab group versus placebo at months 24 and 36 (Table). There were no significant differences between denosumab and placebo groups for W.Th, cortical porosity or cortical thickness. No extensive search for cortical fluorochrome labels was done, unlike prior trabecular analyses1, and cortical labels in at least one cortical envelope were observed in 13 (43%) and 10 (50%) of denosumab biopsies at months 24 and 36, respectively. Though envelope-specific dynamic bone formation and remodeling parameters could not be reliably assessed, the overall labeling findings indicate that densoumab markedly reduced cortical bone turnover.

Conclusion: These data are consistent with the mechanism of action of denosumab. Lower Ec eroded surfaces with denosumab may reflect inhibited bone resorption and refilling of resorption spaces. Reduced cortical labelling and bone formation indices with denosumab may reflect refilling of resorption spaces that were present before fluorochromes were administered, and reduced activation of new remodelling sites. Reduced E.De is a novel finding for denosumab that may contribute to increased bone strength by reducing Ec bone loss and structural vulnerabilities associated with deep resorption cavities.

[1] Reid IR, et al. J Bone Miner Res. 2010;25:2256.

Table: Summary of cortical histomorphometry parameters at months 24 and 36

	Month 24		Month 36	
Parameter	Placebo (n = 37)	Denosumab (n = 30)	Placebo (n = 25)	Denosumab (n = 20)
Ec.Oc.S/BS (%)	0.26 (0.00, 0.59)	0.00 (0.00, 0.00)*	0.14 (0.00, 0.22)	0.00 (0.00, 0.00)†
Ec.ES/BS (%)	4.39 (3.26, 7.60)	1.59 (1.01, 2.17)*	3.53 (1.92, 5.32)	1.39 (0.67, 3.52)†
Maximum Ec.E.De (µm)	16.45 (12.88, 21.61)	11.43 (9.00, 13.11)*	17.40 (12.13, 21.01)	14.04 (6.92, 15.99)†
Mean Ec.E.De (µm)	10.26 (8.32, 13.34)	7.10 (6.03, 8.69)*	10.14 (7.39, 12.87)	7.91 (4.94, 9.65)†
Mean Ec.W.Th (µm)	32.41 (31.16, 33.83)	32.39 (31.68, 34.52)	32.11 (30.48, 33.05)	31.82 (29.30, 33.41)
Cortical thickness (µm)	749.3 (572.0, 904.9)	753.6 (521.3, 1022.0)	689.7 (478.7, 920.4)	605.8 (491.0, 799.3)
Cortical porosity (%)	6.43 (4.56, 9.34)	5.90 (3.83, 8.12)	5.42 (3.77, 7.42)	6.37 (4.39, 7.88)

Data represent median (Q1, Q3); n = number of subjects evaluable for cortical bone histomorphometry

*$P < 0.0001$, †$P < 0.05$, Wilcoxon rank sum test (denosumab versus placebo).

P62

Bone matrix mineralisation after denosumab treatment discontinuation

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Aim: Denosumab discontinuation is associated with reversibility of BMD and bone turnover markers. To understand the effects of reversibility on mineralisation, we evaluated bone matrix mineralisation in transiliac bone biopsy samples.

Methods: A non-interventional cohort study enrolled subjects who received 12 months of denosumab in clinical trials and agreed to undergo bone biopsy after ≥12 and ≤36 months off-treatment. Mean degree of mineralisation (DMB) and the heterogeneity index (HI) were calculated. Bone biopsy samples at 2 or 3 years in the FREEDOM trial from 30 placebo and 42 denosumab treated subjects were used as comparator groups in this analysis.

Results: Fifteen subjects were enrolled from two parent trials. Mean (min, max) age: 62.1 (54, 73) years; years since menopause: 15.8 (6, 30); and time since discontinuation of DMAb: 25 (21, 29) months. Median (min, max) total bone DMB was 1.080 g/cm3 (1.001, 1.123) versus 1.055 g/cm3 (0.985, 1.118) in the FREEDOM placebo group, p-value = 0.029, and 1.081 g/cm3 (0.953, 1.142) in the FREEDOM denosumab group, p-value = 0.89 (Figure). Median (min, max) total bone HI was 0.147 g/cm3 (0.128, 0.179) vs 0.144 g/cm3 (0.118, 0.199) in the FREEDOM placebo group, p-value = 0.11 and 0.128 g/cm3 (0.093, 0.194) in the FREEDOM denosumab group, p value <0.0001 (Figure). Similar results were observed in all bone compartments assessed.

Conclusion: After stopping denosumab for an average of 25 months, the DMB was similar to that observed after 2 or 3 years of denosumab treatment in FREEDOM, indicating a sustained benefit of denosumab after discontinuation. The HI was higher than that observed after 2 or 3 years of denosumab treatment, which may reflect a transient increase in remodelling activity. These data suggest that significant changes in the degree of mineralisation were not observed after denosumab cessation as bone remodelling returned to pretreatment levels.

P63

Meta-analysis of four clinical trials of denosumab compared with bisphosphonates in postmenopausal women previously treated with oral bisphosphonates

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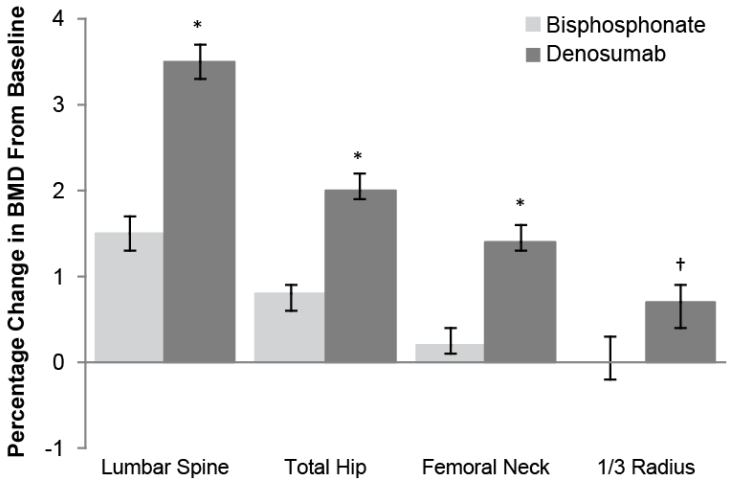
Aim: Meta-analysis of randomised studies comparing the safety and efficacy of transitioning to denosumab (DMAb) versus continuing on oral bisphosphonates (BP).

Methods: Data were pooled from four randomised studies in postmenopausal women with low bone mass or osteoporosis, aged ≥55 years and pretreated with oral BPs, who were randomised 1:1 to DMAb or a BP (oral or IV) for 12 months. Percentage change from baseline in BMD at the lumbar spine, total hip, femoral neck and 1/3 radius at month 12; Percent change from baseline in serum CTX (sCTX) at 1, 6, and 12 months; and safety were assessed. Fractures were collected as adverse events (AEs) and not adjudicated.

Results: 2,850 subjects were included (1,426 DMAb; 1,424 BP). Mean (SD): age 68(±8) years, lumbar spine BMD T-score –2.5(±1.0); duration of prior oral BP use 3.8(±3.6) years. BMD percent change from baseline at month 12 was significantly greater with DMAb vs BPs at all measured skeletal sites (figure) and independent of length of prior BP use (<2 or ≥2 years) at all sites measured (except for 1/3 radius for those with <2 years of prior BP use). Median sCTX (n=1,058) percent decrease from baseline was greater with DMAb than BPs at months 1 (–58% vs –12%), 6 (–36% vs –14%), and 12 (–26% vs 8%; all p<0.0001). Overall AEs/serious AEs were similar between groups. There were no cases of osteonecrosis of the jaw. Three events consistent with atypical femoral fracture were observed (2 DMAb; 1 BP). Osteoporosis-related fractures were reported in 47 (3.3%) DMAb and 43 (3.1%) BP subjects.

Conclusions: This integrated assessment shows greater clinical benefit with increases in BMD and reductions in bone turnover and similar safety profile in transitioning from oral BPs to DMAb, compared with continuing on or cycling through the same therapeutic class.



*p<0.0001 and †p=0.001 denosumab vs bisphosphonate.

Data are least-squared means and 95% confidence intervals based on an ANCOVA model adjusting for treatment, duration of prior bisphosphonate use, baseline BMD, study, DXA machine type, and baseline-BMD-value-by-machine–type interaction.
1/3 radius was assessed in 2 of the 4 studies.

Funding: Amgen Inc.

P64

An In Vitro Model For The Study Of Cell Responses To The Structure And Mechanics Of Tendon Degeneration

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All cells exist within a 3D microenvironment where they are exposed to a multitude of mechanobiological cues, from nano-level cell/matrix interactions, to tissue-level mechanical strain. These cues are fundamental to maintaining tissue homeostasis, but when disrupted during disease these cues can promote pathological outcomes and disrupt healing.

Tendons are load-bearing connective tissue structures, organised in a highly aligned manner, and maintained by tendon cells (tenocytes). During disease, tendon structure and mechanics, which are fundamental to maintaining tendon tissue homeostasis, are severely altered and this changes the mechanobiological stimuli experienced by the tenocytes.

Understanding the contribution these stimuli play in tendon pathology is important when designing treatment strategies. Thus, the aim of this study was to develop an in vitro model of tendon degeneration which mimics the changes observed in patients with tendon disease.

Bovine Achilles tendon was cut into 300µm slices and decellularised in nuclease solution for 48h. Degeneration was induced by progressive treatment with degradative enzymes and compared to degeneration in human tendon obtained from orthopaedic surgery. Atomic force microscopy was used to map changes in substrate Young's modulus, the orientation of collagen fibres within treated slices was measured using ImageJ software and tensile strain was measured using an instron machine. Primary human tenocytes were cultured on the degenerated slices for 24h and cell morphology was assessed using confocal microscopy.

Control slices (no degeneration) retained a high level of aligned fibres, and a Young's modulus similar to healthy human tendon. Progressively degenerated slices structurally and biomechanically mimicked diseased samples obtained from human patients (Fig.). Tenocytes cultured on control slices retained an elongated tenocyte-like morphology, which was lost on the degenerated slices.

This novel in vitro model of tendon degeneration mimics human disease states and allows the study cell responses to tissue matrix with varying structural organisation and substrate stiffness.

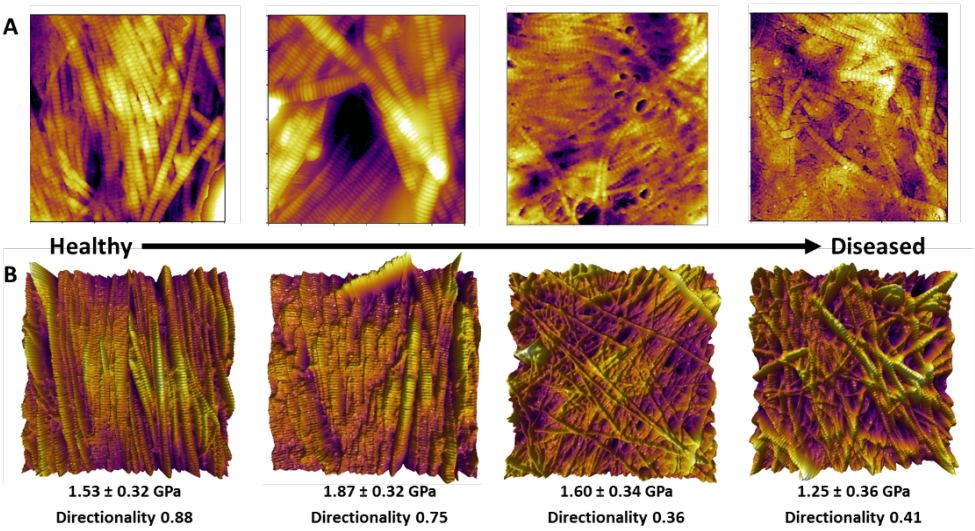

Figure. A: Atomic force microscopy (AFM) images of tendon biopsies obtained from patients undergoing orthopaedic surgery, with increasing degrees of disease severity from left to right. **B:** AMFM images of tendon slices created in our lab aiming to mimic healthy tendon (left) and increasing levels of tendon disease similar to those observed in the clinical samples.
Mean Young's modulus of the tendon slices and directionality measurements are presented below the representative images with a higher directionality indicative of a more organised structure.

