ANZBMS 23rd Annual Scientific Meeting

8 - 11 SEPTEMBER 2013

HILTON ON THE PARK,
MELBOURNE, VICTORIA

Meeting Handbook

ANZBMS

PLATINUM SPONSORS

AMGEN

gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

References: 1. Lewiecki EM, *Women's Health* 2009; 5(1): 15–22. 2. Prolia® (denosumab) Approved Product Information www.amgen.com.au/Prolia.PI 3. Cummings SR *et al. N Engl J Med* 2009; 361: 756–765.

AMGEN® gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

PLEASE REVIEW FULL PRODUCT INFORMATION BEFORE PRESCRIBING.

PBS Information: Authority required (STREAMLINED) as treatment for postmenopausal osteoporosis. Refer to PBS Schedule for full information.

Product Information is available via Prolia® Medical Information 1800 646 998 or www.amgen.com.au/Prolia.PI

For information on Prolia® or to report an adverse event involving Prolia®, please contact Prolia® Medical Information on 1800 646 998.

MINIMUM PRODUCT INFORMATION – INDICATION: Treatment of osteoporosis in postmenopausal women to reduce risk of vertebral, non-vertebral and hip fractures. **CONTRAINDICATIONS**: Hypocalcaemia. Hypersensitivity to denosumab, CHO-derived proteins or any component. **PRECAUTIONS**: Correct hypocalcaemia prior to initiating therapy. Monitor calcium in patients predisposed to hypocalcaemia. Adequate intake of calcium and Vitamin D is important. **ADVERSE EFFECTS**: Hypocalcaemia, skin infections (predominantly cellulitis), pancreatitis, rarely jaw osteonecrosis, #*very rarely atypical femoral fractures.* **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.

#Please note changes in product information.

Amgen Australia ABN 31 051 057 428 North Ryde NSW 2113, Ph 1800 646 998, www.amgen.com.au
GlaxoSmithKline ABN 47 100 162 481 Level 4, 436 Johnston Street, Abbotsford VIC 3067, www.gsk.com.au

DMB-AUS-AMG-527-2013 Approved July 2013 • AM5199/6/13

PBS INFORMATION: Authority required (STREAMLINED).
Refer to PBS Schedule for full authority information.

Please review Product Information before prescribing. To access a copy of the Product Information please go to www.servier.com.au/PI or telephone 1800 153 590. **Minimum Product Information. PROTOS® (strontium ranelate).** Contains aspartame. **Indications:** *Treatment of severe (established) osteoporosis in postmenopausal women at high risk of fracture to reduce the risk of fracture. *Treatment of severe (established) osteoporosis in men at increased risk of fracture. **Contraindications:** Known hypersensitivity to strontium ranelate or to any of the excipients. Severe renal impairment. Current or previous venous thromboembolic events (VTE) incl deep vein thrombosis and pulmonary embolism. Temporary or permanent immobilisation (e.g. post-surgical recovery or prolonged bed rest). *History of ischaemic heart disease, peripheral arterial disease or cerebrovascular disease. *Systolic blood pressure (SBP) ≥160mmHg, or diastolic blood pressure (DBP) ≥90mmHg. **Precautions:** *Treatment should only be initiated by a doctor experienced in treating osteoporosis. Use with caution in patients at risk of VTE. In patients >80 years at risk of VTE re-evaluate ongoing PROTOS treatment. In the event of immobilisation, discontinue PROTOS as soon as possible and take preventative measures for VTE. Do not restart therapy until the VTE has resolved and the patient is mobile. Stop PROTOS if VTE occurs. *In postmenopausal osteoporosis studies, a significant increase in myocardial infarction (MI) was observed with PROTOS compared to placebo. *Evaluate patients for cardiovascular risk prior to commencing PROTOS and during ongoing treatment. *Patients with significant risk factors for cardiovascular events should only be treated with PROTOS after careful consideration. *Stop treatment if the patient develops ischaemic heart disease, peripheral arterial disease, cerebrovascular disease or if SBP is ≥160mmHg, or DBP is ≥90mmHg. Cases of life-threatening SJS, TEN and DRESS have been reported. Discontinue immediately if signs of allergic reaction occur. Monitor these patients closely – See Approved PI for details. PROTOS contains aspartame, a source of phenylalanine, which may be harmful for people with phenylketonuria. Category B3 - do not use in pregnancy. Do not use in children. Do not use while breastfeeding. **Interactions:** PROTOS should preferably be taken >2 hours after: food, milk, milk products, or medicines containing calcium, which may reduce bioavailability. Suspend during treatment with oral tetracycline and quinilone antibiotics, as may reduce absorption of these drugs. Concomitant bisphosphonate treatment is not recommended. No known interaction with oral vitamin D. PROTOS interferes with colorimetric tests to determine blood and urinary calcium levels. **Adverse Reactions:** Common: headache, disturbances in consciousness, memory loss, *MI, nausea, diarrhoea, loose stools, dermatitis, eczema, VTE, blood CPK increase. **Dosage and Administration:** One 2g sachet once daily by mouth, taken as a suspension in a glass containing a minimum of 30ml of water, preferably at bedtime. No dosage adjustment required in the very elderly, mild to moderate renal impairment, or hepatic impairment. **Presentation:** PROTOS 2g sachets contain 2g strontium ranelate as a yellow powder. Boxes contain 7 or 28 sachets. **Date of most recent amendment to full Product Information:** 30 May 2013. Servier Laboratories (Australia) Pty. Ltd. 8 Cato Street Hawthorn, VIC.

*Please note changes in Product Information

This document was prepared in August 2013.

SPONSOR ACKNOWLEDGEMENTS

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

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ANZBMS President's Welcome

On behalf of the Australian and New Zealand Bone and Mineral Society I invite you to attend the 23rd ANZBMS Annual Scientific Meeting at the Hilton on the Park, Melbourne, Australia between September 8–11, 2013. This meeting will continue with ANZBMS mission to deliver an forum of excellence hosting internationally and locally acclaimed experts in the field of bone and mineral research. We are delighted to that Prof Roland Baron and Prof Clifford Rosen will be our keynote attendees at this meeting. The meeting program themes are: evidence-based skeletal medicine, molecular insights and novel therapeutic interventions. These will be intertwined with our overarching goals to foster research collaboration, inform best clinical practice and facilitate networking of early career researchers.

Professor Matthew Gillespie
President ANZBMS

Invited Overseas Speakers

Prof Roland Baron (Department Head and Professor of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine AND Professor of Internal Medicine, Harvard Medical School and Massachusssetts General Hospital, Boston USA), to speak on recent developments in osteoporosis therapy, including targetting of sclerostin, cathepsin K and Dkk1 signalling, as well as speaking on new basic research in osteoblast and osteoclast biology.

Dr Clifford J Rosen (Director of Clinical and Translational Research, Maine Medical Center, USA), to speak on the relationships between glucose metabolism, adipogenesis and bone, and vitamin D deficiency.

INTRODUCTION

ANZBMS Council Members 2013

President	Matthew Gillespie
President Elect	Markus Seibel
Honorary Secretary	Gerald Atkins
Honorary Treasurer	Gethin Thomas
Councillors	Nicholas Pocock
	Mark Bolland
	Charles Inderjeeth
	Natalie Sims
Past President	Rebecca Mason

Past & Present Councillors *1990 – 2013*

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

ANZBMS PROGRAM ORGANISING COMMITTEE

Natalie Sims – Chair
Minghao Zheng
Paul Baldock
Peter Ebeling
Rachel Davey
Mark Bolland
Nathan Pavlos
Rebecca Mason
Matthew Gillespie

LOCAL ORGANISING COMMITTEE

Eleanor Mackie – Chair
Rob Daly
Rachel Davey
Julian Quinn
Nicole Walsh

ANZBMS Secretariat

Ivone Johnson – Executive Officer
Melissa Dupavillon – Administrative Assistant
Tel: 02 9256 5405
Email: ijohnson@anzbms.org.au
Web: www.anzbms.org.au

ANZBMS Meeting Manager

Lara Birchby
The Meeting People Pty Ltd
PO Box 764, Mitcham South Australia 5062
Tel: 08 8177 2215
Email: lara@themeetingpeople.com.au

ANZBMS AWARD WINNERS

ROGER MELICK YOUNG INVESTIGATOR AWARD

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

CHRISTOPHER AND MARGIE NORDIN YOUNG INVESTIGATOR POSTER AWARD

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

CHRISTINE AND T JACK MARTIN RESEARCH TRAVEL GRANT

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*

AMGEN/ANZBMS OUSTANDING ABSTRACT AWARD

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

MSD – ANZBMS CLINICAL RESEARCH EXCELLENCE AWARD

2012		*Belal Khan*

KAYE IBBERTSON AWARD ON METABOLIC BONE DISEASE

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*

SOL POSEN RESEARCH AWARD

2006	-	*Nathan Pavlos*
2007	-	*Aaron McDonald*
2008	-	*Haotian Feng*
2009	-	*Ming-Kang Chang*
2010	-	*Tak Sum Cheng*
2011	-	*Kylie Alexander*
2012		*Julie Quach*

PHILIP SAMBROOK AWARD

2012	-	*Gustavo Duque*

PROGRAM INFORMATION

Speaker Support Centre
A fully equipped and staffed Speaker Support Centre will be located in the back of the Ballrooms on the conference floor of the Hilton on the Park Melbourne. It is important that **all** speakers giving oral presentations check in with the technicians 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 Technical Director located at the back of the ballrooms.

Posters
Plenary posters will be up for the duration of the meeting and the authors of these must attend on Sunday 8th September during the Welcome Reception.
Attended authors discussions for **all posters** will take place on both **Monday 9th September from 15:40 – 16:30.**
All posters must be removed at the end of afternoon tea on Tuesday 10th 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 Ballrooms at the Hilton on the Park Melbourne Hotel on the conference level.

197 Wellington Parade, Melbourne VIC 3000

Registration Desk
The registration desk will be open at the following times:

Sunday 8 September	14:00 - 18:30
Monday 9 September	07:30 - 18:00
Tuesday 10 September	08:00 - 17:30
Wednesday 11 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.

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 in the Grand River Ballroom. If you have requested a special diet please make yourself known to one of the waiting staff.

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

SOCIAL EVENTS
Sunday 8 September
Welcome Reception - 17:30 – 19:00
Exhibition, Hilton the Park Melbourne
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 $70 per person may be purchased from the registration desk.

Monday 9 September
Young Scientists & Students Networking Function 18:30 – 22:30
Offsite – The Kingston Hotel, 55 Highett Street, Richmond
This is an opportunity for students and young researchers to network and enjoy light refreshments together. Tickets are $20 per person and are available from the registration desk until Sunday night. Attendees can meet in the foyer at 18:15 to walk together to the function.