P65

Biomimetic Multilayered Nano-Fibrous Scaffolds with Controlled Delivery of BMP-6 Peptide for Guided Bone Regeneration

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Effective regeneration of large defects associated with trauma and tumour resection in load-bearing bones remains a significant clinical problem. Biomaterial implants resembling biological and structural features of the native bone, with the addition of localised growth factor delivery, offer a viable alternative to bone grafts. In this work, we have utilised a modular tissue engineering approach to firstly fabricate aligned poly(lactic-co-glycolic acid) (PLGA) bioactive electrospun fibers reinforced with rod-shape nano-hydroxyapatite (n-HA), in an effort to replicate osteon-like structures. These aligned PLGA nanofibers, once modified with highly oriented n-HA (PLGA-HA), display significant

improvements in mechanical properties, surface roughness and hydrophilicity. The niche-mimicking architectural features of both PLGA and PLGA-HA modified nanofibers wereconfirmed to promote superior osteogenic commitment from human bone marrow mesenchymal stem cells (hBMSCs) under osteogenic differentiation conditions, with substantial upregulation of osteogenic genes and increased production of osteogenic proteins and mineral deposition, compared to controls. To further enhance differentiation outcomes, we introduced a poly(ethylene glycol) (PEG) based-hydrogel layer atop of the PLGA-based electrospun fibrous scaffold, creating a bilayered construct. The covalent incorporation of a BMP-6 peptide within this hydrogel layer significantly enhanced both early and late osteogenic commitment compared to the mono-layer scaffolds, as confirmed through gene expression, alkaline phosphatase production, protein secretion and mineral deposition. Moreover, the incorporation of the BMP-6 peptide into the bilayered scaffold was able to induce osteoblastic differentiation of hBMSCs in growth medium alone, without the addition of any osteogenic-inducing factors to the media. In vivo assessment of these scaffolds is currently underway. The outcomes of this study demonstrate that these novel multi-layered, composite nanofiber scaffolds presenting native bone mimicking biochemical and biophysical features are promising candidates for engineered bone tissue repair.

P66
Cdc42 plays important roles in postnatal angiogenesis and bone formation

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Aim: Cdc42, Rho family low molecular weight G protein, plays important roles in various functions of cells such as cytoskeletal rearrangement, cell adhesion and cell proliferation. To investigate how Cdc42 is involved in the action of vascular endothelial cells on bone formation, we generated Cdc42 conditional knockout mice in which Cdc42 was time-specifically deficient in vascular endothelial cells.

Methods: We used CadCreER mice expressing the recombinant enzyme Cre specifically in the vascular endothelial cells under the control of the VE-cadherin promoter by tamoxifen administration, and mated with Cdc42 flox mice (Cdc42fl/fl; Cad-CreER: Cdc42 cKO). Tamoxifen was administered intraperioneally at a dose of 100μg/mouse at postnatal day 0 (P0) and P1.

Results and Discussion: Mice in which Cdc42 was deleted in vascular endothelial cells at embryonic stage are lethal (Berry D. Development, 142, 3058-3070, 2015). Therefore, when the Cdc42 gene was deleted after birth, Cdc42 cKO mice were smaller than the control mice, and died between P8 and P10. Our findings confirmed that these mice had various pathological aberrances in the vessels of most organs, such as hemorrhage and blood flow congestion, as well as invasion of blood cells. Electron microscopic observation revealed that capillary endothelial cells became detached from the basement membrane as well as phagocytosis of dead endothelial cells by macrophages. Regarding to bone formation, tissue analysis of the femur showed that the Cdc42 cKO mouse had shorter femur bones, columnar disorganization of chondrocytes in the growth plate, morphological abnormality of the hypertrophic chondrocyte as compared to the control Sin the formation of blood vessels and bone formation after birth.

P67
Are there sex-specific associations with bone mass in relation to muscle parameters in a paediatric population?

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Post puberty, bone mass displays clear sex-specific patterns. However, some research has suggested that a sexual dimorphism in bone mass is evident in younger children and is likely attributable to differences in lean mass. Thus, we

aimed to determine whether the association with both overall muscle mass and/or muscle strength was different between the sexes in a paediatric population.

Participants were children recruited as part of the Vitamin D in Pregnancy Study (median age: 10.9 (IQR 10.2-12.1yr). Of the 402 children measured at birth, 209 (52.3%) returned for 11-year follow-up, and 172 had complete data for the current analyses. Children had an assessment of bone mineral content (BMC), bone mineral density (BMD) and lean mass by DXA (Lunar). Handgrip strength was measured using a dynamometer (JAMAR). Linear regression models were adjusted for height, weight, age and pubertal stage.

Compared to girls, boys had a trend for greater lean mass (29.3 vs 28.1kg, p=0.06), and had greater handgrip strength (15.7 vs 15kg, p=0.01). Sex interaction terms were significant in models predicting bone mass. When stratified by sex, both muscle strength and total lean mass were associated with BMD and BMC for boys and girls. In adjusted models, including both muscle strength and lean mass, the observed association differed between boys and girls. At the spine in boys, BMC and BMD were associated with muscle strength (β 0.34 [95%CI 0.09-0.59] and 0.008 [95%CI 0.003-0.014]; respectively) but not total muscle mass. In girls, spine BMC and BMD were associated with total lean mass (β 0.95 [95%CI 0.61-1.3] and β 0.01 [95%CI 0.005-0.02]; respectively). The same pattern of association remained when those who self-reported being in a higher pubertal stage were removed from the analyses.
A clear sexually dimorphic pattern in bone mass was exhibited in association with muscle parameters in this paediatric cohort.

P68
Effect of phosphorus concentration on osteocyte senescence

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Purpose: Phosphorus, which is requisite for homeostasis, has been thought to acceralate human senescence. Aging has been demonstrated to increase chronic kidney disease, which induces hyperphosphatemia. Consequently, the risk of bone fractures is increased. However, the effects of phosphorus on osteocyte senescence are unclear. The aim of this study was to investigate the effects of phosphorus concentration on osteocyte senescence.

Materials and Methods: C57B6L/6J mice (3-week-old) were used. Mice were divided into 3 groups. A low phosphorus diet (P; 0.2%, Ca; 0.6%) (LPD), normal phosphorus diet (P; 0.6%, Ca; 0.6%) (control), and high phosphorus diet (P; 1.73%, Ca; 0.58%) (HPD)were fed with each group (n=7 per each group). Euthanasia was performed after 1, 2, 3 and 4 weeks after the onset of phosphorus-controlled diet. Histomorphometric and immunohistochemical analyses were performed using long bones.

Results: Growth disturbance were noted in HPD vs. control and LPD. Thinner cortical bone was observed in HPD vs. control and LPD. Moreover, osteocyte cytoplasm in HPD significantly atrophied as compared with control and LPD.

Discussion: High concentration of phosphorus affected osteocyte and growth of long bones. Concentration of phosphorus may contribute to osteocyte function and aging.

P69
Fractures, Fractures, Fractures: But it's not just in the Bones

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Mr SR aged 44 years was reviewed during his hospital stay after sustaining a fragility fracture of his left femur requiring close reduction with internal fixation. The plain film satisfied the criteria for atypical femoral fracture of the left femur.

His history revealed a 15 year duration of bisphosphonate therapy used to treat his osteoporosis with 10 fragility fractures dating back to age 4 years. Mr SR's history also included congenital blindness, and absence seizures in childhood. Mr SR had a short stature of 144cm and was barrel chested with bowing of his tibias. His examination also displayed ligamentous laxity and he had white sclera. There was no family history of blindness and despite his mother and maternal grandmother both having osteoporosis, they had not sustained any fragility fractures. There was no significant identifiable lifestyle factors detrimental on bone health for Mr SR. At this point, one of the dilemmas in Dr SR's management included confirming his actual diagnosis. The working process was that of a genetic condition that linked his blindness and osteoporosis. Through further literature review, it appeared that Mr SR most likely had Osteoporosis Pseudoglioma Syndrome due to a mutation in LRP5 which would explain his presentation entirely. He consent was obtained and his genetic profile is currently awaited. In the interim, his bisphosphonate therapy was ceased and he was commenced on teriparatide. He has not sustained further fractures to date.

P70

Bone Loss Following Risk Reducing Bilateral Salpingo-Oophorectomy in Women with Increased Risk for Breast and Ovarian Cancer: Preliminary Results from WHAM Study

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Aim: This study is examining bone loss and compromised bone strength associated with surgical menopause following risk reducing bilateral salpingo-oophorectomy (RRBSO) in women with increased risk for breast and ovarian cancer.

Methods: Women following RRBSO (HR group) and age-matched controls (Control group) were recruited into the ongoing Women's Health After Surgical Menopause (WHAM) study. Dual energy X-ray absorptiometry (DXA) scans of the lumbar spine and proximal femur and peripheral quantitative computed tomography (pQCT) scans of the tibia were performed at baseline and 24 month (M) follow-up. Finite element models (FEM) based on individual pQCT cross-sections (4% and 66%) were established to assess bone strength and stiffness of different loading cases.

Results: Twenty-seven HR women following RRBSO including 10 given hormone therapy (HR-HT) and 17 with no hormone therapy (non-HT), and 30 Controls have completed 24M follow-up so far. No difference was observed in age, height, weight, DXA, pQCT or pQCT-FEM variables between groups at baseline (all $p>0.05$). Significant changes were observed in non-HT patients in DXA [-0.068 ($p<0.001$), -0.034 ($p=0.01$), -0.037 ($p=0.01$) g/cm2 for lumbar spine, total hip, femoral neck BMD, respectively], pQCT [-19.14 and -18.79 mg/cm3 (both $p=0.01$) for total BMD and cortical BMD, respectively] and pQCT-FEM [-6.71 ($p<0.01$), -4.63 ($p=0.02$), -18.99 ($p=0.02$) N for bending, compression, torsion strength at 4% tibia, respectively] variables, while changes in HR-HT patients and Controls were not statistically significant (all $p>0.05$). While no difference was observed in DXA variables between HR-HT and Non-HT patients, pQCT (2389.09 vs. 1933.54 mg/cm3 for cortical BMD, $p=0.05$) and pQCT-FEM (1673.14 vs. 1339.33 kN/mm for bending stiffness, $p=0.03$) variables were higher in HR-HT than in non-HT patients at 24M.

Conclusion: In this ongoing study, surgical menopause following RRBSO was associated with bone loss and compromised bone strength. Hormone therapy may alleviate decreases in bone density and bone strength. More data are required to validate this benefit.

Table 1. Changes in DXA, pQCT and pQCT-FEM variables between HR-HT, Non-HT and Controls

		Non-HT (n=17)			HR-HT (n=10)			Control (n=30)		
		Mean difference	%change	P	Mean difference	%change	P	Mean difference	%change	P
DXA	LS aBMD (g/cm^2)	-0.068±0.037	-6.19	<0.001	-0.006±0.028	-0.64	0.5	0.002±0.021	0.14	0.7
	TH aBMD (g/cm^2)	-0.034±0.047	-3.49	0.01	0.003±0.028	-0.36	0.7	0.0002±0.026	0.02	>0.9
	FN aBMD (g/cm^2)	-0.037±0.050	-4.11	0.01	0.008±0.028	-0.92	0.4	-0.0001±0.031	-0.01	>0.9
pQCT	Tot vBMD (mg/cm^3)	-19.14±26.87	-6.06	0.01	-8.22±22.90	-2.77	0.3	-2.61±18.19	-0.83	0.4
	Tot CSA (mm^2)	23.37±71.57	2.61	0.1	26.47±111.02	2.58	0.5	-11.16±132.56	-1.10	0.6
	Trb vBMD (mg/cm^3)	-3.55±10.93	-1.46	0.2	0.31±6.66	0.14	0.9	-3.94±24.74	-1.69	0.4
	Trb CSA (mm^2)	12.76±32.24	2.61	0.1	11.91±50.00	2.58	0.5	-5.01±59.64	-1.10	0.6
	Crt vBMD (mg/cm^3)	-18.79±24.91	-1.64	0.01	-2.09±21.22	-0.18	0.8	-1.87±15.32	-0.16	0.5
	Crt Thk (mm)	-0.06±0.37	-1.32	0.6	0.05±0.13	1.20	0.3	-0.02±0.52	-0.52	0.8
	SSI_p (mm^3)	-36.03±239.93	-1.41	0.5	-49.85±78.61	-2.27	0.1	-60.82±429.73	-2.61	0.4
pQCT-FEM	$S_{bending}$_4% (N)	-6.71±5.81	-11.75	<0.01	-1.71±5.88	-3.54	0.4	-0.48±6.92	-0.88	0.7
	$k_{bending}$g_4% (kN/mm)	-248.31±259.41	-10.39	<0.01	-5.68±241.39	-0.29	0.9	21.08±218.04	0.96	0.6
	$S_{compression}$_4% (N)	-4.63±7.31	-13.96	0.02	-5.46±9.14	-19.21	0.1	-1.62±6.15	-5.19	0.2
	$k_{compression}$_4% (kN/mm)	-141.75±166.17	-9.98	<0.01	-27.87±92.99	-2.38	0.4	-6.61±132.58	-0.50	0.8
	$S_{torsion}$_4% (N)	-18.99±29.02	-30.60	0.02	-29.81±48.30	-52.86	0.1	-1.73±34.27	-2.95	0.8
	$k_{torsion}$_4% (kN/mm)	-164.72±169.41	-9.85	<0.01	-17.59±150.43	-1.31	0.7	12.83±152.68	0.85	0.6
	$S_{bending}$_66% (N)	-5.52±14.16	-4.97	0.1	-2.97±7.33	-3.01	0.3	-2.86±10.75	-2.77	0.2
	$k_{bending}$_66% (kN/mm)	-130.46±310.88	-4.29	0.1	-80.33±119.83	-3.13	0.08	0.81±171.31	0.03	>0.9
	$S_{compression}$_66% (N)	-9.00±19.23	-9.72	0.07	-10.90±22.98	-13.38	0.2	-2.90±13.85	-3.20	0.3
	$k_{compression}$_66% (kN/mm)	-199.64±204.91	-4.89	<0.01	-30.80±91.23	-0.87	0.3	23.24±136.84	0.59	0.4
	$S_{torsion}$_66% (N)	-36.57±67.69	-25.38	0.04	-60.35±103.18	-49.96	0.1	-19.01±49.44	-13.88	0.04
	$k_{torsion}$_66% (kN/mm)	-153.46±425.32	-4.78	0.2	-75.08±121.38	-2.97	0.1	0.18±234.74	0.01	>0.9