Tuesday 10 September
25th Anniversary Conference Dinner
19:00 for 19:30 – 23:00
Ballroom, Hilton on the Park Melbourne
Dinner will be held in the Ballroom of the Hilton on the Park Melbourne and will include a three-course dinner, drinks and entertainment by Blue Vinyl Lounge. This function is included in the full registration fee. Extra tickets $150 per person available from the registration desk until lunchtime on Monday. Admission is by ticket only. Dress code: Smart casual

ANZBMS 2013 EXHIBITION

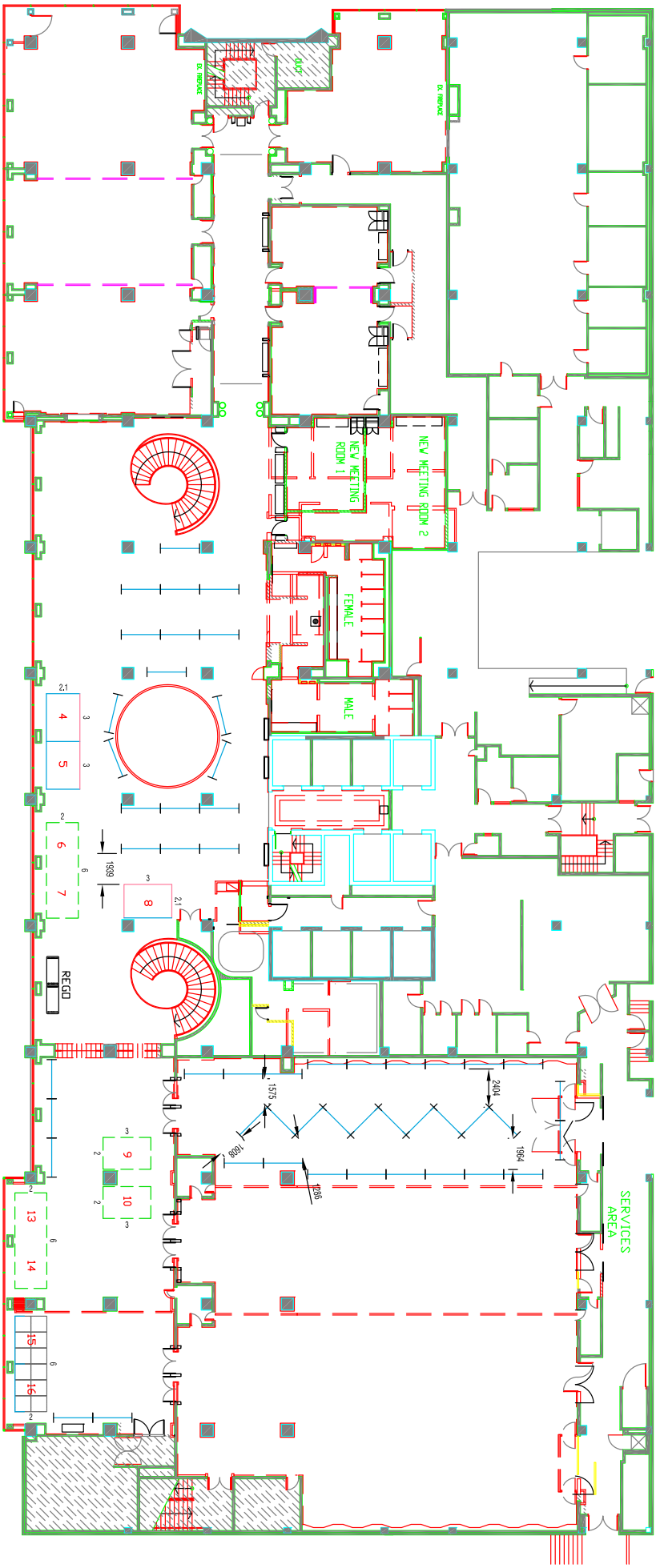
The Trade Exhibition will be located in the foyer of the Hilton on the Park Melbourne. Please visit the booths during refreshment breaks in recognition of the generous support this conference has received from the sponsors.

Exhibition Hours

Sunday 2 September 2012	1400 – 1900 (Welcome Reception and Exhibition Opening)
Monday 3 September 2012	0800 – 1700
Tuesday 4 September 2012	0800 – 1700
Wednesday 5 September 2012	0800 – 1030

Exhibitors (see floor plan for location of booths)

Booth No	***Company***
13 & 14	Amgen – PLATINUM SPONSOR
Internet Café	Eli Lilly Australia – Internet Café Sponsor
15 & 16	Hologic Inc.
5	InMed
4	Medtel
10	Merck Sharp & Dohme
9	Sanofi – SILVER SPONSOR
6 & 7	Servier Laboratories – SILVER SPONSOR
8	Thomson Scientific Instruments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Sunday 8 September 2013

14:00 **Registration opens** **Mezzanine Level, Hilton on the Park**

14:30 **Official Opening of the 23rd Annual Scientific Meeting** *Ballroom B/C*

14:40 **SESSION 1**
Bone and Energy Metabolism
Chairs: John Eisman, Tania Winzenberg

14:40 **IS1** Taking the Temperature of Fat-Bone Cross-Talk
Clifford Rosen (USA)

15:20 **IS2** Glucocorticoid Effects on Bone and Energy Metabolism
Tara Brennan-Speranza (ANZAC Research Institute)

15:44 **IS3** The interaction between inflammation and glucocorticoids in glucocorticoid-induced osteoporosis
Mark Cooper (ANZAC Research Institute)

16:08 **OR1** **Amgen-ANZBMS Outstanding Abstract - Clinical Presentation**
Higher dietary calcium intakes are associated with reduced risks of vertebral fractures and severe abdominal aortic calcification in older Australians
Peter Ebeling (The University of Melbourne)

16:20 **OR2** **Amgen-ANZBMS Outstanding Abstract - Biomedical Presentation**
gp130 in osteocytes is required for PTH-induced bone formation
Narelle McGregor (St. Vincent's Institute)

16:32 **OR3** **Amgen-ANZBMS Outstanding Abstract - Biomedical Presentation**
2-photon imaging reveals myeloid cells as the cellular targets for the anti-tumour activity of bisphosphonates *in vivo*
Simon Junankar (Garvan Institute)

16:44 **Professor Philip Sambrook Award Presentation**
with support from Osteoporosis Australia

17:00 – 19:00 **Welcome Cocktail Party, Exhibition and Plenary Posters (attended)** *Foyer*

Monday 9 September 2013

08:30 **SESSION 2** *Ballroom B/C*
Emerging Therapies
Chairs: Ego Seeman, Natalie Sims

08:30 **IS4** Emerging therapeutic options in osteoporosis: Cellular basis and challenges
Roland Baron (USA)

09:10 **IS5** Emerging therapies in orthopaedics
David Little (The Children's Hospital at Westmead)

09:34 **IS6** What is new in OA therapy?
Flavia Cicuttini (Monash University / Alfred Hospital)

09:58 **OR4** Is modulation by strontium of osteoclastogenic signals in human osteoblasts entirely through the calcium-sensing receptor?
Mark Rybchyn (The University of Sydney)

10:10 – 10:40 **Morning Tea** *Mezzanine*

10:40 **SESSION 3** *Ballroom B/C*
Chairs: Rory Clifton-Bligh, Allison Pettit

10:40 **OR5** **Amgen-ANZBMS Outstanding Abstract – Biomedical**
Tamoxifen-induced deletion of the glucocorticoid receptor in chondrocytes enhances K/BxN serum-induced arthritis in mice
Jinwen Tu (ANZAC Research Institute)

10:52 **OR6** **Amgen-ANZBMS Outstanding Abstract - Clinical**
A novel primary school exercise intervention for bone and fat outcomes: the CAPO Kids trial
Rossana Candiota Nogueira (Griffith University)

Roger Melick Young Investigator Award Finalists:

11:04 **OR7** Sclerostin exerts a coordinated pro-osteoclastogenic effect via its action in osteocytes
Asiri Wijenayaka (The University of Adelaide)

11:16 **OR8** Adherence to oral bisphosphonate therapy in a Fracture Liaison Service: A randomised controlled trial
Kirtan Ganda (ANZAC Research Institute)

11:28 **OR9** RANKL-induced Myo1b occupies sites of dynamic actin-membrane remodeling and is required for osteoclast formation and function
Pei Ying Ng (University of Western Australia)

11:40 **OR10** Quadriceps weakness is a predictor of fracture risk: a time-dependent analysis
Hanh Pham (Garvan Institute)

11:52 **OR11** The Vitamin D receptor promotes MCF-7 cell growth *in vitro* and *in vivo* via a ligand independent cytoplasmic function
Trupti Trivedi (ANZAC Research Institute)

12:04 **OR12** The IFITM5 mutation c.-14C>T results in an elongated transcript expressed in human bone and causes varying phenotypic severity of osteogenesis imperfecta type V
Syndia Lazarus (University of Queensland)

12:16 **OR13** Uncoupling protein 1 protects bone mass under cold stress conditions
Amy Nguyen (Garvan Institute)

12:30 – 14:00 **Lunch, networking and poster viewing** *Mezzanine*

13:00 – 14:00 **AMGEN SYMPOSIUM (Supported by Amgen)** *Ballroom B/C*

Anti-resorptives in postmenopausal osteoporosis: mechanisms and effects

Speaker: Professor Roland Baron (Department Head and Professor of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine; Professor of Internal Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, USA)
Panel members:
Professor Markus Seibel (Professor and Chair of Endocrinology, University of Sydney; Head, Department of Endocrinology & Metabolism, Concord Hospital; Director, Bone Research Program, ANZAC Research Institute, Sydney, NSW)
Professor Ego Seeman (Professor of Medicine, University of Melbourne; Consultant Endocrinologist, Endocrine Centre of Excellence, Austin Health, VIC)

14:00 **SESSION 4** *Ballroom B/C*
Cancer and Bone
Chairs: Michelle McDonald, Jeffrey Zajac

14:00 **IS7** Anti-tumour effects of bisphosphonates: looking beyond the skeleton
Michael Rogers (Garvan Institute)

14:25 **IS8** Bone and Cancer
Andrew Zannettino (The University of Adelaide)

14:50 **IS9** FGF23 and Tumour induced osteomalacia
Nobuaki Ito (The University of Adelaide)

15:15 **IS10** Refining murine models of osteosarcoma
Carl Walkley (St. Vincent's Institute)

15:40 – 16:30 **Afternoon Tea and Attended Poster Session (All Posters)** *Mezzanine*

16:30 – 18:06 **PARALLEL ORAL PRESENTATIONS**

16:30 **SESSION 5A** *Ballroom B*
Clinical Presentations
Chairs: Grahame Elder, Fran Milat

16:30 **OR14** Denosumab versus risedronate in women with postmenopausal osteoporosis suboptimally adherent to alendronate: efficacy and safety results from a randomised, open-label study
John Wark (on behalf of Amgen Australia)

16:42 **OR15** Dietary, supplemental and total calcium intake in relation to common carotid artery intima medial thickness and atherosclerosis in older women
Joshua Lewis (University of Western Australia)

16:54 **OR16** Vertebral Modic changes on lumbar spine MRI: a characterisation of bone remodelling and osteocyte morphology
Julia Kuliwaba (SA Pathology)

17:06 **OR17** Cortical Bone Fragility Contributes to Fractures in Children
Sandra Iuliano (University of Melbourne)

17:18 **OR18** Longitudinal relationships of antiepileptic drug (AED) therapy with bone mineral and balance measures
John Wark (University of Melbourne)

17:30 **OR19** Maternal vitamin D status during pregnancy and peak bone mass attained at 20 years in offspring
Kun Zhu (Sir Charles Gairdner Hospital)

17:42 **OR20** Late menarche is not associated with decreased bone strength in adulthood: A 7-year longitudinal study
Timo Rantalainen (Deakin University)

17:54 **OR21** Cortical exceeds trabecular bone loss before menopause but net bone loss is modest because periosteal apposition occurs
Åshild Bjørnerem (University of Tromsø)

16:30		**SESSION 5B** ***Biomedical Presentations*** *Chairs: Paul Baldock, Yu Zhang*	*Ballroom C*
16:30	**OR22**	CHKB mutant mice display an osteoporotic phenotype with altered osteoclast and osteoblast activities Jennifer Tickner (University of Western Australia)	
16:42	**OR23**	Visualisation of tumour cell dormancy and activation in the skeleton by two-photon intra-vital imaging Michelle McDonald (Garvan Institute)	
16:54	**OR24**	Interleukin-6 family cytokines maintain bone material strength through osteocyte gp130 signalling Rachelle Johnson (St. Vincent's Institute)	
17:06	**OR25**	Roquin is a novel regulator of bone homeostasis Bay Sie Lim (University of Western Australia)	
17:18	**OR26**	Neuropeptide Y signalling in osteoblasts controls glucose metabolism in mice Nicola Lee (Garvan Institute)	
17:30	**OR27**	Mice lacking expression of Oncostatin M Receptor (OSMR) show exacerbated bone loss in response to inflammatory arthritis Nicole Walsh (St. Vincent's Institute)	
17:42	**OR28**	Superoxide dismutase 2 deficiency in osteocytes causes bone loss and fragility in mice Keiji Kobayashi (Chiba University, Japan)	
17:54	**OR29**	Osteomacs are closely associated with osteoclasts *in vivo* and regulate osteoclast formation *in vitro* via cardiotrophin-like cytokine factor 1 Allison Pettit (Mater Research, Translational Research Institute)	
18:06		**Sessions Close**	
18:30		**Young Scientists and Students' Dinner**	*The Kingston Hotel*
19:00		**President's Dinner**	

Tuesday 10 September 2013

08:20 **SESSION 6** *Ballroom B/C*
Exercise and the Muscle-Tendon-Bone Unit
Chairs: Yu Zheng, Mark Kotowicz

08:20 **IS11** Muscle as an Endocrine Organ
Mark Febbraio (Baker IDI)

08:45 **IS12** Exercise and bone strength in children
Belinda Beck (Griffith University)

09:10 **IS26** Exercise prescription for falls and fracture prevention – Is there an optimal type and dose?
Robin Daly (Deakin University)

09:35 **IS27** The skeleton and glycaemic control: do we have a link?
Itamar Levinger (Victoria University)

10:00 – 10:30 **Morning Tea** *Mezzanine*

10:30 – 12:30 **PARALLEL ORAL PRESENTATIONS**

10:30 **SESSION 7A** *Ballroom B*
Clinical Presentations
Chairs: Kerrie Sanders, Tuan Nguyen

10:30 **OR30** Low fracture rates maintained with 7 years of Denosumab treatment for postmenopausal osteoporosis: results from the first 4 years of the FREEDOM Extension
Daniel Thiebaud (Amgen Australia)

10:42 **OR31** Increased bone loss is associated with post-fracture mortality
Dana Bliuc (Garvan Institute)

10:54 **OR32** Hip cortical porosity predicts non-vertebral fractures in postmenopausal women
Åshild Bjørnerem (University of Tromsø)

11:06 **OR33** Body mass index (BMI) and fracture risk: a bone mineral density (BMD)-dependent and site-specific association
Mei Chan (Garvan Institute)

11:18 **OR34** Associations of accelerometer-determined moderate and vigorous physical activity with sarcopenia two years later in community-dwelling older adults
David Scott (The University of Melbourne)

11:30 **OR35** The pathophysiology of subchondral cancellous bone in postmenopausal knee osteoarthritis: diffuse microdamage accumulation and osteocyte cell network deficiency
Julia Kuliwaba (SA Pathology)

11:42 **OR36** Multicentric carpotarsal osteolysis may be a disorder of bone modelling and not osteolysis
Syndia Lazarus (University of Queensland)

11:54 **OR37** Femoral neck DXA parameters and pQCT tibial cortical density are predictive of survival in dialysis patients
Natalie Yap (The University of Sydney)

12:06 **OR38** Cross sectional correlations between hip muscles, muscle strength and bone mineral density
Harbeer Ahedi (Menzies Research Institute, University of Tasmania)

12:18 **OR39** Calcium plus Vitamin D supplementation: a meta-analysis of risk and benefit
Steven Frost (University of Western Sydney)

10:30 **SESSION 7B** *Ballroom C*
Biomedical Presentations
Chairs: Mark Forwood, Hong Zhou

10:30 **OR40** Variance in 3D structure of femoral shaft intracortical void volume: a high-resolution micro-CT study
Egon Perilli (Flinders University)

10:42 **OR41** Disulfiram attenuates osteoclast differentiation via suppression of key RANKL-mediated signaling cascades
Hua Ying (University Of Western Australia)

10:54 **OR42** Intrinsic PTH secretion from the human parathyroid via a prostanoid-dependent autocrine mechanism
Arthur Conigrave (University of Sydney)

11:06 **OR43** Subchondral bone mineral and osteocyte changes in osteoarthritis
Anjali Jaiprakash (Institute of Health and Biomedical Innovation, QUT)

11:18 **OR44** Gender-related differences in the skeletal phenotype of adult global vitamin D receptor (VDR) knockout mice
Jackson Ryan (University of South Australia)

11:30 **OR45** Protease-activated receptor-1: common pathways in bone and muscle repair?
Charles Pagel (The University Of Melbourne)

11:42 **OR46** Osteocyte cell death in subchondral bone in a mouse model of post-traumatic osteoarthritis correlates with the severity of aggrecan loss in overlying cartilage
Brett Tonkin (St. Vincent's Institute)

11:54 **OR47** Stage-specific effects of ephrinB2 in the osteoblast lineage on bone strength
Christina Vrahnas (St. Vincent's Institute)

12:06 **OR48** Targeting Class I HDACs to suppress both inflammation and bone loss in arthritis
Melissa Cantley (University of Adelaide)

12:18 **OR49** Inhibition of PDGFRα increases OPG production from osteoblastic cells - an indirect mechanism by which tyrosine kinase inhibitors inhibit osteoclastogenesis
Mei Lin Tay (University of Auckland)

12:30 – 13:30 **Lunch, poster viewing and networking** *Mezzanine*

13:30 **SESSION 8** *Ballroom B/C*
Clinical Cases Panel Discussion
Chair: Rory Clifton-Bligh *Faculty: Peter Ebeling, Jackie Center*

13:30 **Case 1** Not your typical atypical femur fracture
Sarah Catford (Western Hospital)

13:50 **Case 2** Paediatric bone disease
Andrew Biggin (Sydney Children's Hospital Network)

14:10 **Case 3** Trauma fracture on androgen deprivation therapy for (non-metatastic) prostate cancer
Mark Ng Tang Fui (Austin Health)

14:30 **Case 4** A Case of Osteoporosis
Syndia Lazarus (Royal Brisbane and Women's Hospital)

14:50 **Case 5** Paget's Disease
Ramanamma Kalluru (The University of Auckland)

15:10 **Case 6** Low Turnover; Bystander or Abettor?
Grahame Elder (Westmead Hospital)

15:30 – 16:00		**Afternoon Tea**	**Mezzanine**
16:00		**SESSION 9** **Mini Poster Oral Presentations** **Selected from Finalists of the Chris And Margie Nordin Poster Prize Judging** *Chair: David Findlay, Julie Pasco*	**Ballroom B/C**
16:00	**OR50**	mini oral (to be selected from Poster Prize Judging)	
16:07	**OR51**	mini oral (to be selected from Poster Prize Judging)	
16:14	**OR52**	mini oral (to be selected from Poster Prize Judging)	
16:21	**OR53**	mini oral (to be selected from Poster Prize Judging)	
16:30		**Light of the Southern Cross – Icons in Bone Memorial Oration** *Chair: Markus Seibel* Brain, Bone, Fat: Unraveling the Role of AP1 Transcription Factors Roland Baron (USA)	
17:15		**ANZBMS AGM**	**Ballroom B/C**
19:00 for 19:30 – 11:30		**Conference Dinner**	**Ballroom B/C**

Wednesday 11 September 2013

08:40 **SESSION 10** **Ballroom B/C**
Bone Cell Blology
Chairs: Gerald Atkins, Emma Baker

08:40 **IS15** Osteocytes and PTH
Paola Divieti-Pajevic (USA)

09:15 **IS16** Cell stress, MITF and the control of osteoclast formation
Julian Quinn (Prince Henry's Institute)

09:40 **IS17** Long-chain fatty acids – another player in the fat/bone mass relationship
Jillian Cornish (The University of Auckland)

10:05 **IS18** Deciphering the osteoclastome
Nathan Pavlos (University of Western Australia)

10:30 – 11:00 **Morning Tea** **Mezzanine**

11:00 **SESSION 11:**
Vitamins and Bone
Chairs: Lyn March, Rachelle Buchbinder

11:00 **IS19** Vitamin D and the skeleton: the paradox of a long-standing paradigm
Clifford Rosen (USA)

11:40 **IS20** What is the optimal 25-Hydroxyvitamin D [25(OH)D] level?
Ian Reid (The University of Auckland)

12:05 **IS21** Photoprotection by Vitamin D compounds
Rebecca Mason (The University of Sydney)

12:30 **Conclusion**

Draft program date: 19 August 2013

PLENARY POSTER PRESENTATIONS

Plenary Poster P1
Knockdown of PTH/PTHrP receptor PTH1R in osteosarcoma cells decreases invasion and increases mineralization *in vivo*
Patricia WM Ho, Megan R Russell, Ankita Goradia, Blessing Crimeen-Irwin, Pece Kocovski, Alvin JM Ng, Alistair M Chalk, Kristi Milley, Janine A Danks, John L Slavin, Ross A Dickins, T John Martin, Carl R Walkley

Plenary Poster P2
Down-regulation of GLI2 prevents metastasis of osteosarcoma via regulation of ribosomal protein S3
Takao Setoguchi, Setsuro Komiya

Plenary Poster P3
OSMR-/- mice show reduced synovial inflammation and cartilage damage, but increased bone loss in response to joint injury
Brett Tonkin, Natasha Jansz, Joshua Johnson, Evange Romas, Natalie Sims, Nicole Walsh