Abbreviations LS: lumbar spine, TH: total hip, FN: femoral neck, Tot: total, Trb: trabecular, Crt: cortical, S: strength, k: stiffness

Table 2. Comparison between women on HT (HR-HT) and non-HT at 24M

		HR-HT (n=10)	Non-HT (n=17)	P
DXA	LS aBMD (g/cm^2)	1.099±0.119	1.004±0.199	0.2
	H aBMD (g/cm^2)	0.982±0.115	0.884±0.131	0.07
	FN aBMD (g/cm^2)	0.909±0.112	0.818±0.115	0.07
pQCT	Tot vBMD (mg/cm^3)	296.94±41.82	288.15±36.92	0.6
	Tot CSA (mm^2)	239.59±38.53	219.24±32.98	0.2
	Trb vBMD (mg/cm^3)	1142.18±32.93	1128.95±29.45	0.3
	Trb CSA (mm^2)	57.10±11.99	48.23±11.35	0.08
	Crt vBMD (mg/cm^3)	2389.09±526.22	1933.54±552.92	0.05
	Crt Thk (mm)	33.18±8.04	28.43±5.98	0.1
	SSI_p (mm^3)	1419.81±291.93	1172.01±199.27	0.03
pQCT-FEM	$S_{bending}$_4% (N)	62.06±14.81	56.40±17.73	0.4
	$k_{bending}$g_4% (kN/mm)	1673.14±350.46	1339.33±343.50	0.03
	$S_{compression}$_4% (N)	111.13±24.26	98.67±13.81	0.2
	$k_{compression}$_4% (kN/mm)	6041.60±913.27	2570.38±563.10	0.2
	$S_{torsion}$_4% (N)	92.51±12.95	81.44±9.46	0.03
	$k_{torsion}$_4% (kN/mm)	4083.18±515.33	3559.66±217.32	0.008
	$S_{bending}$_66% (N)	144.12±33.59	128.52±21.66	0.2
	$k_{bending}$_66% (kN/mm)	3208.19±788.69	2524.87±375.73	0.02
	$S_{compression}$_66% (N)	1.10±0.12	1.00±0.20	0.2
	$k_{compression}$_66% (kN/mm)	0.98±0.12	0.88±0.13	0.07
	$S_{torsion}$_66% (N)	0.91±0.11	0.82±0.12	0.07
	$k_{torsion}$_66% (kN/mm)	296.94±41.82	288.15±36.92	0.6

P71
Osteomalacia in sub-tropical Auckland

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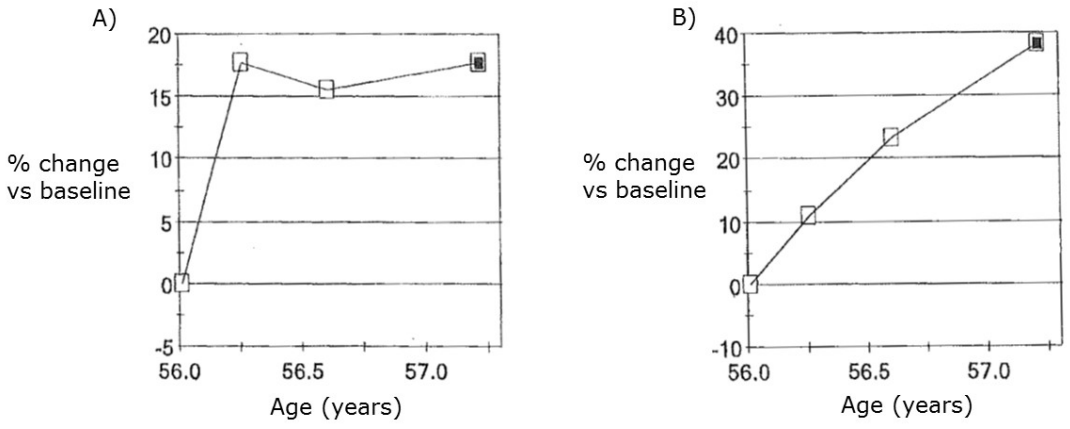
Introduction: Vitamin D deficiency is the most common cause of osteomalacia worldwide. We present a patient with typical features of osteomalacia, who also had markedly reduced bone mineral density (BMD).

Case summary: A 56 year-old man of Indian descent was referred with left sided hip pain after a fall. MRI scans demonstrated an undisplaced stress fracture in the inferomedial femoral neck and subchondral oedema within the femoral head. Bone densitometry showed T-scores of -2.0 at the spine, -3.5 at the femoral neck, and -2.4 for the total hip. Laboratory tests revealed: 25-hydroxyvitamin D < 10nmol/L (reference range 50-150nmol/L), parathyroid hormone (PTH) 35pmol/L (1.7-7.3pmol/L), calcium (albumin adjusted) 1.92mmol/L (2.10-2.55mmol/L), alkaline phosphatase 132U/L (40-120U/L), and procollagen-1 N-terminal propeptide (P1NP) 118µg/L (20-85µg/L).

A diagnosis of vitamin D deficiency-related osteomalacia was made. He was prescribed a ten-day course of calciferol 1.25mg (50,000IU)/day and started on calcium carbonate 1.25g twice daily. 14 months following treatment, his BMDs increased by 33.4% and 19.5% at the hip and spine respectively (Figure). His laboratory tests, including PTH and P1NP, normalised.

Conclusion: Our patient presented with osteomalacia associated with markedly reduced BMD. Following correction of the vitamin D deficiency his symptoms resolved, and bone biochemistry and BMD normalised. A striking feature of this patient was the presence of "osteoporosis" at presentation and the complete reversal of this within 14 months with vitamin D and calcium supplementation. Such changes are never seen with conventional pharmacological management of osteoporosis. In patients with osteoporosis, evaluation of their risk of severe vitamin D deficiency is important as treatment can rapidly reverse musculoskeletal morbidity.

Figure: % change in bone mineral density at the spine (A) and total hip (B)



P72

Dairy intakes and fractures in Australian women: a population based cohort study

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Aims: Although dairy consumption is recommended for the maintenance of healthy bones at any stage of life, evidence on the association between milk intake and risk of fracture is conflicting. This study assesses the association between milk or total dairy intake and fractures in women.

Methods: Women (n=1057, aged 20-94yr) enrolled in the Geelong Osteoporosis Study, were followed from baseline (1993-1997), to date of first fracture, death or December 31, 2017, whichever occurred first (median 20.6 years, IQR 10.9-22.1). A Cox proportional hazards model was used to determine the association between different categories of milk consumption (measured in cups per day, where 1 cup=250mL) or total dairy intakes (grams) and major osteoporotic fractures (MOF; hip, clinical spine, forearm and proximal humerus). Total dairy product intake was calculated by considering milk, cheese, yogurt and ice-cream consumption.

Results: During follow-up (14,592 person-years), 238 women had a MOF. Higher rates for fractures were found in groups who consumed < ½ cup milk per day (18.80, 95% CI 12.70-27.82) and > 4 cups of milk per day (18.08, 95% CI 6.78-48.17). Compared to women who consumed ½-1 cup milk per day, hazard Ratios (HRs) were higher in those who consumed more than 4 cups (1.60, 95%CI 0.58-4.36) and less than half a cup of milk per day (1.46, 95%CI 0.94-2.29), however, these associations did not reach significance (Table 1). Similarly, fracture rates and hazard ratios for fractures were high in grpups who consumed higher amount of total dairy produts, however, no significant aasociations were detected between these groups (Table 1).

Conclusion: Although we observed a dose-response link between high milk and dairy intakes for increasing risk of fracture, the associations was not significance. This study shows that neither high or low milk nor total dairy intakes are associated with increased fracture risks.

Table 1: Fracture rates(per 1000), unadjusted and age-adjusted hazard ratios (HR) for major osteoporotic fractures in different milk consuming categories and total dairy products consuming categories with their 95% confidence interval.

Milk					
Categories	Rate (per 1000)	Unadjusted HR	*P values*	Age adjusted HR	*P values*
Less than half	18.80 (12.70-27.82)	1.34 (0.86-2.10)	0.19	1.46(0.94-2.29)	0.09
½-1 cup	14.10(11.44-17.37)				
1-2 cups*	14.93(11.98-18.61)	1.06(0.79- 1.44)	0.69	1.03(0.76- 1.40)	0.86
3-4 cups	17.90(12.27-26.11)	1.28(0.83- 1.98)	0.26	1.18(0.77- 1.81)	0.46
>4 cups	18.08(6.78-48.17)	1.33(0.49-3.65)	0.56	1.60(0.58-4.36)	0.36
Toatl Dairy Proucts					
Refernce range	Rate	Unadjusted HR	*P values*	Age adjusted HR	*P values*
<200 g	14.55(11.00-19.52)	0.95(0.67- 1.35)	0.78	0.98(0.69- 1.40)	0.92
200-300 g*	15.07(12.21-18.59)				
400- 799 g	15.52(12.34-19.52)	1.04(0.76-1.42)	0.82	1.19(0.87- 1.63)	0.27
>800 g	17.76(10.31-30.58)	1.17(0.65-2.08)	0.60	1.19(0.66- 2.13)	0.56

½ cup- 1-cup* - reference category for milk consumtion
200-300 g* - reference category for total dairy consumtion

P73

In Vitro and In Vivo Assessment of Poloxamers as a Drug Delivery System for Bone Regeneration

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Poloxamers are copolymer hydrogels used for a wide range of applications in clinic, and are commonly studied for targeted drug delivery. Lactoferrin (LF) is a large glycoprotein which is proven to enhance bone regeneration. However, no studies have assessed the ability of poloxamers to deliver LF. The aim of this study was to determine the potential of poloxamers as a delivery system for the LF, both in vitro and in vivo.

The release of LF from poloxamers was measured by HPLC. LF-loaded poloxamers (LF/poloxamers) were placed in tissue culture inserts suspended above cultures of primary rat osteoblasts, and viability assessed after 72hrs using alamarBlue® assay. Immune response was assessed by qPCR with THP-1 cells after 24 and 48hrs with LF/poloxamers. For in vivo, 5mm diameter critical-size defects were created over the parietal bone in 40 Sprague–Dawley rats, which were randomised into empty defects, and defects grafted with LF/poloxamers (20 ug/gel). The rats were sacrificed at 4 or 12 weeks post-operation, calvariae were dissected and scanned by microCT.

During the first 8hrs, 50% of the LF was released from the poloxamers, with the remaining released over 72hrs. Poloxamers, with and without LF, increased cell viability at 72hrs ($p<0.001$). LF/poloxamers had no effects on the IL1b and IL8 gene expressions, whereas TNF expression was higher than the controls group at 24hrs, but was back to control levels at 48hrs. MicroCT analysis showed that the LF/poloxamers had no effect on bone volume (BV) and tissue mineral density at 4 weeks post-operation, however, at 12 weeks the LF/poloxamers group had lower BV than the controls ($p<0.05$).

LF/poloxamers were cytocompatible and support cell viability in vitro. However, LF/poloxamers did not improve bone regeneration in vivo. Therefore, the current formulation of Poloxamers may not be suitable for LF delivery in rat calvarial defect model.

P74

Effects of increased calcium intake on mouse serum profile and gene expression.

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Aim: Calcium supplements are associated with an increased risk for cardiovascular disease in postmenopausal women. To elucidate the mechanism we investigated whether calcium supplements increased vascular calcification, altered the serum profile and produced changes in gene expression in a mouse model of accentuated inflammation.

Method: Adiponectin knockout mice were randomized into 2 studies and then into 2 treatment groups: Calcium Supplement study and Diet study each comprising 38 mice. In the former mice were fed a calcium supplemented or placebo jelly 5 days per week. In the latter mice were fed high calcium chow (HCC) or normal chow (NC). After 32 weeks we analysed sera and genes implicated in vascular calcification. We looked at between group differences and then to understand whether anatomical heterogeneity was present we analysed gene expression in each mouse with anatomical site as a repeated measure using a mixed model two-way analysis of variance.

Results: In the Diet study there were significant between group differences in both serum calcium (HCC 2.68, NC 2.37; $p=0.0036$) and phosphate (HCC 1.41, NC 2.07 $p=0.0015$). In the Calcium Supplement study there were significant between group differences in serum phosphate (calcium 1.61, placebo 2.28; $p=0.0007$) but not calcium. There were no significant between group differences in calcium deposition in the aorta or heart in both studies. Heterogeneity in gene expression was observed in all genes analysed. Furthermore, in the calcium supplement study there were significant allocation by treatment interactions for ENPP1 and MGP and cardiac MGP expression in the calcium group was 3-4x that of the placebo.