Plenary Poster P4
Assessing not only bone loss but also soft tissue swelling in a murine inflammatory arthritis model: a novel 3D micro-CT method
Egon Perilli, Melissa D Cantley, Victor Marino, Tania N Crotti, Malcolm D Smith, David R Haynes, Anak SSK Dharmapatni

Plenary Poster P5
Androgen action directly via the androgen receptor in osteoblasts is dependent on the stage of osteoblast maturation
Rachel Davey, Patricia Russell, Michele Clarke, Kristine Wiren, Jeffrey Zajac

Plenary Poster P6
Bidirectional regulation of osteoclast formation by ephrinB2/EphB4 signaling in the osteoblast lineage
Stephen Tonna, Patricia W. M Ho, Farzin M Takyar, Carl R Walkley, T John Martin, Natalie A Sims

Plenary Poster P7
Completing the evaluation of the skeletal regulation by the NPY system: the Y5 receptor
Ee Cheng Khor, Yan-Chuan Shi, Ronaldo Enriquez, Herbert Herzog, Paul Baldock

Plenary Poster P8 / Christopher and Margie Nordin Young Investigator Poster Award Finalist
SQSTM1/p62 mutant proteins associated with Paget's disease of bone lead to increased autophagy markers, while attenuating autophagosome maturation
Sarah Rea, Melanie Sultana, Nathan Pavlos, Robert Layfield, Jiake Xu, John Walsh, Thomas Ratajczak

Plenary Poster P9
Spatial control of bone formation using a porous polymer implant
Nicole Yu, Marie Gdalevitch, Aaron Schindeler, Ciara Murphy, Kathy Mikulec, Lauren Peacock, Jane Fitzpatrick, Justin Cooper-White, Andrew Ruys, David Little

Plenary Poster P10
Changes in proximal femur structure with age: A cross-sectional study of 719 Caucasian females aged between 20 and 89 years
Benjamin Khoo, Keenan Brown, Christopher Cann, Richard Prince

Plenary Poster P11
Effects of individualised bone density feedback and educational interventions on osteoporosis knowledge and self-efficacy in young women: a 12-yr prospective study
Feitong Wu, Laura Laslett, Karen Wills, Brian Oldenburg, Graeme Jones, Tania Winzenberg

Plenary Poster P12
Two novel mutations in the osteoprotegerin encoding gene, *TNFRSF11B*, in patients with juvenile Paget's disease
Ally Choi, Dorit Naot, Pelin O Simsek Kiper O, Linda Di Meglie, Tim Cundy

Plenary Poster P13
Parental socioeconomic status and childhood fractures: data from the Geelong Osteoporosis Study Fracture Grid
Natalie K Hyde, Julie A Pasco, Sharon L Brennan

Plenary Poster P14

Selective serotonin reuptake inhibitors (SSRIs) decrease serum markers of bone turnover: Geelong Osteoporosis Study

Lana J Williams, Michael Berk, Jason M Hodge, Mark A Kotowicz, Fiona Collier, Julie A Pasco

Plenary Poster P15

Comparative bone densitometry and anthropometry of the Indian and Nigerian female students, graduated in Ukrainian Medical University

Lubov Stklyanina, Vladyslav Luzin, Helen Nuzna, Vasily Tarasov

Plenary Poster P16

Prevention of aromatase inhibitor-induced bone loss with alendronate in postmenopausal women: The Batman Trial

Anna Lomax, Saw Yee Yap, Karen White, Jane Beith, Ehtesham Abdi, Adam Broad, Sanjeev Sewak, Chooi Lee, Philip Sambrook, Nick Pocock, Margaret Henry, Elaine Yeow, Richard Bell

Plenary Poster P17

Changes in bone mineral density related to incidence of fracture with 6 years of denosumab treatment for postmenopausal osteoporosis

Miller PD, Cummings SR, Reginster JY, Franchimont N, Bianchi G, Bolognese MA, Chapurlat R, Hawkins FG, Kendler DL, Oliveri B, Zanchetta JR, Daizadeh N, Wang A, Wagman R, Papapoulos S

Plenary Poster P18 / Christopher and Margie Nordin Young Investigator Poster Award Finalist

Predictors of refracture in patients managed within a Fracture Liaison Service: A 7-year prospective study

Kirtan Ganda, Markus Seibel

POSTER PRESENTATIONS

Christopher and Margie Nordin Young Investigator Poster Award Finalists

P20 **Osteopontin and the response to muscle injury: requirement for expression by inflammatory or muscle cells**
Dimuthu K. Wasgewatte Wijesinghe, Eleanor J. Mackie, Charles N. Pagel

P21 **Neurotrophin-3 (NT-3) attenuates chondrogenesis but enhances osteogenesis during growth plate bony repair**
Yu-Wen Su, Rosa Chung, Tina Vincent, Fiona H Zhou, Alice M Lee, Xin-Fu Zhou, Cory, J Xian

P22 **Adipogenesis occurs at the expense of osteoblast differentiation in primary osteoblasts deficient in protease-activated receptor-2**
P Kularathna, C.N. Pagel, J.D. Hooper, E.J. MacKie

P23 **Clinical manifestation of rickets is enhanced in low calcium/phosphate fed osteoblast-specific Vitamin D Receptor (OSVDR) transgenic mice: Evidence for direct negative effects of Vitamin D on mineralisation**
Rahma Triliana, Paul H Anderson, Howard A Morris

P24 **Bone volume increases with loading, but is not altered with unloading, in the homozygous SOST knockout mouse**
Alyson Morse, Michelle McDonald, Ina Kramer, Michaela Kneissel, Natalie Kelly, Katherine Melville, Marjolein Van Der Meulen, David Little

P25 **Associations between lean and fat mass and bone structure: a co-twin study**
Negar Shahmoradi, Xiaofang Wang, Sandra Iuliano, Ali Ghasem Zadeh, Åshild Bjørnerem, Ego Seeman

P26 **Relationship between body composition and osteoarthritis**
Mei Chan, Jacqueline Center, John Eisman, Tuan Nguyen

P27 **Is Osteoporosis-related Quality of Life (QoL) associated with fracture-free mortality: Geelong Osteoporosis Study (GOS)**
Yu Zhang, Mark Kotowicz, Julie Pasco, Kerrie Sanders

P28 **Frequency distribution of voxels with their varying content of mineralized bone and void volume captures variance in microstructure better than trait means**
Yohann Bala, Egon Perill, Sandra Iuliano, Ali Ghasem-Zadeh, Xiao-Fang Wang, Ego Seeman, Roger Zebaze

P29 **Fracture and utilisation of residential aged care in Australia**
Haslinda Gould, Sharon Brennan, Robert MacInnis, Mark Kotowicz, Geoff Nicholson, Julie Pasco

P30 **Generation of highly purified osteocytes by fluorescence activated cell sorting (FACS)**
Ling Yeong Chia, Nicole Walsh, Carl Walkley, Emma Baker, T John Martin, Natalie Sims

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P31 **Local synthesis of 1,25-dihydroxyvitamin D (1,25D) promotes osteocyte maturation**
Andrew Turner, Maarten Hanrath, Gerald Atkins, Paul Anderson, Howard Morris

P32 **The effects of recombinant undercarboxylated osteocalcin on insulin-stimulated glucose uptake following *ex vivo* muscle contraction**
Itamar Levinger, Xuzhu Lin, Xinmei Zhang, Alan Hayes, George Jerums, Mathieu Ferron, Gerard Karsenty, Ego Seeman, Glenn McConell

P33 **Vitamin D Receptor in mature osteoblasts and osteocytes is required for normal bone formation and resorption**
Helen Tsanagri[1], Trish Russel[2], Michele Clarke[2], Andrew Turner[1], Jackson Ryan[1], Yolandi Straczak[1], Kate Barratt, Rebecca Sawyer, Howard Morris, Gerald Atkins, Rachel Davey, Paul Anderson

P34 **Biphasic calcium-dependent control of cyp27b1 expression mediated by the calcium-sensing receptor**
Vimesh A. Avlani, Alice Huang and Arthur D. Conigrave

P35 **Analysis of vitamin D metabolism gene expression in human bone: evidence for autocrine control of bone remodelling**
Renee Ormsby, Masakazu Kogawa, David Findlay, Paul Anderson, Howard Morris, Gerald Atkins

P36 **EGFL7 expressed in bone microenvironment mediates the migration of endothelial cells via ERK, STAT3 and integrin signaling cascades**
Shek Man Chim, Vincent Kuek, Siu to Chow, Bay Sie Lim, Jennifer Tickner, Rosa Chung, Yu-Wen Su, Ge Zhang, Wendy Erber, Vicki Rosen, Cory Xian, Jiake Xu

P37 **The role of EGFL6 in bone homeostasis**
Shek Man Chim, Jennifer Tickner, Baysie Lim, Benjamin Ng, Jiake Xu

P38 **Anabolic action on bone cells by the coiled-coil domain of fasting-induced adipose factor ($FIAF_{CCD}$)**
JM Lin, D Naot, JL Costa, AB Grey, J Cornish

P39 **Osteoclast gp130 signalling stimulates periosteal bone formation**
Rachelle Johnson, Narelle McGregor, Holly Brennan, Ingrid Poulton, T John Martin, Natalie A Sims

P40 **Cell death is augmented in ephrinB2-deficient osteoblasts**
Blessing Crimeen-Irwin, Stephen Tonna, Patricia WM Ho, T John Martin, Natalie A Sims

P41 **End-products of a tryptophan degradation pathway have an osteo-anabolic effect on differentiating mesenchymal stem cells**
Christopher Vidal, Sandra Bermeo, Wei Li, Krishanthi Gunaratnam, Gilles Guillemin, Chai Lim, Gustavo Duque

P42 **Analysis of bone formation activity utilizing transgenic mice by *in vivo* bioluminescence imaging**
Tomoko Nakanishi, Kazuo Kokubun, Haruka Oda, Mika Aoki, Makoto Taniguchi, Atsumi Soma, Yasuhiro Kazuki, Mitsuo Oshimura

P43 **Selective serotonin reuptake inhibitors (SSRIs) inhibit human osteoblastogenesis and decrease bone development and mineralization in Zebrafish**
Jason Hodge, Daniel Fraher, Fiona Collier, Janine McMillan, Lee Kennedy, Megan Ellis, Ken Walder, Seetal Dodd, Michael Berk, Julie Pasco, David Ashley, Yann Gibert, Lana Williams

P44 **A novel mouse cortical bone derived mesenchymal stem cell-like cell line**
Dongqing Yang, Sandra Isenmann, Stan Gronthos, Paul Anderson, Howard Morris, Gerald J. Atkins

P45 **Molecular mechanisms underlying accelerated bone remodeling during hyperhomocysteinemia**
Viji Vijayan, Mayuri Khandelwal, Kapil Manglani, Rajiv Ranjan Singh, Sarika Gupta, Avadhesha Surolia