Conclusion: Altered serum calcium and phosphate levelswere observed in all mice fed calcium but only bolus calcium supplemented jelly meaningfully altered expression of genes related to calcification, suggesting that calcium intake has the potential to change vascular health.

P75
Reframe Osteoporosis Clinical Audit: Study Design and Methods

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Aims: The aim of the audit is to assess Australian GPs' management of osteoporosis compared with RACGP guidelines (i.e. Osteoporosis risk assessment, diagnosis and management algorithm published by the Royal Australian College of General Practitioners).

Method: ReEvaluating Fracture Risk Assessment, Management & Education (REFRAME) is a chronic disease management (CDM) program that is designed to help primary care professionals identify patients at risk of osteoporosis and manage them appropriately. Practices implementing the CDM program can choose to participate in the REFRAME Clinical Audit.

The patients (men and women) being studied fall into two groups:
Group 1: Patients over the age of 70 who have not received a DXA scan in the past 5 years
Group 2: Patients over the age of 50, who have had a previous fragility fracture since the age of 50, and who have not received a DXA scan in the past 5 years.

Eligible patients are identified by screening utilising practice management software, and then recalled for a bone health check. The resulting actions (e.g. referral for DXA scan, osteoporosis diagnosis and management strategy) will be captured and compared to those recommended in the relevant clinical standard (RACGP guideline).

Results: At the time of submission over 100 GPs had provided audit data for over 500 patients.

Conclusion: The REFRAME Clinical Audit is underway, and the data collection period will continue throughout 2018.

P76
Longitudinal associations of dietary patterns with muscle strength, balance and falls in a cohort of Australian middle-aged women

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Aims: Influences of dietary patterns on falls risk are poorly understood, particularly in younger adults. This longitudinal analysis aimed to examine associations between dietary patterns and muscle strength, balance and falls in middle-aged women.

Methods: Of the 345 women aged 36-57 years at baseline, 268 were followed for a mean of 5.3 years. Baseline diet was assessed by the Cancer Council of Victoria food frequency questionnaire. Exploratory factor analysis was used to identify dietary patterns and scores for each pattern calculated by summing the food intakes and their factor loadings. Lower limb muscle strength (LMS) and balance (timed up and go, step, functional reach (FRT) and lateral reach tests) were measured at baseline and follow-up and retrospective falls incidence in the last year measured at follow-up. Associations between dietary pattern scores and these outcomes were assessed using linear mixed-effects and log-binomial regressions respectively.

Results: 67 women (28%) reported falling at least once in the previous year. Three dietary patterns were identified: 'healthy' (high intakes of vegetables, legumes, fruit, tomatoes, nuts, snacks, garlic, whole grains and low of high-fat dairy), 'high protein, high fat' (red meats, poultry, processed meats, potatoes, cruciferous and dark-yellow vegetables, fish, chips, spirits and high-fat dairy) and 'processed foods' (high intakes of meat pies, hamburgers, beer, sweets, fruit juice, processed meats, snacks, spirits, pizza and low of cruciferous vegetables). In both univariable analyses and after adjustment for potential confounders, there were no associations between any dietary pattern and any outcome.

Conclusions: This is the first study examining longitudinal influences of dietary patterns on major falls risk factors and falls incidence. While no associations were observed, confirmation of these findings in studies with larger samples using prospective measurement of falls incidence are needed before dietary influences on falls-related outcomes can be ruled out.

Table Adjusted models for associations between three dietary patterns ('Healthy, 'high protein, high fat' and 'Processed foods') and lower limb muscle strength, balance measures and falls after a mean of 5.3 years

	Healthy	High protein, high fat	Processed foods
	β (95%CI)	β (95%CI)	β (95%CI)
Lower limb muscle strength (kg)[a]	-2.3 (-5.0, 0.28)	-1.5 (-4.1, 1.1)	0.3 (-2.3, 3.0)
Timed up and go test (second)[b]	-0.004 (-0.08, 0.07)	0.04 (-0.04, 0.12)	-0.05 (-0.13, 0.02)
Step test (step)[b]	0.15 (-0.13, 0.44)	0.01 (-0.28, 0.29)	-0.07 (-0.36, 0.21)
Functional reach test (cm)[b]	-0.25 (-0.90, 0.41)	-0.04 (-0.69, 0.61)	0.72 (0.08, 1.37)
Lateral reach test (cm)[b]	0.16 (-0.28, 0.61)	-0.19 (-0.63, 0.25)	0.14 (-0.30, 0.59)
	RR (95% CI)	RR (95% CI)	RR (95% CI)
Retrospective incident falls [a]	1.11 (0.94, 1.32)	0.92 (0.73, 1.15)	0.85 (0.66, 1.10)

RR, relative risk; CI, confidence interval.
Number of participants were 268 for incident falls and 342-345 for the other outcomes (as linear mixed-effects models could use all available data).
Beta coefficients are the change in the outcome for a standard deviation-unit increase in each factor score (i.e., 122, 81 and 57 for dietary pattern 1, 2 and 3, respectively).
[a] Adjusted for age, weight, height, strenuous physical activity, employment status, hours of watching TV, total energy intake, and calcium and vitamin D supplement.
[b] Adjusted for age, weight, height, strenuous physical activity, educational level, hours of watching TV, total energy intake, and calcium and vitamin D supplement.

P77

Promotion of osteoclast differentiation by reactive sulfur species.

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Osteoclasts, multinucleated cells specialized for bone resorption, are differentiated from monocytes/macrophage-lineage cells under stimulation by RANKL, a cytokine produced by osteoblasts and osteocytes. While many studies indicated that reactive oxygen species (ROS) promotes osteoclastogenesis, its suppression by oxidative stress has also been reported. Persulfides and polusufides, compounds having consecutive multiple sulfur atoms produced in various types of cells, are called reactive sulfur species (RSS) for their high reactivity, especially with ROS. In this study, we examined the effects of RSS on osteoclastogenesis in vitro to clarify the effects of cellular redox state on osteoclastogenesis. Mouse bone marrow macrophages were cultured for 3 days in the presence of RANKL and M-CSF with or without addition of a hydrogen sulfide donor (NaHS) and RSS donors having 2 to 4 sulfur atoms (Na2S2, Na2S3, and Na2S4). Osteoclast formation was evaluated by activity staining for tartrate-resistant acid phosphatase (TRAP) as well as determination of TRAP activity in the cultures. Expression of the marker genes of osteoclasts was quantitatively evaluated by real-time PCR. NaHS did not have an effect on differentiation of macrophages into osteoclasts induced by RANKL. On the other hand, persulfides promoted it in concentration-dependent manners. The activity of persulfides to enhance osteoclast differentiation was in the order of Na2S4 > Na2S3 > Na2S2. In addition, the first 24-hour incubation of macrophages with the Na2S4 was sufficient for the achievement of the enhanced osteoclast differentiation. In addition, Na2S4 accelerated the mRNA expression of NFATc1, one of the master transcription factors of osteoclastogenesis, during the first 24 hours after addition of RANKL to the cultures. Since treatment of osteoclast precursors with RSS donors enhanced osteoclast differentiation, it is suggested that reductive condition in the precursor cells at their early stage in differentiation is preferable for their differentiation into osteoclasts.

P78

Bone Mineral Density by Dual X-ray Absorptiometry and Trabecular Bone Score in Primary Hyperparathyroidism

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Western Health

Patients with Primary Hyperparathyroidism (PHPT) have reduced Bone Mineral Density (BMD) by Dual X-ray Absorptiometry (DXA) at cortical sites with relatively preserved trabecular BMD. However, increased fracture risk at all sites suggests that BMD is not capturing all determinants of bone strength. Trabecular bone score (TBS), a grey level textural index derived from lumbar spine (LS) DXA images, is validated as an indirect measure of trabecular microarchitecture, and may have a role in fracture risk assessment in this disorder.

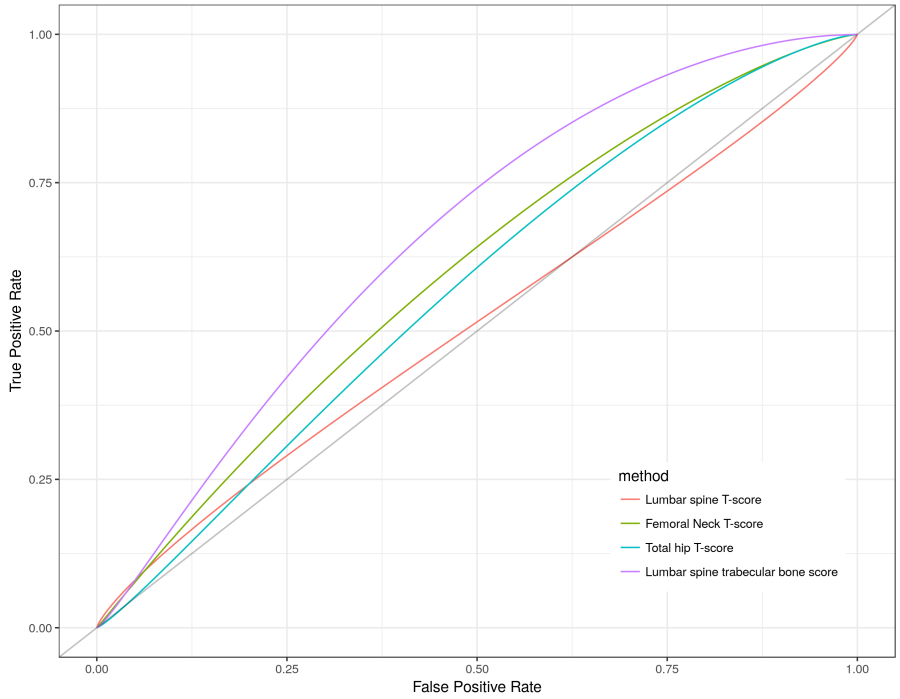
Aims: We assessed DXA data in patients with PHPT admitted to a Tertiary Teaching Hospital to determine whether TBS is a more accurate marker of fracture risk than areal BMD.

Methods: Patients admitted between 01/04/2007 and 01/07/2017 diagnosed with PHPT, with at least one DXA, were analysed. Data were extracted manually from electronic medical records, and fractures from radiology and clinical history. TBS was derived from LS DXA. Data was analysed via R statistical program.

Results: 204 patients had a diagnosis of PHPT, 68 had DXA. Mean age was 65.2 years (range 26-96), 78% were female and 78% of Caucasian background. 23 (35%) had prevalent fractures at diagnosis. Mean (SD) T-scores were -1.51 (1.63) at LS, -2.07 (0.99) at FN, -1.32 (1.17) at TH. Mean TBS was 1.19 (0.12). Only 16%, 26% and 10% of LS, FN and TH T scores respectively, were below the WHO osteoporosis threshold (T<-2.5), while 53% of TBS were <1.2 which defines degraded microarchitecture. ROC for fracture prediction showed AUC (95% CI) of 0.52 (0.37,0.52) for LS T score, 0.60 (0.45,0.60) for FN, 0.55 (0.41,0.55) for TH and 0.65 (0.51,0.65) for TBS, the differences not reaching statistical significance (p-value=0.19) (figure 1).

Conclusion: TBS was indicative of osteoporosis in a higher percentage than BMD T-scores, but did not significantly improve prediction of prevalent fractures in this cohort.

Figure 1: Smoothed receiver operating curve (ROC) analysis comparing lumbar (L) spine T score, femoral neck (FN) T-score, total hip T-score and lumbar spine (Lspine) trabecular bone score.



P79

The effect of varying the doses of cocktail monoclonal antibodies to collagen type II on joint destruction and mechanical allodynia in a murine model of inflammatory arthritis

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Rheumatoid arthritis (RA) is associated with joint destruction and hyperalgesia. Murine models are extensively used to elucidate RA pathogenesis, however limited studies have assessed pain. The collagen antibody-induced arthritis (CAIA) model reflects the effector phase of disease with histological characteristics of the affected joints mimicking RA. Most studies initiate disease with 250-400µl of a monoclonal antibody cocktail against collagen type II followed by E.coli lipopolysaccharide (LPS; 25µg). This results in severe joint damage, making analysis problematic. We use 150µl of antibody followed by 10µg LPS to induce a mild form of the disease. The joint destruction and pain induced by different antibody dose has not been explored. We propose the higher antibody dose (300µl) with 10µg LPS will lead to greater disease severity and pain.

Mice were allocated to 3 groups; control (n=5), low dose CAIA (150µl Ab+10µg LPS; n=5) and moderate dose CAIA (300µl Ab+10µg LPS; n=8). Inflammation was scored daily in all paws. Mechanical allodynia was assessed in the rear paws using the von Frey paw withdrawal test. At endpoint, bone (BV) and paw volume (PV; inflammation) was assessed in the front paws by micro-CT. Inflammatory cell infiltration, cartilage and bone damage and pannus formation were assessed histologically.

From day 5 until endpoint the moderate dose group had greater paw scores compared to the low dose group ($p<0.05$, at day 5). PV was significantly greater in the moderate dose group ($p<0.05$) and had a greater response threshold compared to control mice. The moderate dose group had a greater PV and histological scores compared to low dose group, however these were not significant. There was no significant difference in BV between all groups.

Overall there was no clear difference in BV and paw withdrawal response between the low (150 µl Ab) and moderate (300 µl Ab) doses inducing CAIA.

P80
Palmitoleic acid is a promising bone health molecule

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Palmitoleic acid, an omega-7 mono-unsaturated fatty acid (C16:1), is found in the diet and is endogenously synthesised by adipocytes. Saturated palmitic acid (C16:0) has been demonstrated to be potently anti-resorptive, inhibiting osteoclasts, at low concentrations although saturated fatty acids at higher concentrations have also been reported to be cytotoxic and are usually considered to be associated with adverse health conditions. In contrast, unsaturated palmitoleic acid has demonstrated a wide array of positive effects on health, being anti-inflammatory and down-regulating and inhibiting lipogenesis. In this study, we have investigated the skeletal effects of palmitoleic acid.

Using primary osteoblasts, palmitoleic acid significantly stimulated the osteoblasts viability (by 33 % at 20 µg/mL; $P < 0.002$, ANOVA) and 3H-thymidine incorporation (by 12 and 14%, at 1 and 10 µg/mL respectively; $P < 0.001$). In addition, palmitoleic acid inhibited osteoclast formation with significant effect seen at 10 and 20 µg/mL in bone marrow cultures (by 27% and 33%, respectively; $P <0.002$) and in RAW264.7 cells (by 29 and 52%, respectively; $P < 0.0001$). The inhibition was not due to non-specific cytotoxicity, since palmitoleic acid stimulated the viability of bone marrow cells and RAW264.7 cells as measured with AlamarBlue®. Real-time PCR results showed that palmitoleic acid reduced the expression of M-CSF, DC-STAMP, NFATc1, TNF-α and TRAP. In addition, this acid reduced the expression of RANKL and OPG at similar rates, suggesting the independence of RANKL/OPG pathway for its action on osteoclastogenesis.

In summary, palmitoleic acid is a promising factor in increasing bone health, stimulating osteoblasts and inhibiting osteoclasts.

P81
Prevalence of cardiovascular risk factors in a population-based cohort of Danish bisphosphonate users: The Odense Bisphosphonate Safety Study

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Background: Here we report on the CV profile in a nationally representative cohort of oral bisphosphonate (BP) users as part of a population-based study into the long-term safety of BPs, the Odense Bisphosphonate Safety Study (OBSS).

Participants: BP users over 45 years who had areal bone mineral density (aBMD) assessed by dual-energy x-ray absorptiometry [DXA] after 1 January 2003.

Measurements: Clinical information on anthropometry, renal function, prior fractures, Charlson co-morbidity index, prescription history were obtained from medical records compiled through Statistics Denmark [Project #5079]. Continuous outcomes are expressed as median and interquartile range (IQR) and categorical variables expressed as frequency and percentage.

Results: 7,533 individuals [women, n=5,917 [88.6%] were identified [Table 1]. Median age was 69 years [62, 77]. A large proportion of individuals were in the normal weight range [48.6%] on body mass index though approximately a third [33.3%] were overweight and approximately 13% were obese. CV disease was prevalent in half of the individuals [50.3%] and CV medication use was common [lipid-lowering therapy – 35.5% of the whole cohort and blood pressure-lowering therapy – 43.3% of the whole cohort]. Mild kidney disease was common [58.6] and anti-diabetic use was infrequent [6.2%].

Discussion: In this preliminary report of the OBSS, the prevalence of important CVD risk factors appears to be substantial and management of these risk factors alongside BMD and fracture risk factors should be encouraged. There is a need to understand the cardiovascular safety of BP use in the context of multiple co-morbidities and polypharmacy which may predispose to CV disease and to further identify individuals with osteoporosis at increased CV risk. A comparative study against matched non-users with adjustment for differences in BMD is currently underway to determine if BP use influences CVD risk.

Table 1. Clinical characteristics of bisphosphonate users in Odense Bisphosphonate Safety Study (OBSS) cohort

n		7533
Age (years)		69 [62, 77]
Women		5917 [88.6]
BMI (kg/m^2)		24.6 [22.0, 27.6]
	<18	360 [4.7]
	18-25	3660 [48.6]
	25-30	2512 [33.3]
	30-35	752 [9.9]
	35+	249 [3.3]
aBMD (g/m^2)	Total hip	0.711 [0.632, 0.792]
	Femoral neck	0.595 [0.531, 0.664]
	Lumbar spine	0.769 [0.707, 0.859]
T-score	Total hip	-1.94 [-2.54, -1.33]
	Normal	18 [0.2]
	Osteopenia	5484 [72.8]
	Osteoporosis	2031 [27.0]
	Femoral neck	-2.32 [-2.87, -1.74]
	Normal	12 [0.2]
	Osteopenia	4383 [58.1]
	Osteoporosis	3138 [41.7]
	Lumbar spine	-2.94 [-3.50, -2.00]
	Normal	260 [3.5]
	Osteopenia	2577 [34.2]
	Osteoporosis	4696 [62.3]
CVD history		3791[50.3]
Charlson co-morbidity index (one-year look-back)	0	3498 [46.4]
	1	1177 [15.6]
	2	970 [12.8]
	3	588 [7.81]
	4	397 [5.2]
	5	315 [4.2]
	6+	588 [7.8]

Medication use (one-year look-back)	Lipid-lowering	2678 [35.5]
	Anti-hypertensive	3260 [43.2]
	Anti-thrombotic/coagulant	2124 [28.2]
	Anti-inflammatory	1990 [26.4]
	Anti-diabetic (including glucose lowering and insulin sensitising)	472 [6.27]
	Prednisolone	2110 [28.0]
Fracture history	Any	5373 [71.3]
	Hip	2100 [27.8]
	Lumbar spine	1097 [14.56]
	Any arm	4055 [53.8]
	Humerus	2007 [26.6]
Estimated glomerular filtration rate [mL/min/1.73m^2] n=7,263		83.0 [72.2, 92.3]
	CKD1	2321 [32.0]
	CKD2	4257 [58.6]
	CKD3	665 [9.1]
	CKD4	18 [0.3]
	CKD5	2 [0.0]
Bisphosphonate type	Alendronate	7218 [96.0]
	Other oral	315 [4.0]

P82

Repeated childhood fracture is not related to abnormal bone or body composition in middle age.

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Although personal fracture history is one of the strongest predictors of future fractures, current guidelines ignore fractures that occur during childhood. However, it is not clear if multiple childhood fractures are related to transient reductions in bone during growth or skeletal deficits that will track into adulthood. Furthermore because of the close relationship between skeletal muscle mass and bone mass throughout the lifecourse skeletal deficits in childhood may be able to predict abnormal adult body composition. We studied 660 men and women from a birth cohort, of which 39 females and 67 males suffered from two or more childhood (age <18 years) fractures. Dual x-ray absorptiometry was used to examine body composition (fat mass, lean mass, % body fat), total bone mineral content, total bone mineral density, hip bone mineral content and hip bone mineral density at age 45. Compared to those who did not repeatedly fracture as children, those with multiple childhood fractures did not differ in terms of body composition, total body bone mineral density, or bone mineral content ($p>0.05$). However, there were small differences in hip bone density in those that repeatedly fractured compared to those that did not. Total hip BMD was 3% (95%CI: -6%, -1%; p=0.041) lower, T-scores were 0.24 standard deviations (95% CI: -0.47, -0.01; p=0.036) lower and Z-scores were 0.22 standard deviations (95% CI: -0.43, -0.02; p=0.034) lower than those without repeat fractures. These associations were not moderated by sex. Further investigation is required to determine other early childhood factors that predict abnormal adult bone and body composition.

P83

Early middle-age prevalence of sarcopenia, osteopenia and osteosarcopenia in a birth cohort.

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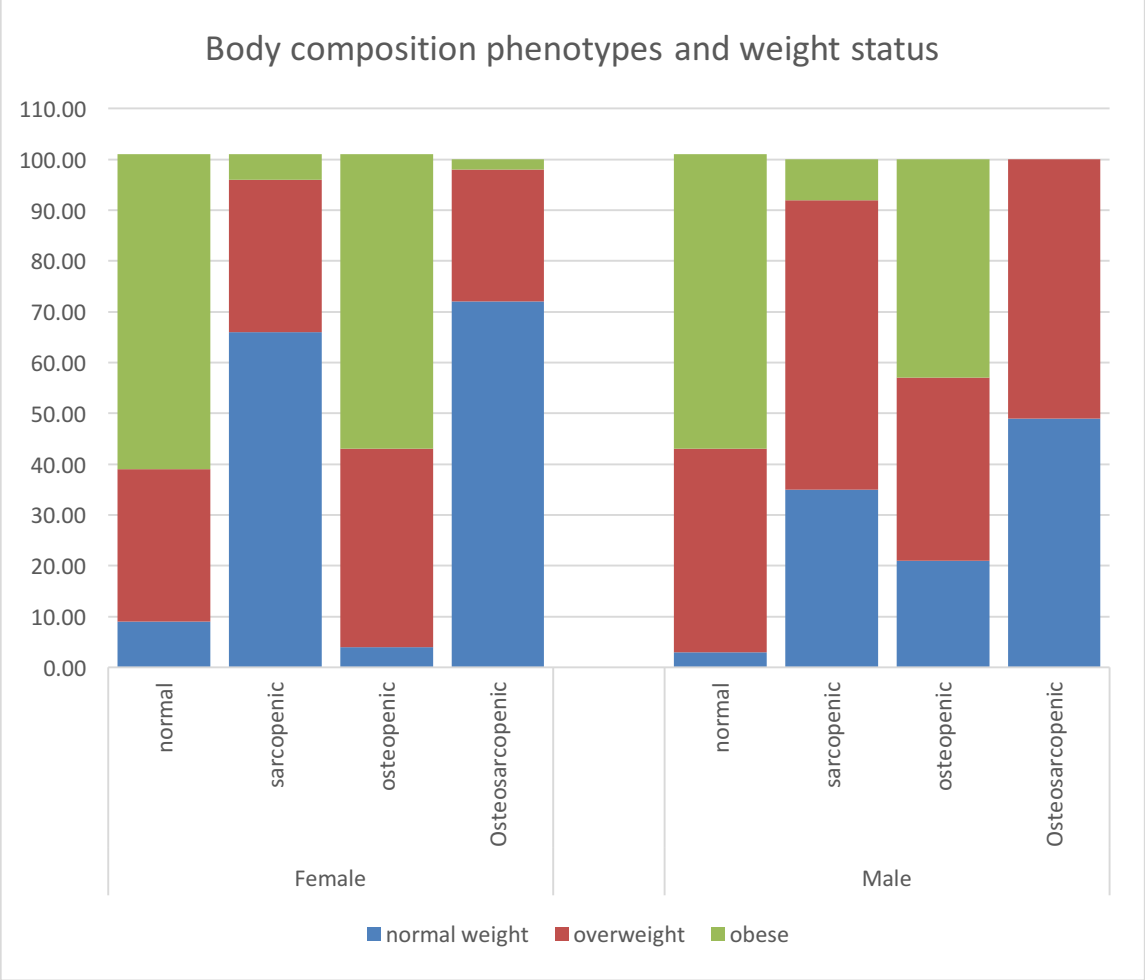
Despite limited evidence that pre-sarcopenia is apparent in middle age, identifying the prevalence of this condition earlier in the lifecourse has rarely been undertaken and there are currently no estimates of the prevalence of osteosarcopenia in a middle aged cohort. The lack of prevalence data in this age group may explained by the cutpoints used to identify low muscle mass earlier in the lifecourse. Prado et al developed cutpoints using a population-based approach, but estimates of the prevalence of sarcopenia in a middle-aged cohort using these cutpoints are limited. In our preliminary analysis of the Dunedin Study cohort at Phase 38 using bioelectrical impedance we found 35.9% of the cohort were pre-sarcopenic at age 38. Logistic regression showed the odds ratio of having low muscle mass, but high fat mass in the overweight group was 30 times more likely relative to the 'normal' BMI group. At phase 45, using DXA and sex-specific medians to define sarcopenia a preliminary analyses of 660 males and females revealed an overall prevalence of sarcopenia of 50% (49.8% males; 50.1% females), osteopenia of 21.8% (18.1% males; 25.2% females) and sarcoosteopenia of 15.8%(13.7% males; 17.7% females). Sarcopenia, was more common in normal weight females (66.1%), and overweight males (57%). In both males and females osteopenia was more prevalent in those with obesity. Sarcoosteopenia was only slightly more common in overweight vs normal weight males (51.2% vs 48.8%), but much more prevalent in normal weight (72.1%) females. The prevalence of these abnormal bone and body composition phenotypes is high in early middle age and associations between BMI categories and sex suggest the relationship between muscle mass and bone may differ between sexes as adiposity increases.