P46 **The effects of 17β-estradiol on cellular proliferation and calcification of human mesenchymal stem cell during osteogenesis**
Jenny Wang, Joshua Lewis, Lawrence Liew, Anthony Buzzai, Rodney Dilley, Jeremy Tan, Gerard Hardisty, Jeffery Hamdorf, Minghao Zheng, Richard Prince

P47 **MicroRNAs & cell signaling pathways: orchestrating osteogenesis and osseointegration**
Nishant Chakravorty, Anjali Jaiprakash, Ross Crawford, Adekunle Oloyede, Saso Ivanovski, Yin Xiao

P48 **The roles of Schnurri family in differentiation of osteoblasts and chondrocytes**
Katsuyuki Imamura, Shingo Maeda, Ishidou Yasuhiro, Masahiro Yokouchi, Setsuro Komiya

P49 **The direct anti-anabolic effect of sclerostin on the mechanical loading response in bovine bone *ex vivo***
Masakazu Kogawa, Kamarul A Khalid, Asiri R Wijenayaka, Renee T Ormsby, David M Findlay, Gerald J Atkins

P50 **The characterisation of SaOS2 osteosarcoma cells as an in vitro osteocyte-like cell model**
Matthew Prideaux, David M. Findlay, Asiri R. Wijenayaka, Duminda Kumarasinghe, Gerald J. Atkins

P51 **What determines osteocyte density in bone matrix? A computational model**
Pascal R Buenzli, C David L Thomas, John G Clement

P52 **Global deletion of the cyp27b1 gene results in impaired osteoclastogenesis and activity in splenocyte cultures generated *ex vivo* from mouse knockout models**
Daniel Reinke, Masakazu Kogawa, Paul Anderson, Howard Morris, Gerald Atkins

P53 **Histological localization of 15N-minodronate by isotope microscopy and its biological effects on bone cells**
Muneteru Sasaki, Hiromi Hongo, Sachio Kobayashi, Hisayoshi Yurimoto, Norio Amizuka

P54 **Antipsychotics inhibit human osteoclast formation and function**
Alice Torpy, Jason Hodge, Fiona Collier, Julie Pasco, Michael Berk, David Ashley, Lana Williams

P55 **Chemotherapy agents induce Microphthalmia Transcription Factor (MITF) in osteoclast progenitors**
A. Gabrielle Van Der Kraan, Ryan Chai, Michelle Kouspou, Ben Lang, Preetinder Singh, Matthew Gillespie, John Price, Julian Quinn

P56 **Luman, an ER stress transducer, is involved in osteoclastogenesis through the regulation of DC-STAMP expression**
Soshi Kanemoto, Atsushi Saito, Yasuhiro Kobayashi, Teruhito Yamashita, Takeshi Miyamoto, Naoyuki Takahashi, Kazunori Imaizumi

P57 **Interleukin-33 may stimulate functional osteoclast formation in the absence of RANKL**
Damien Eeles, Jason Hodge, Preetinder Singh, Brian Grills, Johannes Schuijers, Matthew Gillespie, Damian Myers, Julian Quinn

P58 **Generation and characterisation of the osteoclast-specific Vitamin D Receptor knock out mice**
Yolandi Starczak, Patricia K Russell, Michele V Clarke, Masakazu Kogawa, Howard A Morris, Rachel A Davey, Gerald J Atkins, Paul H Anderson

P59 **The bone has an endogenous and cell-autonomous circadian clock**
Naoki Okubo, Yoichi Minami, Hiroyoshi Fujiwara, Ryo Oda, Kazuhiro Yagita, Toshikazu Kubo

P60 **Neonatal leptin treatment partially reverses developmental programming of bone morphology**
Maureen Watson, Elwyn Firth, Mark Vickers, Greg Gamble, Karen Callon, Jill Cornish

P61 **Fluorescent tracking of vascular and myogenic cells during BMP-2 induced bone formation**
Mille P Kolind, Alastair Aiken, Kathy Mikulec, Lauren Peacock, David G Little, Aaron Schindeler

P62 **Leucovorin prevents methotrexate chemotherapy-induced bone loss and bone marrow adiposity in rats potentially by modulating Wnt/β-catenin signalling and folate metabolism**
Kristen R Georgiou, Nadhanan Rethi Raghu, Cory J Xian

P63 **Expression of novel cartilage genes in growth cartilage and during maturation of cultured ATDC5 cells**
Babatunde Awodele, Michiko Mirams, Charles Pagel, Eleanor J. MacKie

P64 **Differential gene expression in growth cartilage and osteochondrosis**
Babatunde Awodele, Eleanor MacKie, Charles Pagel, Michiko Mirams

P65 **Disrupted signaling of FGF23/klotho induces not only vascular calcification but also vascular ossification**
Tomoka Hasegawa, Ichiro Ohkido, Shigeichi Syoji, Tamaki Yamada, Kimimitue Oda, Keitaro Yokoyama, Norio Amizuka

P66 **Tenocyte viability and function following treatment with the protein component extracted from a hydroxyapatite-based product**
Ashika Chhana, David Musson, Pirashanthini Maruthayanar, Karen Callon, Dorit Naot, Jillian Cornish

P67 **A novel pathway of hedgehog signaling modification by primary cilia in odontoblasts**
Kazumi Kawata, Keishi Narita

P68 **Assessment of the rate of decalcification and histological outcome of microwave-assisted and traditional methods of bone decalcification**
Gemma Diessel, Mark Forwood, Wendy Kelly, Scott Little

P69 ***In vivo* monitoring of bone and fat in mice using the Quantum Fx scanner**
Natalie Wee, Nancy Mourad, Ee Cheng Khor, Ronaldo Enriquez, Natasa Kovacic, Michelle McDonald, Herbert Herzog, Peter Croucher, Paul Baldock

P70 ***In vitro* tenocyte mechanobiological studies mimicking *in vivo***
David Musson, Jungjoo Kim, Karen Callon, Iain Anderson, Vickie Shim, Jillian Cornish

P71 **The features of ultrastructure biomineral of dentin lower incisors in intact rats of different ages**
Vitaly Morozov, Vladyslav Luzin, Dmitry Astrakhantsev, Marin Zhernovaya, Pavel Golubkov

P72 **Lateral-projection areal bone mineral density (aBMD) predicts vertebral failure load better than posterior-anterior aBMD**
Andrew M Briggs, Egon Perilli, Susan Kantor, John Codrington, Ian H Parkinson, Karen J Reynolds, John D Wark

P73 **Utility of reference-point indentation for characterization of brittle murine bones.**
Susan Millard, Anneliese Dickson, Roland Steck, Nicholas M Fisk

P74 **Integrating micro CT indices, CT imaging and computational modelling to assess the mechanical performance of fluoride treated bone**
Dharshini Sreenivasan, Maureen Watson, Michael Dray, Raj Das, Andrew Grey, Jillian Cornish, Justin Fernandez

P75 **Atypical femoral fractures are associated with physiological patterns of bone tensile deformations**
Saulo Martelli, Peter Pivonka, Peter R. Ebeling

P76 **Inter- and intra-individual variation in human osteon geometry across age**
Christina Jovanovic, Hannah Kim, Min Kim, John Clement, David Thomas

P77 **Validation of ultrasound transit time spectroscopy of solid volume fraction in bone:marrow replica models by comparison of geometric calculation, computer simulation, and experimental studies**
Marie-Luise Wille, Christian M. Langton

P78 **The specific surface of cortical bone and its influence on the temporal evolution of cortical porosity during age-related bone loss**
Chloe Lerebours, Peter Pivonka, David Thomas, John Clement, Pascal Buenzli

P79 **Biophysical properties of rat humerus after implantation into the tibia of biogenic hydroxyapatite, saturated with zinc**
Vladimir Koveshnikov, Vladyslav Luzin, Vladimir Korotun, Vitaly Morozov, Vitaly Stry

P80 **Racial differences in cortical and trabecular microstructure arise during puberty**
Xiao-Fang Wang, Ali Ghasem-Zadeh, Qingju Wang, Jiawei Teo, Sandra Iuliano, Roger Zebaze, Yohann Bala, Ego Seeman

P81 **Up-regulation of 11beta-hydroxysteroid dehydrogenase 2 decreases the sensitivity of cancer cells to glucocorticoids**
Sarah Kim, Yu Zheng, Colette Fong-Yee, Mark Cooper, Markus Seibel, Hong Zhou

P82 **Molecular mechanisms of bone invasion by oral squamous cell carcinoma (OSCC)**
Jingjing Quan, Nigel Morrison, Newell Johnson, Jin Gao

P83 **Assessment of structural and remodeling indices in periarticular bone tissues in the osteoarthritic femoral head: influence of age and gender**
Guangyi Li, Yuanchen Ma, Euphemie Landao, Zhen Lin, Nathan Pavlos, an Qin, Tak Cheng, Minghao Zheng

P84 **Impaired muscle function in a mouse model of osteoarthritis**
Chris Van Der Poel, Pazit Levinger, Brett Tonkin, Itamar Levinger, Nicole Walsh

P85 **Post-traumatic osteoarthritis and lower limb muscle functions**
Hossein Mokhtarzadeh, Chen Hua Yeow, Denny Oetomo, Fatemeh Malekipour, Peter Pivonka, Peter Lee

P86 **Rheumatoid arthritis and incident fracture in women: A prospective study**
Sharon Brennan, Leisje Toomey, Mark Kotowicz, Margaret Henry, Hedley Griffiths, Julie Pasco

P87 **The xIAP inhibitor Embelin suppresses inflammation and bone loss in Collagen Antibody Induced Arthritis (CAIA) mice**
Anak SSK Dharmapatni, Melissa D Cantley, Victor Marino, Egon Perilli, Tania N Crotti, Malcolm D Smith, David R Haynes

P88 **Subchondral bone injury precedes cartilage degeneration when articular surfaces undergo fatigue loading**
Ebrahim Bani Hassan, Liliana Tatarczuch, Su Toulson, Eleanor J MacKie, Robert C Whitton

P89 **The relationship between serum and synovial tissue levels of osteoclast associated receptor (OSCAR) in early rheumatoid arthritis**
Tania Crotti, Roxanne Coleman, Anak Dharmapatni, Mihir Wechalekar, Helen Weedon, Michelle Lorimer, Andrew Zannettino, David Haynes, Malcolm Smith

P90 **An injectable formulation for co-delivery of BMP, bisphosphonate, and hydroxyapatite microparticles for bone tissue engineering**
Aaron Schindeler, Peter Valtchev, Tegan L Cheng, Ciara Murphy, Fariba Dehghani, David G Little

P91 **$PTH_{(1-34)}$ treatment for improved healing of bisphosphonate associated stress fractures**
Ciara M Murphy, Aaron Schindeler, Nicole YC Yu, Kathy Mikule, Lauren Peacock, David G Little

P92 **The effect of parthenolide treatment on a murine calvarial model of peri-prosthetic osteolysis as assessed by live-animal micro-computed tomography**
Muhamad Syahrul Fitri Zawawi, Victor Marino, Melissa Cantley, Egon Perilli, Jiake Xu, Anak Dharmapatni, David Haynes, Tania Crotti