P84

Endotoxin and bone: cross-sectional evidence from a population-based sample of men

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Background: Increased risk of osteoporosis and related fractures are associated with gastrointestinal diseases, including ulcerative colitis and irritable bowel syndrome. The direct action of bacterial components such as endotoxin (lipopolysaccharides) on intestinal serotoninergic activity and potential downstream effects on bone remain unknown. We aimed to investigate lipopolysaccharide binding protein (LBP) levels and bone mineral density (BMD) in a randomly selected population-based cohort of men.

Methods: Serum LBP (ng/mL) was measured using enzyme-linked immunosorbent assay (ELISA; R&D Systems) for 1149 men (ages 20-96yr, median 61yr) enrolled in the Geelong Osteoporosis Study (GOS). BMD (g/cm2) was measured at the PA-spine, hip, total body and forearm using dual-energy X-ray absorptiometry (Lunar). Weight and height were measured; medication use, physical activity and smoking status were self-reported. LBP values were natural log transformed (ln-LBP) and associations between ln-LBP and bone measures were tested using Pearson's correlation. Multivariable linear regression models were developed to test associations after adjusting for age, weight, height, physical activity and medications affecting bone (thyroid medication, bisphosphonates).

Results: Ln-LBP (median 16.5ng/mL, IQR:11.5-23.1) was positively correlated with age (r=0.06, p=0.04) yet negatively with weight (r=-0.08, p=0.005), total body (r=-0.09, p=0.003), spine (r=-0.05, p=0.08), distal- (r=-0.02, p=0.003) and mid-forearm BMD (r=-0.15, p=<0.001), but not hip BMD (r=-0.04, p=0.14). After adjustments, LBP was associated with decreased BMD at the mid-forearm (β -0.013, SE ± 0.004, p<0.001) and showed a trend with decreased spine (β -0.012 ± 0.009, p=0.19) and total body BMD (β -0.006 ± 0.004, p=0.13). No associations between LBP and hip (β-0.002 ± 0.006, p=0.77) and distal-forearm BMD (β 0.001 ± 0.003, p=0.66) were evident.

Conclusion: While acknowledging potential unrecognised confounding, these data suggest LBP is independently associated with mid-forearm, and potentially spine and total body BMD, in adult men. Given the paucity of data, replication and future studies are warranted.

P85

Utility and predictors of grip strength in women presenting to an Osteoporosis Refracture Prevention Clinic with a minimal trauma fracture

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Aim: To examine the utility of grip strength and its significance in women with a minimal trauma fracture (MTF) and as an emerging tool in the assessment of sarcopaenia.

Method: Participants were women >50yrs with MTF presenting to Royal North Shore Hospital Osteoporosis Refracture Prevention Clinic.

Grip strength was measured using a Jamar Dynamometer. BMD of hip, spine and forearm was measured using Hologic QDR 4500W and Hologic Horizon Dual Energy Absoptiometry Xray (DEXA).

Results: Seventy-seven women with a mean age of 66.9+8.9 years were included. The mean grip strength was 23.7+5.1kg for the dominant hand and 23.1+5.6 kg for the non- dominant hand. Participants' fracture sites included 52 upper limb (67.5%), 19 lower limb (24.7%), 5 vertebrae (6.5%) and 1 rib (1.3%).

Twenty (26%) patients met the criteria for sarcopaenia. according to grip-strength cut-offs defined by the European Working Group for Sarcopenia in Older People (EWGSOP).
Patients with sarcopaenia had significantly lower wrist BMD (0.527 + 0.06 vs 0.580 + 0.09g/ cm 2, p=0.03); demonstrated a trend towards lower hip BMD (0.635+0.113g/cm2 vs 0.689+0.116, p=0.09) and had significantly higher mean PTH (64.3 + 23.4ng/L vs 46.7 ng/L + 15.6, p=0.005).

In multivariate regression analysis, the association between reduced grip strength and higher PTH remained significant. Older age, poorer renal function and lower BMI were also independent predictors of lower grip strength.

Conclusion: Grip strength measurement identified a higher than expected prevalence of sarcopaenia in this relatively young group of patients with MTF predominantly affecting the upper limb. Lower grip strength was significantly associated with lower wrist BMD and higher PTH levels. Whilst the significance of these findings requires further analysis, grip strength may have utility in identifying those at greater risk of refracture, requiring resistance training and specific therapies targeting muscle mass.

P86
1,25(OH)D3 abrogates palmitic acid-induced lipotoxicity in normal human osteoblasts in vitro

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Introduction: Bone loss begins in the third decade of life and involves decreased bone formation associated with a progressive reduction in osteoblast (Ob) survival, function and number. With ageing, MSC number and differentiation potential decrease due to reduced capacity to transform into Ob, leading instead to increased adipogenesis and lipid accumulation in the bone marrow of osteoporotic bones. Adipocytes produce palmitic acid (PA), a fatty acid (FA) known to be toxic to Ob in vitro. Potential mechanisms include induction of dysfunctional autophagy and reduced differentiation. Vitamin D (1,25(OH)2D3) induces osteoblastogenesis and has an anti-apoptotic effect on Obs. We therefore hypothesised that 1,25(OH)2D3might rescue Obs from PA-induced lipotoxicity in vitro.

Methods: Initially we compared the capacity of human Obs (Lonza, CC2538) to differentiate and mineralize in the presence of PA or 1,25(OH)2D3 or in combination. Cell survival was assessed using a 3-(-2,5-diphenylterazolium bromide (MTT) assay. Autophagy was assessed via LC3-II expression, confocal microscopy, and monitoring of live autophagosomes at different time points. RT-PCR was performed to test the palmitoylation process in the presence of PA and 1,25(OH)2D3.

Results: As expected, PA decreased mineralisation, differentiation and affected autophagy in human Obs. In contrast, cells treated with a combination of PA and 1,25(OH)2D3recovered their capacity to differentiate and mineralise. 1,25(OH)2D3 increases Ob survival ($P<0.01$) in the presence of PA. Furthermore, 1,25(OH)2D3 inhibited the autophagy process which was induced by PA at 24 and 48 hrs

Conclusion: This study has identified potential mechanisms to explain how 1,25(OH)2D3might abrogate PA-induced lipotoxicity, including normalization of mineralization, survival and autophagy in Obs exposed to a strong lipotoxic milieu that mimics the human bone marrow environment. Future work should characterise the mechanisms of this effect as a step towards development of novel therapies to overcome lipotoxicity in the bone setting.

P87
Risk of fracture in Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM) may increase fracture risk despite increased bone mineral density (BMD). Predictors for fracture in T2DM are unclear.

We aimed to determine the fracture risk in T2DM and the role of BMD in fracture prediction.

In the Dubbo Osteoporosis Epidemiology Study, a population-based study, radiologically-confirmed clinical fractures and BMD by DXA were collected from 1989-2015. T2DM was defined by self-reported diagnosis or diabetes medication use, after age 30 years.

There were 265 participants with, and 3303 without, T2DM at the time of recruitment. T2DM was of relatively short duration (median 6.3 years (IQR: 3.2-10.7) and patients were managed with diet in 32.5%, oral agents in 60.4% and insulin in 16.6%. T2DM participants were more likely to be male, have higher body mass index and BMD.

Over 10.5±7.0 person-years, there were 711 incident fractures in women (23 in T2DM) and 215 in men (11 in T2DM). T2DM did not increase fracture risk in either gender after adjusting for age, BMI, FNBMD, prior osteoporosis treatment and falls. Although FNBMD was higher in T2DM overall, FNBMD was lower in those who fractured compared to those who did not, although this was only significant in men. Within T2DM men, FNBMD (HR 2.20/SD decline, p=0.0078) and age (HR 1.13/year, p=0.0245) predicted incident fracture. Neither FNBMD nor age predicted incident fracture in T2DM women. There was a trend to increased fractures in men, but not women, with longer duration T2DM (HR 1.64, p=0.18).

In summary, there was no increased fracture risk in this cohort of mostly non-insulin treated T2DM of relatively short duration. Within T2DM participants, FNBMD was lower in those who fractured and remained a predictor for incident fractures in men. Longer duration of T2DM may increase fracture risk. Further studies to determine the mechanisms contributing to fracture risk are required.

P88
Visceral adipose tissue is associated with increasing insulin resistance and decreased bone turnover, independent of total body fat

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Poor bone health has been increasingly recognised in those with type 2 diabetes (T2DM) and obesity. Both conditions are closely linked and are associated with higher bone mineral density (BMD). However, not all obese people have insulin resistance (IR) or T2DM, and visceral adipose tissue (VAT), rather than subcutaneous fat, is a potential marker of IR. The relative effects of obesity, fat distribution and IR on bone health remain unclear.

The aim was to assess the relationship between bone turnover markers (BTM), BMD and body composition in insulin-sensitive lean (IS-L), insulin-sensitive overweight (IS-O), insulin resistant (IR) and T2DM participants.

From the Dubbo Osteoporosis Epidemiology Study, 612 participants had fasting plasma samples analysed for IR (Homeostasis Model Assessment [HOMA] ≥2.5) and BTM (osteocalcin [OC], procollagen type 1 N-propeptide [P1NP] and collagen type 1 cross-linked C-terminal telopeptide [CTx]), and concurrent whole body DXA scans for BMD, body composition and VAT.

Among these participants (mean age 68.4±4.8 years), BMD was lower in IS-L compared with IS-O, IR and T2DM, with no differences within the obese groups (see table). Total body fat mass (FM) and lean mass (LM) followed a similar pattern. Abdominal FM and particularly VAT increased progressively from IS-L to IS-O, IR and T2DM. BTMs were lower in T2DM compared with the 3 other groups.

All fat parameters were negatively associated with BTM. However, VAT remained the only independent predictor for CTX (-0.03/kg VAT [-0.05 – -0.01], p=0.0031) and P1NP (-3.69/kg VAT [-5.67 – -1.72], p=0.0002) in a multivariate model with body composition parameters.

Variable	IS-Lean (HOMA<2.5, BMI<25kg/m^2)	IS-Overweight (HOMA<2.5, BMI≥25kg/m^2)	IR (HOMA ≥2.5)	T2DM
Number	131	193	129	85
BMI (kg/m^2)	**22.9±1.6***	28.3±2.7*#	28.8±3.7^#	29.9±3.7#
FN BMD (g/cm^2)	**0.83±0.12**	0.90±0.13#	0.90±0.14#	0.92±0.14#
Total body FM (kg)	**19.9±5.6**	28.2±6.7#	29.7±8.2#	30.2±7.5#
Abdominal FM (kg)	**13.0±4.0**	**19.7±4.9*#**	22.0±5.3#	23.4±6.0#
VAT (kg)	**5.8±3.7***	**12.4±6.3*#**	**14.6±7.4*#**	**18.1±8.8#**
OC (ng/mL)	22.0±8.3*	**19.5±7.0*#**	20.0±7.7*	**16.2±6.5**
P1NP (ng/mL)	43.72±18.8*	40.3±16.5	43.4±19.6*	**34.1±13.6**
CTX (ng/mL)	0.34±0.18*	0.30±0.14^	0.30±0.19^	**0.24±0.12**

*p<0.01 when compared to T2DM, ^p<0.05 when compared to T2DM, #p<0.01 when compared to IS-L

(Repeated measures ANOVA with Bonferroni adjustment used to compare between groups)

In summary, although obesity was associated with higher BMD, BTM were lower only in T2DM. VAT increased with increasing severity of IR. Thus, VAT and IR, not total body fat, is associated with reduced bone turnover and may drive poor bone health in T2DM despite the preserved BMD.

P89

Proton Pump Inhibitors (PPIs) are Associated with Distal Radial Microstructural Deterioration

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Background: Proton Pump Inhibitors (PPIs) are commonly used in elderly people. As studies have linked PPIs use with increased fracture risk, we hypothesised that PPIs use is accompanied by microstructural deterioration and reduced bone mineral density (BMD).

Methods: We identified 51 women receiving PPIs and 36 women without exposure to PPIs in this cross-sectional study of aged-care residents aged 73 to 99 years. The non-dominant distal radius and tibia were scanned using high-resolution peripheral quantitative computed tomography of the non-dominant side (Scanco XtremCT, Switzerland). BMD at the lumbar spine and femoral neck were determined using dual-energy X-ray Absorptiometry (DXA, Lunar Prodigy). Fracture history were obtained from the medical records. Robust regression was used to compare the two groups and to adjust for covariates.

Results: Mean age of PPIs users differed from non-users (mean ± SD: 86.5 ± 5.4 vs. 89.2 ± 5.1 years respectively, p = 0.024). In PPIs users, distal radial total cortical area was 0.37 SD lower (p=0.077), compact-appearing cortical area was 0.46 SD lower (p=0.052), cortical porosity was 0.45 SD higher (71.0% vs. 68.2%, p=0.024) compared to non-users, after adjustment for age and total bone cross-sectional area. The PPIs users had similar matrix mineral density and trabecular bone volume/total volume, number and thickness compared non-users. No differences were observed for distal tibial structure or BMD. The odds ratio (OR) for fracture was 2.4 (95% CI: 0.92 – 7.00) in PPIs users relative to non-users (p=0.075).