P93 **The effect of programmable mechanical stimulation on tendon repair in a bioreactor system**
Tao Wang, Zhen Lin, Robert E Day, Bruce Gardiner, Jonas Rubenson, Thomas B. Kirk, David W. Smith, David G. Lloyd, Allan Wang, Ming H Zheng

P94 **Can bone quality markers predict nonunion?**
Koji Nozaka, Yoichi Shimada, Naohisa Miyakoshi, Shin Yamada, Michio Hongo, Yuji Kasukawa, Hidetomo Saito, Hiroaki Kijima

P95 **Tenofovir therapy decreases bone mineral density in hepatitis B: implications for clinical management**
Phillip Wong, Suong Le, Adam Doyle, Ilani Shochet, Frances Milat, William Sievert

P96 **The association between gonadal status, body composition and bone mineral density in transfusion-dependent thalassemia**
Phillip Wong, Peter Fuller, Matthew Gillespie, Vicky Kartsogiannis, Donald Bowden, Boyd Strauss

P97 **Is transfusion-dependent thalassemia associated with an increased risk of antiresorptive agent-induced Osteonecrosis of the Jaw (ONJ)?**
Jeremy Chan, Phillip Wong, Donald K Bowden, Peter J. Fuller, Frances Milat

P98 **Prior use of bone-related medication in adults with recent low trauma fracture**
Kerrie Sanders, Geoffrey Nicholson, Amanda Stuart, Yu Zhang, Sandra Iuliano, Ego Seeman, Tania Winzenberg, Laura L Laslett, Catherine Shore-Lorenti, Peter R Ebeling

P99 **Predictors of bisphosphonate uptake in Australian adults following a low trauma fracture**
Kerrie Sanders, Julie Abimanyi-Ochom, Jenny J Watts, Catherine Shore-Lorenti, Geoffrey Nicholson, Amanda Stuart, Yu Zhang, Ego Seeman, Sandra Iuliano, Richard Prince, Lyn March, Marita Cross, Tania Winzenberg, Laura L Laslett, Gustavo Duque, Peter R Ebeling, Fredrik Borgstrom

P100 **Non-osteoporotic low-trauma fractures and the burden of re-fracture and mortality**
Jacqueline Center, Dana Bliuc, Nguyen Nguyen, Tuan Nguyen, John Eisman

P101 **Osteoporosis management among Australians at high risk of osteoporotic fractures: an overview from the 45&Up Study cohort**
Jian Sheng Chen, Judy M Simpson, Fiona Blyth, Lyn M March

P102 **Risedronate slows or partly reverses microarchitecture deterioration depending on whether remodelling is perturbed or in steady state**
Yohann Bala, Roland Chapurlat, Dieter Felsenberg, Thierry Thomas, Michel Laroche, Edward Morris, Jose Zanchetta, Angela M. Cheung, Ali Ghasem-Zadeh, Roger Zebaze, Ego Seeman, René Rizzoli

P103 **Relationships between serum sclerostin levels and bone metabolism-related indices as well as bone fragility**
Mika Yamauchi, Masahiro Yamamoto, Kiyoko Nawata, Ken-Ichiro Tanaka, Noriko Ogawa, Toshitsugu Sugimoto

P104 **Distributions and reference intervals for serum osteocalcin, N-propeptide of Type 1 collagen and C-terminal cross-linking telopeptides of Type 1 collagen in older men. The Health In Men Study.**
Paul Chubb, Elizabeth Byrnes, Laurens Manning, John Beilby, Kieran McCaul, Peter Ebeling, Samuel Vasikaran, Leon Flicker, Bu Yeap

P105 **Diabetes-related foot complications and their treatment are associated with bone deficits**
Qian Sun, Paul Wraight, Stephen Tsimos, Sue Kantor, Amar Lakhani, John Wark

P106 **Vertebral Fractures are under-recognised and rarely actioned by hospital clinical teams**
Samantha Donaldson, Emma Duncan, Melissa Clarke

P107 **Effects of Denosumab in men with low bone mineral density (BMD), men with prostate cancer receiving androgen deprivation therapy and women with Postmenopausal Osteoporosis (PMO)**
Lauren Leahy (Amgen), Michael R McClung, Jean-Pierre Devogelaer, David L Kendler, Ãsten Ljunggren, Michael A Bolognese, Henry G Bone, E Michael Lewiecki, Paul D Miller, Ugis Gruntmanis, Matthew R Smith, Yuqing Yang, Andrea Wang, Rachel B Wagman, Jesse W Hall, Steven Boonen

P108 **Mild to moderate chronic kidney disease is associated with vertebral fracture independent of albuminuria in patients with Type 2 diabetes**
Masahiro Yamamoto, Toru Yamaguchi, Shozo Yano, Mika Yamauchi, Toshitsugu Sugimoto

P109 **The Orthogeriatrics Model: Testing the effectiveness of an integrated model of care in older patients with hip fracture**
Gustavo Duque, Oddom Demontiero, Luis Sardinha, Griselda Loza-Diaz

P110 **GPs' perceptions of the usefulness of absolute fracture risk assessment in their practice: barriers, facilitators and other issues for implementation.**
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P131 **Associations between serum 25-hydroxy vitamin D concentrations and multiple health conditions, physical performance measures, disability and all-cause mortality: The Concord Health and Ageing in Men Project**
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P135 **Osteocalcin, muscle strength and indices of bone health in older women**
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P137 **PTH in the elderly: are the normal ranges method dependent? Preserved renal function estimation by using different indexes**
Andrea Kozak, Ana Maria Sequera, Viviana Mesch, Patricia Otero, Paula Esteban, Graciela Astarita, Monica Saavedra, Isabel Teres, Patricia Pagano, Maria Jose Iparaguirre, Mirta Gurfinkiel, Marta Torres

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Invited and Oral Presentations

Sunday 8 September 2013
SESSION 1: Bone and Energy Metabolism

IS1

Taking the Temperature of Fat-Bone Cross-Talk

Clifford J Rosen
Professor of Medicine Tufts University School of Medicine, USA

The relationship of obesity to bone mass and fractures is complex and it is difficult to discern whether fat mass is a potential risk factor or is indeed protective of osteoporosis. To try and understand this inter-relationship requires a basic understanding of the adipocyte, its origin and its implications for the skeleton. Both osteoblasts and adipocytes arise from a common mesenchymal progenitor cell in the bone marrow and stromal vascular fraction of adipose depots. In states of low bone mass, marrow adiposity is enhanced while osteoblast differentiation is often suppressed. This has led to the concept that there is an all or none differentiation process whereby the progenitor can become either an osteoblast or adipocyte but not both. However, there are in vivo models that do not support that tenet and suggest that the cross-talk between cells may be more complex than purely an adipocyte induced inhibitory action. Recent work from our lab and other groups have examined the role of brown-like adipogenesis on skeletal function. Indeed, the volume of pre-formed brown adipose tissue is directly related to bone mineral density whereas activation of brown like adipogenesis through the sympathetic nervous system correlates with bone loss. This has led us to hypothesize that temperature may mediate body composition and skeletal remodeling. In this talk, I will present preliminary data to support the importance of both systemic and local temperature in regulating skeletal remodeling. I will also examine the role of new agents in stimulating browning of white fat depots and their off target effects on the skeleton.

IS2

Glucocorticoid Effects on Bone and Energy Metabolism

Tara C Brennan-Speranza[1], Holger Henneicke[1], Sylvia Gasparini[1], Caren Gundberg[2], Colin R. Dunstan[1,3], Hong Zhou[1], Markus J Seibel[1,4]
[1]*Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, Australia,*
[2]*Department of Orthopaedics, Yale University School of Medicine, New Haven, CT, USA*
[3]*Biomedical Engineering, Faculty of Engineering, University of Sydney, Sydney, Australia*
[4]*Dept of Endocrinology & Metabolism, Concord Hospital, The University of Sydney at Concord, Sydney, Australia*

Glucocorticoids have significant beneficial effects in the treatment of inflammatory conditions. However, long-term use of glucocorticoids is associated with numerous adverse outcomes which are often chronic in nature and difficult to treat. These include osteoporosis, sarcopenia, impaired glucose metabolism and obesity. In bone, glucocorticoids induce a rapid and profound suppression of osteoblast function, which results in significant bone loss over time. In contrast to these well documented skeletal effects, the pathways by which glucocorticoids exert their detrimental effects on energy metabolism are less well established.
Recent research has revolutionised our understanding of the way the skeleton interacts with other organs and body functions. Mouse data suggests that the osteoblast-specific peptide, osteocalcin, may be involved in the control of fuel metabolism via its actions on pancreatic insulin production and secretion as well as insulin sensitivity.[1-3] The skeleton may therefore have a role in the control of fuel metabolism under normal physiological conditions.
We recently demonstrated that osteoblast-targeted disruption of glucocorticoid signaling in transgenic mice not only prevents suppression of osteoblast function and serum osteocalcin levels but also attenuates metabolic dysfunction and obesity in corticosterone-treated mice. We therefore investigated the role of osteocalcin in the pathogenesis of glucocorticoid-induced insulin resistance, glucose intolerance and obesity. We found that heterotopic (hepatic) expression of osteocalcin through gene therapy prevented glucocorticoid-induced dysmetabolism and fatty liver disease in corticosterone-treated animals[4].
These data provide evidence that the suppression of osteoblast function plays a central role in mediating the effects of exogenous GC on systemic energy metabolism.

1. Ferron, M., Hinoi, E., Karsenty, G. & Ducy, P. Osteocalcin differentially regulates β cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proceedings of the National Academy of Sciences* **105**, 5266-5270 (2008).
2. Ferron, M.*, et al.* Insulin Signaling in Osteoblasts Integrates Bone Remodeling and Energy Metabolism. *Cell* **142**, 296-308 (2010).
3. Lee, N.K.*, et al.* Endocrine Regulation of Energy Metabolism by the Skeleton. *Cell* **130**, 456-469 (2007).
4. Brennan-Speranza, TC.*, et al.* Osteoblasts mediate the adverse effects of glucocorticoids on fuel metabolism. *J Clin Invest.* **122**, 4172-4189. doi: 4110.1172/JCI63377. Epub 62012 Oct 63324. (2012)

Sunday 8 September 2013
SESSION 1: Bone and Energy Metabolism

IS3

The interaction between inflammation and glucocorticoids in glucocorticoid-induced osteoporosis
Mark Cooper (Australia)

Therapeutic glucocorticoids are widely used to treat patients with inflammatory disorders but bone loss is a common and troublesome problem in these patients. Glucocorticoids and inflammation are both detrimental to bone due to an uncoupling of bone formation from resorption in favour of excessive resorption. However, the relative contributions of glucocorticoids and inflammation to bone damage in patients with inflammatory disease are unclear. In contrast to the clinical situation, in vitro studies and animal models examining glucocorticoid effects on bone rarely consider the role of inflammation. Recent studies have linked bone loss associated with joint inflammation to the production of wnt inhibitors such as DKK1 by cells within the inflamed synovial (joint lining) tissue. These paracrine factors are also induced by glucocorticoids in this tissue indicating that glucocorticoid induced bone damage could be partly through actions on the joint rather than through direct actions on bone. The sensitivity of bone and synovial tissue to glucocorticoids is regulated by the glucocorticoid metabolising enzyme 11b-hydroxysteroid dehydrogenase type 1 (11b-HSD1) which converts inactive cortisone to active cortisol. In both synovium and bone tissue the activity of this enzyme increases dramatically in response to inflammation heightening the sensitivity of bone to endogenous and therapeutic glucocorticoids. Furthermore, the inflamed synovium itself appears to be a significant source of active glucocorticoids. These studies highlight the complex interaction between inflammation and glucocorticoids in bone loss during inflammatory disease. A better understanding of these interactions should lead to better approaches to target inflammation-associated bone loss.

OR1 Amgen-ANZBMS Outstanding Abstract - Clinical Presentation

Higher dietary calcium intakes are associated with reduced risks of vertebral fractures and severe abdominal aortic calcification in older Australians
Belal Khan[1], Caryl Nowson[2], Robin Daly[2], Dallas English[3], Peter Ebeling[1]
[1]Northwest Academic Centre, the University of Melbourne, Western Health, [2]School of Exercise and Nutrition Sciences, Deakin University, [3]School of Population Health, the University of Melbourne

Introduction: Higher dietary calcium intakes (DCI) prevent age-related bone loss; however, data on fracture prevention are equivocal. Evidence on the influence of DCI on the association between vertebral fractures and abdominal aortic calcification (AAC) is scant.
Aims and Methods: We assessed associations between long-term DCI and prevalent vertebral fractures and AAC, measured by lateral thoraco-lumbar radiographs, and hip and spine BMD, by DXA, in 407 men and women, followed for a mean±SD of 17.7±1.1 years, randomly selected from a large prospective cohort study (MCCS). DCI was assessed using a food frequency questionnaire at entry (1990-1994).
Results: The mean (SD) baseline age was 53±5.4 years. 103 (29.8%) participants had 172 prevalent vertebral deformities between T5-L4, while 229 participants (66.2%) had prevalent AAC between L1-L4. Compared with the low DCI group, the risk of vertebral deformities was significantly lower (OR 0.46; 95% CI, 0.27-0.78) in the high DCI group, and there was a non-significant 50% reduction in the risk of AAC (OR 0.50; 95%CI, 0.23-1.08). When only severe vertebral deformities (OR 0.31; 95%CI 0.12-0.78) or severe AAC (OR 0.35; 95%CI, 0.13-0.94) were considered, the risk of both was significantly decreased with high DCI. High DCI tended to be associated with higher spine and hip BMD, but was only significantly related to higher femoral neck BMD in women ($\beta_{FemoralNeck}$ 0.04; 95%CI, 0.01-0.07).
Conclusion: High DCI is associated with decreases in both vertebral fracture risk and severe AAC, and maintenance of hip BMD in postmenopausal women. Associations were strongest for decreased vertebral fracture risk.

Sunday 8 September 2013
SESSION 1: Bone and Energy Metabolism

OR2 Amgen-ANZBMS Outstanding Abstract - Biomedical Presentation

gp130 in osteocytes is required for PTH-induced bone formation

Narelle McGregor, Therese Standal, Rachelle Johnson, T John Martin, Natalie Sims
St Vincent's Institute of Medical Research

PTH acts via its receptor (PTH1R) to increase osteoblast proliferation and differentiation, an effect utilized for osteoporosis anabolic therapy. Members of the IL-6/gp130 cytokine superfamily are expressed by the osteoblast lineage, are rapidly increased by PTH and share similar gene targets, such as inhibiting osteocytic sclerostin, but their role in the anabolic action of PTH is unknown.

To determine whether osteocytic gp130 is required for PTH anabolic action, male mice with conditional deletion of gp130 in osteocytes (Dmp1Cre.gp130f/f) and controls (Dmp1Cre.gp130w/w) were treated with PTH(1-34) (30ug/kg 5x/week for 5 weeks). In w/w mice this treatment dramatically increased osteoblast number/bone surface (BS) (p=0.001), osteoid surface/BS (p=0.009), mineralizing surface/BS (p=0.03) and increased serum *N-terminal propeptide of type I collagen (P1NP) levels by 30% (p= 0.006)*. However, in f/f mice, PTH treatment changed none of these parameters. *The only effect of PTH common to* w/w and f/f mice was a similar increase in trabecular mineral apposition rate, suggesting that although PTH does not increase osteoblast proliferation in the absence of osteocytic gp130, osteoblasts that are present do respond to PTH.

Impaired PTH anabolic action was associated with a 47% reduction in PTH1R mRNA levels in f/f femora compared to w/w (p=0.03), and was reduced by 85% (p=0.03) in gp130f/f primary calvarial osteoblasts infected with lentiviral-cre recombinase compared to infected C57Bl/6 cells.

In conclusion, osteocyte gp130 signalling plays a key role in regulating PTH1R expression; promoting this pathway could enhance the anabolic action of PTH.

OR3 Amgen-ANZBMS Outstanding Abstract - Biomedical Presentation

2-photon imaging reveals myeloid cells as the cellular targets for the anti-tumour activity of bisphosphonates *in vivo*

Simon Junankar[1], Tri Phan[1], Charles McKenna[2], Shuting Sun[2], Michael Rogers[1]
[1]Garvan Institute of Medical Research, [2]University of Southern California

Bisphosphonates (BPs) target rapidly to bone and are the gold standard anti-resorptive treatment for metastatic bone disease. BPs also have anti-tumour effects in preclinical cancer models and increased survival in some clinical trials of patients with breast cancer and myeloma. However, the exact mechanisms underlying these anti-tumour effects are still unclear since it is generally considered that BPs have little effect on cells other than osteoclasts.
To determine the cell types capable of internalising BP *in vivo* in the 4T1 murine breast cancer model, we examined the distribution and cellular uptake of fluorescently-labelled BP (AF647-RIS) in mammary tumours in live mice. Within minutes after tail vein injection, intravital 2-photon imaging revealed the flow of BP into mammary tumour via the tumour vasculature, with slow diffusion into tumour tissue. 2 hours after injection, tumour-associated macrophages (labelled *in vivo* with anti-F4/80) could be seen to contain the fluorescent BP. Flow cytometric analysis of the tumours 24-hours later confirmed that CD11b+F4/80+ macrophages and CD11b+Gr1+ immature myeloid cells, but not tumour cells, had internalised the BP i*n vivo*.
These studies provide conclusive evidence that BPs can be rapidly internalised *in vivo* by myeloid cells completely outside the skeleton. The particular vascular organisation of tumours may facilitate the local diffusion of BP and endocytic uptake by tumour-associated myeloid cells. Given the important role of macrophages and immature myeloid cells (MDSC) in promoting tumour progression and metastasis, our studies suggest that the anti-tumour activity of BPs occurs via effects on these myeloid cell types.

Monday 9 September 2013
SESSION 2: Emerging Therapies

IS4

Emerging Therapeutic Options in Osteoporosis: Cellular Basis and Challenges

Roland Baron
Department Head and Professor of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine AND Professor of Internal Medicine, Harvard Medical School and Massachusssetts General Hospital, Boston USA

Osteoporosis is the result of an imbalance between bone formation and bone resorption during bone remodeling. Therapeutic intervention therefore targets either osteoclasts with anti-resorptive drugs or osteoblasts with anabolic drugs.
Because bone resorption and bone formation are coupled within each remodeling unit, decreasing osteoclast numbers leads to a delayed but profound decrease in bone formation and low bone remodeling activity and turnover. In contrast, a novel therapeutic approach targets the activity of osteoclasts through inhibition of Cathepsin K, which does not decrease the number of osteoclasts. Pre-clinical and clinical results show that inhibition of Cathepsin K maintains coupling and bone formation remains within the normal range. This leads to prolonged increases in bone density. The other main approach to increase bone mass and strength to treat osteoporosis is to directly increase bone formation by targeting the osteoblast lineage with anabolics. Teriparatide is currently the gold standard but has limitations. The discovery that the Wnt signaling pathway is the main regulator of bone mass in humans led to the development of antibodies that prevent the Wnt inhibitory action of sclerostin. These compounds have been tested in the clinic and demonstrate a modest anti-resorptive activity associated with a potent induction of bone formation, the combined result of these actions being a rapid and pronounced increase in bone density. The emergence of these therapeutic options opens the door to combined or sequential approaches to treat osteoporosis.

IS5

Emerging Therapies in Orthopaedics

David G Little
The Children's Hospital at Westmead

Exciting developments of novel anabolic and anti-catabolic therapies for bone disorders may have several implications in the field of orthopaedics. Bone homeostasis and remodelling share many common pathways with bone repair, while there are also important differences. Loading and unloading responses have also been investigated.
PTH remains the anabolic mainstay for intervention in osteoporosis, while Wnt pathway modulators such as sclerostin antibody are under intensive investigation due to potent anabolic bone effects. These agents are specific bone anabolic drugs which may not have any favourable effect on early stages of fracture repair which are more in tune with non-specific wound repair phenomena.
Further these drugs also modulate catabolic pathways which may or may not be key in certain bone healing scenarios. These differ from traditional anticatabolic approaches. Likewise, novel anticatabolics may interact in bone repair differently than our classic bisphosphonate model.
Lastly, there is avid interest in the potential utility of anabolic drugs in those sustaining or at risk of sustaining adverse skeletal effects associated with prolonged anti-catabolic therapy.

Monday 9 September 2013
SESSION 2: Emerging Therapies

IS6

What is new in OA therapy?

Flavia Cicuttini
School of Public Health and Preventive Medicine/Monash University/Alfred Hospital, Victoria

Osteoarthritis (OA) is a major clinical and public health problem for which therapies are limited. Although the hallmark of disease progression in OA is progressive articular cartilage loss, it is increasingly clear that OA is a disease of the whole joint. Major advances in our understanding of the pathological processes involved in OA have been possible through improved methods of assessing joints non-invasively using imaging methods such as magnetic resonance imaging (MRI). This has resulted in a greater appreciation of the role of bone in OA. Bone is a dynamic tissue undergoing constant remodeling. The importance of bone marrow lesions as well as changes in bone shape and structure are now well established. Bone and cartilage degradation occur simultaneously. However whether OA starts in cartilage or bone is unclear, but it may vary in different situations. However bone has proved to be an important therapeutic target in the treatment of OA. In this talk the findings from the recent clinical trials of strontium ranelate, zolendronic acid and vitamin d in OA will be presented as well as how anti-sclerostin/Dkk1 and cathepsin K inhibition might be applied to OA.

OR4

Is modulation by strontium of osteoclastogenic signals in human osteoblasts entirely through the calcium-sensing receptor?

MS Rybchyn[1], W Green[1], T Brennan-Speranza[2], AD Conigrave[3], RS Mason[1]
[1]Bosch Institute, Physiology, School of Medical Sciences, [2]Anzac Institute, [3]School of Molecular Bioscience, University of Sydney, NSW, Australia.