Conclusion: The increased fracture risk associated with exposure to PPIs may be the result of cortical rather than trabecular microstructural deterioration and remain undetected using BMD.

P90

Gene mining for novel molecular determinants of dento-skeletal homeostasis and disease in the Collaborative Cross.

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The Gene Mine (Geniad) consists of an octo-parental recombinant inbred panel of approximately 1000 mice from over 80 strains that is housed in Perth, WA. The Gene Mine forms a component of the Collaborative Cross (CC). A primary aim of the CC is to provide a stable, reproducible genetic reference platform for the qualitative and quantitative analyses of the causative gene-variants, epistatic mechanisms, and environmental factors that determine disease, including osteoporosis, cardiovascular disease, cancer and diabetes. Integration of phenotypic and genomic data over time and across a variety of fields is vital to advancing our understanding of disease susceptibility. Research into the phenotypic and genomic inter-relationships of the osteoporosis, osteoarthritis, and scoliosis fields of the CC has been initiated by our group. The current project is investigating determinants of dento-skeletal phenotypes within the Geniad CC population. It aims to identify novel molecular determinants of dento-skeletal homeostasis and disease in mice and to correlate these findings with concomitant disease phenotypes, including osteoporosis, osteoarthritis, and scoliosis in the CC mice. To achieve these aims, Geniad mice will be screened by conventional x-ray and micro-CT for dento-skeletal, scoliosis and kyphosis phenotypes. Mapping of QTLs will be performed to identify candidate genes regulating these phenotypes. In vitro gene expression and bioinformatics analyses will then be used to verify and characterize candidate gene involvement. We have completed initial whole body x-ray screening of 952 Geniad mice across 84 strains and observed a range of phenotypes including scoliosis (4.3%), kyphosis (5.2%), kyphoscoliosis (1.7%), and dento-skeletal abnormalities (21.6%). In conclusion, the x-ray screening results are consistent with the incidence of these pathologies in human populations, validating this approach, and indicates the need for further micro-CT analysis, identification and mapping of QTLs, and determination of candidate gene involvement for dento-skeletal, scoliosis and kyphosis phenotypes.

P91

Fracture risk calculation does not improve osteoporosis treatment initiation compared with PBS-prompting in a randomised controlled cross-over trial in Australian General Practice

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A potential barrier to appropriate initiation of osteoporosis treatment is failure to recognize magnitude of risk. Fracture Risk Calculators (FRCs) might address this. We tested the hypothesis that routine inclusion of actuarial fracture risk with graphical representation (using the Garvan FRC) in bone mineral density (BMD) reports would increase initiation of appropriate osteoporosis treatment to patients at high risk of fracture.

In a cluster-randomized cross-over trial, 58 General Practitioners (GPs) were recruited into the study from 8 different medical practices in Sydney. Four practices (29 GPs) were initially randomized to an intervention group (FRC-based BMD reports) and the other four practices (29 GPs) to the control group (standard BMD reports); each report for both groups also included whether Pharmaceutical Benefits Scheme (PBS) criteria for treatment were met. Participating GPs remained in their original randomised group for 6 months before crossing over to the other group for the final 6 months. A novel data capture web-based platform was used to assess GP decisions to treat patients for osteoporosis when provided with actuarial fracture risk (displayed graphically) compared to usual BMD reports with PBS-prompts, in real-time.

Among 424 patients whose risk was shown, treatment was initiated in 34% (n=146), which was not statistically different (P=0.13) from the 30% (n=144) treatment initiation among 487 patients whose risk was not shown. In multivariable logistic regression model, treatment initiation was significantly associated with 10-year risk of fracture (P<0.0001), but not with gender (P=0.15) and the presentation of risk (P=0.14). For treatment-naïve patients with a ten-year fracture risk of ≥20%, there was no difference in treatment intention between the active (70.1%) and control (69.9%) groups (p = 0.17).

We conclude that compared with BMD reports with PBS-prompts, the presentation of risk estimates did not improve the initiation of osteoporosis treatment.

P92

A trial sequential meta-analysis to assess the current evidence for the ability of osteoporosis re-fracture prevention services to reduce re-fracture rates.

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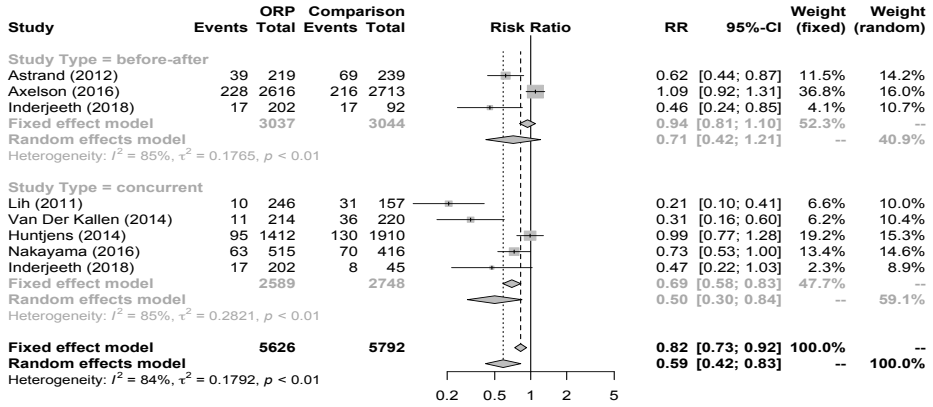
Background: The lifetime risk of an osteoporotic fracture (from the age of 60) for a man and woman is 25% and 44%, respectively. Any a low-trauma fracture increases the risk of subsequent fracture, but timely diagnosis and optimal treatment is recommended as a means to reduce the risk of re-fracture. It has been proposed that an organised approach to managing an initial fracture, such as an Osteoporotic Refracture Prevention (ORP), or Fracture Liaison Service (FLS), will reduce the risk of subsequent fracture. To date, the evidence for the effectiveness of a ORP services to reduce the risk of re-fracture has come from non-randomised (concurrent) comparisons of hospitals with and without ORP, as well as before and after ORP implementation studies. The aim of this study is to determine whether the current evidence is strong enough to support the effectiveness of introducing ORP services into the hospital setings without the need for a randomized controlled trial?"

Methods: Using a Trial Sequential (TSA) meta-analysis approach, this study will assess if superiority, inferiority, or futility has been reached, based on cumulative data of studied assessing the effectiveness of ORP services to reduce refracture rates.

Results: Seven studies have reported the effectiveness of the introduction of a ORP service to reduce re-fracture rates (Random Effects Relative Risk = 0.59, 95% confidence interval, 0.42, 0.83) (upper panel of Figure). In the TSA analysis (lower panel of Figure), Superiorirty, inferiority boundaries have not been crossed.

Conclusion: TSA analysis suggests further trials are needed to demonstrate the re-fracture benefit of ORP services. Continued use of TSA analysis for future trials will indicate when enough evidence has accumulated to definitively conclude that ORP services reduce re-fracture rates.



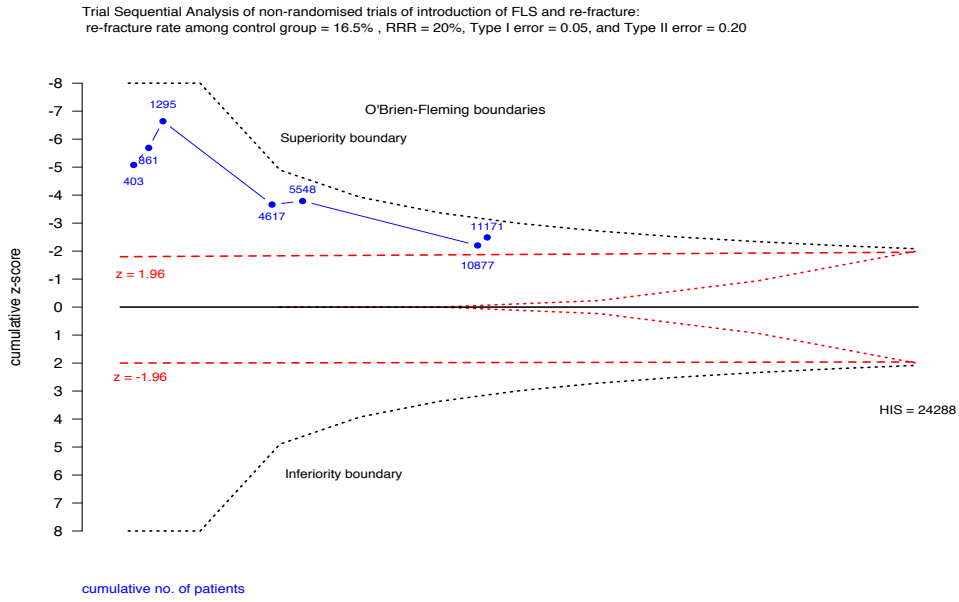


Figure. Forest plot of published trials (upper panel). Trial Sequential Analysis (lower panel). RRR = Relative Risk Reduction, and HIS = Heterogeneity adjusted Information Size (estimated total sample size needed).

P93
Hospital Bed Use after Non-Hip Non Vertebral Fractures

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Introduction: Most analyses of hospital bed use following osteoporotic fractures concentrate on hip or vertebral fractures, but there are very few data on non-hip non-vertebral (NHNV) fractures.

Objective: To examine the hospital bed utilisation of all fracture types in a large NSW sample.

Design, Setting, and Participants: Prospective population-based cohort study of 267,043 women and men from the 45 and Up Study, NSW, Australia. Baseline questionnaire data were linked to hospital administrative and all-cause mortality data from February 1, 2006 to December 31, 2013. Lengths of stay for the acute care episode and total length of stay including type separations and transfers were calculated for all fracture types.

Results: During the 1,490,651 person-years of follow-up, women experienced 7,571 fractures whilst men experienced 4,571 fractures. Of these, there were 2,156 hip fractures (mean age: 78.8 (SD: 9.8)) and 9,087 NHNV fractures (mean age: 66.2 (SD: 12.5)). Mean acute care stay was highest for hip fractures at 7.1 (SD: 9.2) days. For other fracture types, the mean length of acute care ranged between 1 and 9 days, with the longer stays for proximal fractures. Overall, the mean for all NHNV fracture sites was 3.0 (SD: 5.6) days.

Similarly, the means of total length of stay were the highest for hip fractures at 24.8 (SD: 34.4) days. NHNV fractures were associated with shorter duration of stays (mean: 6.1 (SD: 13.2)). However, as hip fractures only accounted for 18% of all fractures, NHNV fractures, as a group accounted for over 50% of the total number of acute bed days (49,172 days) and total bed days (109,117 days) for all fractures.

Conclusion: Non-hip non-vertebral fractures are a major, largely unrecognised, contributor of acute hospital bed use with important implications for health care expenditure.

P94
Health literacy and use of calcium and Vitamin D supplements in a population-based sample of Australian women

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Aims: Previous data suggest an association between poorer health literacy abilities relating to finding, understanding and using health information and greater uptake of anti-fracture medication. The current study aims to investigate associations between health literacy and use of supplements promoted as beneficial to bone health in a population-based sample of women.

Methods: Data was utilised from women (n=672, median age 59.2 years [range 29.9-92.8 years]) participating in the Geelong Osteoporosis Study (GOS), a population-based cohort based in south-eastern Australia. Health literacy was ascertained using the Health Literacy Questionnaire (HLQ), a multi-dimensional tool that generates scores across nine scales. Calcium and vitamin D supplementation was self-reported. One-way Analysis of Variance (ANOVA) and Cohen's d effect sizes (ES [95%CI]) (categorised; Small >0.2-0.5, Moderate >0.5-0.8, Large >0.8) were calculated for differences in mean HLQ scale scores between participants who did vs. did not self-report taking supplements.

Results: Calcium supplements were used by 81 (12.1%) women, and vitamin D supplements by 105 (15.6%) women. Using one-way ANOVA, women who self-reported taking vitamin D supplements demonstrated higher mean scores for the HLQ scale 'Feeling understood and supported by healthcare providers' (p-value <0.0001). Small Cohen's d effect sizes were also observed for this scale (ES 0.40 [95%CI 0.61, 0.19]). No significant differences were seen for the remaining eight domains, or for analyses investigating health literacy and use of calcium supplements. Results did not change when analyses were restricted to women with low BMD.

Conclusion: These results suggest that women who report feeling understood and supported by healthcare providers are more likely to use vitamin D supplements. These results differ from previous findings regarding health literacy and uptake of anti-fracture medications. Further research is required to understand how health literacy interacts with other factors, such as perceived risk vs benefits, to influence medication use.

P95

Resolvin E1 inhibits Cathepsin K gene expression during rankl included osteoclastogenesis

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Resolvin E1 (RvE1) is a specialised pro-resolving lipid mediator involved in the resolution of inflammation. Pathogenic bone loss in periodontitis and rheumatoid arthritis is a consequence of chronic dysregulated inflammation. In animal models, RvE1 is also suggested to play a regulatory role in bone metabolism. We investigated the direct effects of RvE1 on human osteoclast and osteoblast differentiation in vitro.

Methods: RANKL induced osteoclasts were cultured from human peripheral blood mononuclear cells isolated from whole blood donations (n=4). Whereas, osteoblasts were derived from human mesenchymal stromal cells isolated from marrow donations and differentiated in osteogenic media. Cells were treated with RvE1 (1, 5, 10ng/ml) to assess its effect on osteoclast and osteoblast differentiation. Osteoclast formation and activity was assessed by immunohistochemical analysis of tartrate-resistant acid phosphatase (TRAP) and quantification of dentine resorption respectively. Levels of osteoblast mineralisation was assessed by alizarin red stain. Expression of osteoclast and osteoblast specific genes were determined by real-time polymerase chain reaction.

Results: A direct inhibitory effect of RvE1 on osteoclast differentiation was observed with significant reductions in TRAP+ cells and area of dentine resorption compared to controls ($p<0.05$). Additionally, RvE1 significantly downregulated osteoclast cathepsinK gene expression ($p<0.05$). Osteoblast mineral deposition was unchanged in the presence of RvE1, with osteoblast genes RUNX2, OPN, OCN, and Col1a expression also similar to controls ($p>0.05$). There were no additional significant differences in other osteoclast specific genes in the presence of RvE1 compared to control ($p>0.05$).

Conclusions: Our observations support a direct osteoimmunological link between RvE1 activity and bone metabolism. RvE1 inhibition of osteoclastogenesis via gene expression and osteoclast activity could play an important role in chronic inflammatory diseases where significant bone loss is evident.

P96

Bone microarchitecture damage due to press-fit femoral knee implantation: evaluation using HR-pQCT and digital volume correlation (DVC)

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Introduction: During prostheses press-fit implantation, high compressive and shear stresses at the implant-bone interface are generated. Permanent bone damage occurs, however, the extent remains unknown.

Study aim: to quantify, using high-resolution peripheral quantitative computed tomography (HR-pQCT, 61µm/voxel) and Digital Volume Correlation (DVC), permanent bone deformation due to press-fit femoral knee implantation.

Methods: Six human cadaveric distal femora (age 85±3 years, fresh-frozen) were resected using standard intramedullary instrumentation and scanned with HR-pQCT (XtremeCT II, SCANCO) at 60.7µm/voxel, isotropic. Femurs were fitted with cementless Sigma® cruciate retaining femoral knee implants. Implants were then removed without damaging the bone and femurs rescanned.

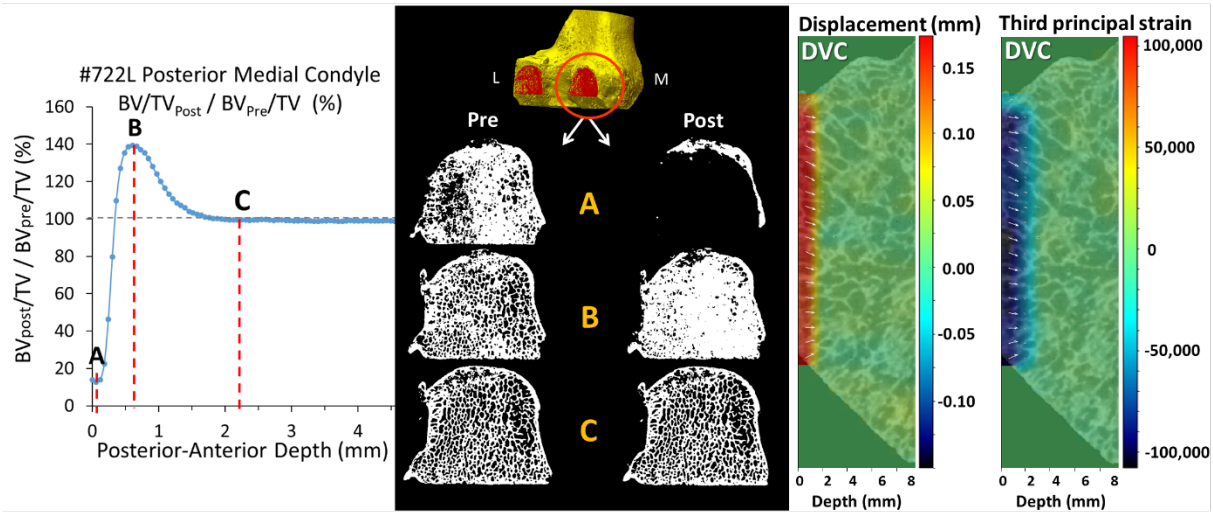
Pre- and post-implantation HR-pQCT cross-section images were co-registered in 3D. For each femur, volumes of interest (VOI) were defined to examine regions where permanent bone deformation was expected to have occurred: the posterior-medial and posterior-lateral condyles for 10mm of depth, from the first posterior coronal cross-section image in the pre-implantation data set.

The bone volume fraction (BV/TV) for the VOIs in pre- and post-implantation images and their ratio (BV/TVpost/BV/TVpre) were calculated, slice by slice, at increasing depth. DVC direct correlation (DaVis, LaVision, Germany) was applied on the VOIs, to assess trabecular bone displacements and plastically accumulated strains.

Results: The "BV/TVpost/BV/TVpre ratio vs. depth" graphs (see Fig.) showed, consistently among the six femurs, three consecutive points of interest ($p<0.05$), indicating: (A) bone removal (BV/TVpost/BV/TVpre ratio<100%), (B) compaction (ratio>100%) and (C) correspondence (ratio=100%).

Correspondingly, the trabecular bone displacement computed by DVC, for the 6 femurs, suggested bone compaction up to 2.4±0.7mm in depth, with peak third principal strains of -150,500±39,500µstrain, well above the yield strain of bone (7,000-10,000µstrain).

Conclusions: Combining 3D-HR-pQCT imaging and DVC provides insight into the extent of permanent deformation of bone due to femoral press-fit implantation. This data can be used to inform surgeons and manufacturers, advancing bone implants development.



P97

Picolinic acid as a novel osteoanabolic: Possible mechanisms of action

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Aims: Picolinic acid (PIC) is one of the end-products of the kynurenine pathway, and is a potent bone anabolic. We have earlier reported that Pic has a concentration-dependent osteogenic effect on human mesenchymal stem cells (hMSCs), in vitro. This led to the hypothesis that PIC may play a role in activating essential osteogenic pathways. Therefore, the aim of this study was to investigate whether PIC regulates Wnt/ -catenin signalling and the NO pathway in hMSCs.

Methods: hMSCs were cultured under standard osteogenic conditions in the presence of osteoanabolic concentration of PIC (100 M) or vehicle control. Nitrite levels in supernatants were determined using Greiss' method. Western blotting was performed to detect -catenin (stabilised form), CK1, GSK3- and eNOS.

Results: We found that Pic significantly enhanced stabilised -catenin levels (non-phosphorylated ser45) during early osteogenesis in hMSCs. In comparison to their respective controls, levels of CK1 decreased during early osteogenesis. However, PIC treatment did not alter the expression of GSK-3 in hMSCs. We also found a notable increase in expression of eNOS in Pic treated cells by day seven.

Conclusion: Our results demonstrate the influence of PIC on two separate pathways. On the -catenin pathway, PIC induces early stabilisation of -catenin and a concomitant decline in CK1 levels. As CK1 is an important component of the -catenin degradation complex, its decrease could explain the increased stability of -catenin observed. Since eNOS is a key player in osteogenesis, PIC-associated increase in its levels suggests a secondary important mechanism of PIC-induced osteogenesis in hMSCs.

These data therefore support the role for PIC as a novel osteoanabolic agent that acts through various osteogenic pathways. Regarding the significant in vivo effects of Pic on the bone mass of lab animals, and low toxicity of the substance, human trials are warranted.

P98

Accuracy variations in DXA bone mineral density: A Muticentre phantom study.

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Dual energy X ray (DXA) absorptiometry is the gold standard for the diagnosis of osteoporosis. To date in Australia there are no studies assessing the diagnostic accuracy of different DXA Imaging Centres. We have conducted a multi-site study of spine and hip phantoms to compare the accuracy of 13 GE-Lunar and 6 Hologic DXA scanners. Both phantoms were measured at each site 3 times on the same occasion with all in clinical use no repositioning between scans. The observed average of the 3 scans were then used as a unbiased estimate of the "true mean" BMD at the lumbar spine (L1-L4), femoral neck and total proximal femur.

The within-centre variation was less than 0.01 g/cm2 (or less than 1% relative to the mean) for BMD at all three sites. The between-centre variation for BMD at the three sites was larger than the within-centre variation, and the largest between-centre variabilities are observed at the femoral neck site measured using Hologic scanners (Table).

Scanner	Site	Mean BMD (g/cm^2)	Largest Between-Centre Absolute Difference -(g/cm2)	Largest Between-Centre Absolute Difference (%)
Lunar	L1-L4	1.208	0.0127	1.5
Lunar	Femoral neck	0.800	0.0247	3.1
Lunar	Total Proximal Femur	0.888	0.0122	1.4
Hologic	L1-L4	1.035	0.043	4.2
Hologic	Femoral neck	0.675	0.060	8.8
Hologic	Total Proximal Femur	0.778	0.033	4.2

Thus, while the within-centre variation in measured values of BMD at all sites was small, the between-centre site variation was potentially clinically relevant at the femoral neck. The magnitude of the observed difference in measured femoral neck BMD could potentially affect therapeutic decisions and assessment of serial changes in BMD, when patients are measured at different centres. The results indicate a need for further studies of DXA accuracy and underline the need for a standardization of BMD measurements across scanners.

P99

Resveratrol ameliorates bone loss in non-obese diabetic (NOD) mice by promoting bone formation

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Resveratrol (RESV) is a polyphenol found in Chinese herbal medicines. In previous study, we demonstrated that RESV improved glucose intolerance and insulin sensitivity in non-obese diabetic (NOD) mice. Aims: To investigate the protective effect and mechanism of RESV on bone damage in diabetic mice. Materials and Methods: The NOD mice were administrated with placebo (NOD+PLAC), or insulin (NOD+INS), or RESV (NOD+RESV), and compared to their non-diabetic litters (CTRL). After 8-weeks treatment, bone mineral density (BMD) and bone properties were detected. In vitro, osteoblasts (MC3T3-E1) were treated with 30mM glucose, combined with PBS, 1nM insulin or 10uM RESV, respectively. The expression of osteogenic genes and the signaling pathway was analyzed using RT-PCR and Western blotting. Results: BMD was reduced in NOD mice, suggesting the diabetic bone loss. Both insulin and RESV increased BMD after 8-weeks administration, and RESV almost reversed the BMD loss in DM mice. Trabecular number (Tb.N), trabecular thickness (Tb.Th), and bone volume (BV/TV) exhibited consistent results. However, trabecular separation (Tb.Sp) showed no difference in each groups. Compared to placebo or insulin groups, RESV significantly increased the expression of bone formation markers OCN and Runx2 mRNA in femur. In addition, the enhanced expression of Trap mRNA in NOD mice was suppressed by RESV. Studies in vitro exhibited similar results. The expression of osteogenic genes OCN and Runx2 in osteoblasts was significantly upregulated by RESV, while it showed no difference in insulin group. Western blot analysis showed that the expression of TGF-β enhanced in insulin-treated cells. RESV promoted osteoblast differentiation and triggered the activation of phosphorylation of ERK1/2 level in osteoblast. Conclusion: These results suggested that RESV promoted bone formation and protected from the diabetes associated bone loss in NOD mice via the activation of ERK1/2 signaling pathway. Thus, RESV may be a promising therapy for diabetes mellitus associated osteoporosis.

P100

The essential role of zinc transporter ZIP10 in skeletal formation

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Aim: Zinc deficiency during development causes severe skeletal defects including deformity and a decrease in bone mass. However, the underlying molecular mechanisms have remained unclear. The aim of this study is to investigate the role of zinc transporter ZIP10, an SLC39 family member that facilitates an increase of the intracellular zinc level during skeletal development.

Methods: We generated bone- and cartilage-specific ZIP10 conditional knockout (Zip10-cKO) mice using Cre/loxP system; namely, the mice were expressing Cre recombinase under the control of type I- and type XI- collagen promoter (Zip10-cKOColI-Cre and Zip10-cKOColXI-Cre mice, respectively). Histological analysis and gene expression profiling using RNA-sequence technique were conducted by using control and Zip10-cKO mice.

Results: Both Zip10-cKOColI-Cre and Zip10-cKOColXI-Cre mice exhibited low birth rates, possibly due to dying at delivery. The low production was more evident for Zip10-cKOColI-Cre mice than Zip10-cKOColXI-Cre mice. Zip10-cKOColI-Cre and Zip10-cKOColXI-Cre embryos (E16.5-17.5) showed dwarfisms with short limbs, narrow chest space, and deformation of calvaria with decreasing calcification. The GO enrichment analysis of the RNA-sequence results using control and Zip-10 deficient osteoblasts, articular superficial cells, and chondrocytes demonstrated that cell viability, cell death, and transcellular transport pathways were significantly altered by loss of Zip10.

Conclusion: These results suggest that ZIP10 is essential for skeletogenesis during embryonic development and that ZIP10 plays important roles in the regulation of cell viability/survival and substance transport in skeletal cells, likely via zinc homeostasis.