The anti-fracture agent strontium ranelate reduces resorption without suppressing formation. Exposure of primary human osteoblasts (HOBs) to strontium results in increased osteoprotegerin (OPG) and decreased receptor-activator-of-NFκB-Ligand (RANKL) expression. The present study investigated whether the calcium-sensing receptor (CaSR) and/or a related class C GPCR, GPRC6A, mediate these responses. HOBs were transfected with siRNA directed at the CaSR (HOB/CaSR^{kd}) or GPRC6A (HOB/GPR^{kd}) or with a scrambled sequence (HOB/SCR) for 24h. Transfected cells were then exposed to zero or 1 mM strontium in the presence of 1 mM calcium for 24h. OPG and RANKL mRNA were measured by qRT-PCR. Strontium induced ~2-fold increase in OPG expression and ~2.5-fold decrease in RANKL expression in HOB/SCR cells ($p<0.05$; $p< 0.01$) but had little effect in HOB/CaSR^{kd} cells. There was no significant difference in the strontium-induced increase in OPG mRNA expression between HOB/SCR and HOB/GPR^{kd} cells. However, the strontium-induced reduction in RANKL mRNA expression was not observed in HOB/GPR^{kd} cells and, instead, a paradoxical increase in RANKL expression was observed when compared to HOB/SCR cells ($p<0.001$). PI-3-kinase inhibitor wortmannin inhibited strontium-induced changes in both OPG and RANKL, but only changes in RANKL were inhibited by ERK-inhibitor peptide. CaSR^{kd} reduced strontium-stimulated Akt phosphorylation, but had no effect on ERK1/2 phosphorylation, (though this is reportedly reduced by GPR^{kd}.) These findings indicate that the classical CaSR is a key mediator of the strontium signal to alter OPG secretion but that the strontium signal for RANKL reduction is more complex and requires contributions from both the CaSR and GPRC6A.

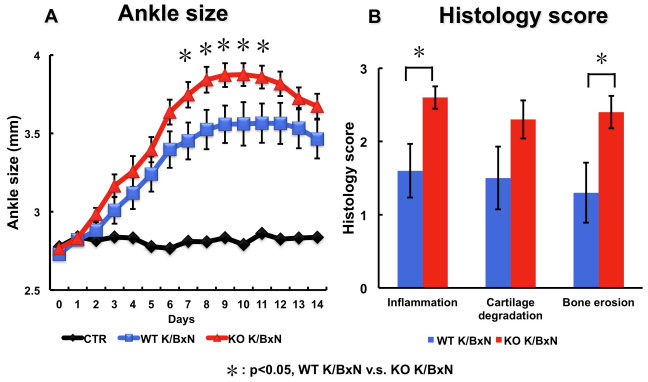
Monday 9 September 2013
SESSION 3

OR5 Amgen-ANZBMS Outstanding Abstract – Biomedical

Tamoxifen-Induced Deletion of the Glucocorticoid Receptor in Chondrocytes Enhances K/BxN Serum-Induced Arthritis in Mice

Jinwen Tu[1], Yaqing Zhang[1], Shihani Stoner[1], Edgar Wiebe[1], Tazio Maleitzke[1], Jan Tuckermann[2], D Chen[3], Markus J. Seibel[4], Hong Zhou[1]
[1]Bone Research Program, Anzac Research Institute, University of Sydney, Sydney, [2]Institute of General Zoology and Endocrinology, University of Ulm, Ulm, Germany, [3]Tissue Department of Biochemistry, Rush University Medical Center, USA, [4]Department of Endocrinology Metabolism, Concord Hospital, Sydney, Australia

Aim: Exogenous glucocorticoids (GCs) are widely used in the treatment of rheumatoid arthritis (RA), however, the function of endogenous GCs in RA remains unclear. This study aimed to investigate the impact of cartilage-specific glucocorticoid receptor knockout (GRKO) on joint inflammation, cartilage damage, and bone metabolism in the K/BxN mouse serum transfer model of autoimmune arthritis.
Methods: GRKO mice were generated by breeding GRflox mice with tamoxifen inducible Cre (Col2a1-CreER^{T2}) mice. Arthritis was induced in 8-week-old male GRKO mice and their Cre-negative GR$^{fx/fx}$ littermates (WT) by injection of K/BxN serum ("KO-K/BxN" and "WT-K/BxN"). KO and WT mice receiving PBS served as controls (CTR).
Results: Both KO-K/BxN and WT-K/BxN mice developed acute arthritis. However, the peak inflammatory response was significantly more pronounced in KO-K/BxN mice (Fig. A). In keeping with this phenoptype, histological analysis revealed significantly increased inflammatory activity and greater bone erosions in KO-K/BxN than WT-K/BxN mice (Fig. B). Spleen weight was significantly increased in KO-K/BxN mice compared to WT-K/BxN and CTR mice. Flow cytometry analysis revealed that the spleen weight changes were associated with a significantly expansion of CXCR2 positive cells in KO-K/BxN mice. In contrast, these changes in spleen were not observed between WT-K/BxN and CTR mice. Micro-CT and histomorphometry showed reduced tibial bone volume (BV/TV) in both groups of arthritic mice compared to CTR, with increased osteoclast activity observed in KO-K/BxN compared to WT-K/BxN mice.
Conclusion: Disruption of endogenous glucocorticoid signaling in cartilage aggravates K/BxN serum-induced arthritis. Our data suggests that chondrocytes modulate local and systematic inflammation via a GR-dependent pathway.



Monday 9 September 2013
SESSION 3

OR6 Amgen-ANZBMS Outstanding Abstract - Clinical

A novel primary school exercise intervention for bone and fat outcomes: the CAPO Kids trial

Rossana Nogueira, Benjamin Weeks, Belinda Beck
Griffith University

The combined increasing incidence of chronic bone and obesity-related disease places a heavy burden on the health economy. Both conditions may stem from childhood. While exercise in youth is beneficial for both bone and metabolism, the nature of exercise recommendations for each traditionally differs. **Aim:** To determine the effect of a brief, novel, enjoyable, school-based exercise regimen targeting both bone and fat in primary school children. **Methods:** A nine-month, school-based, controlled exercise intervention trial was conducted. The intervention (EX) comprised 10 minutes of thrice weekly capoeira and jumping activities. Anthropometrics, waist circumference, calcaneal ultrasound (Lunar Achilles, GE), vertical jump (VJ), cardiovascular endurance (beep test), resting heart rate (HR), blood pressure (BP) and maturity (YAPHV) were measured at baseline and 9 months. A subset of WB, LS and FN BMD, lean and fat mass (DXA, XR800, Norland) was also collected. Change in outcome variables was compared between groups using one-way ANOVA. **Results:** 296 children, including 130 control (CON) (aged 10.7±0.6 yrs; YAPHV -1.9±0.9y) and 166 EX (aged 10.5±0.5yrs, YAPHV -2.1±0.9y) participated. EX improved waist circumference (EX: 1.95±2.82cm; CON: 4.09±4.05cm; $p<0.001$), HR (EX: -4.11±3.35 BPM; CON: 0.22±3.8 BPM; $p=0.001$), VJ (EX: 3.47±4.01; CON: -0.59±5.16; $p<0.001$), beep test (EX: 0.8±1.25; CON: -0.03±0.96; $p<0.001$), stiffness index (EX: 6.25±10.04%; CON: 4.09±6.99%; $p<0.05$) and broadband ultrasound attenuation (EX: 3.99±9.06 dB/MHz; CON: 1.33±8.3 dB/MHz; $p=0.01$) compared to control. Sex-specific effects largely mirrored those findings. **Conclusion:** School-based capoeira improved markers of metabolic and musculoskeletal health in primary school children. The exercise program was safe, feasible and enjoyable.

Monday 9 September 2013
Roger Melick Young Investigator Award Finalists

OR7

Sclerostin Exerts a Coordinated Pro-osteoclastogenic Effect via its Action in Osteocytes

Asiri Wijenayaka[1], Masakazu Kogawa[1], Lynda Bonewald[2], David Findlay[1], Gerald Atkins[1]
[1]*The University of Adelaide,* [2]*University of Missouri Kansas City*

Sclerostin (SCL) is a potent negative regulator of bone formation.(1) We have reported that SCL stimulates RANKL mRNA expression in both human and MLO-Y4 osteocyte-like cells and promotes osteoclast activity in co-cultures of MLO-Y4 cells and osteoclast precursors.(2) The aim of the current study was to further examine this pro-osteoclastogenic effect of SCL.
To examine the individual contributions of osteocytes and osteoclast precursors, MLO-Y4 cells were co-cultured with human peripheral blood mononuclear cell (PBMC) derived osteoclast precursors in the absence or presence of human recombinant sclerostin (rhSCL). We used species-specific PCR primers to amplify resulting osteocyte or osteoclast-derived markers. We performed siRNA mediated knockdown of known receptors to SCL (*Lrp4*, *Lrp5*, and *Lrp6*) in MLO-Y4 cells to elucidate which receptors were important for the pro-osteoclastogenic effect. In addition, human trabecular bone samples were obtained from patients undergoing hip arthroplasty and cultured in the absence or presence of rhSCL; mRNA expression and histology was then analysed.
rhSCL induced mRNA levels of pro-osteoclastogenic mediators, such as *RANKL, CSF1* and *IL6* in human bone and in MLO-Y4/PBMC co-cultures. In the co-culture osteoclast fraction, *CA2, ACP5* (TRAP) and *CATK* mRNA increased with rhSCL treatment. siRNA knockdown studies indicated that all of LRP4, LRP5 and LRP6 receptors were required for the pro-osteoclastogenic response to SCL in MLO-Y4 cells. This study extends our previously reported findings (2) and suggests that sclerostin exerts a coordinated pro-osteoclastogenic effect via its action on the osteocyte, in a manner consistent with inhibition of Wnt signalling.

1. Li *et al*. 2009 J Bone Miner Res **24**:578-588.
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Roger Melick Young Investigator Award Finalists

OR8

Adherence to oral bisphosphonate therapy in a Fracture Liaison Service: A randomised controlled trial

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Aim: To determine predictors of long-term adherence to oral bisphosphonate therapy in patients initiated on treatment by a Fracture Liaison Service (FLS).
Methods: This 2-year randomised controlled trial included 102 patients initiated on oral weekly bisphosphonate therapy within an FLS following an incident osteoporotic fracture. Patients were randomised to six-monthly visits with the FLS (Group A) or referral to their GP with only one FLS follow-up visit after 24-months (Group B). Compliance (medication possession ratio, MPR) and persistence were determined using the Pharmaceutical Benefits Scheme (PBS) database. Subjects with complete PBS data who attended at least one clinic appointment were included in the intention-to-treat (ITT) analysis (n=94), while those attending all appointments were included in the per-protocol (PP) analysis (n=74).
Results: Baseline characteristics were well matched across both groups. In the ITT analysis, median MPR over 24-months was 0.78 (IQR 0.50-0.93) and 0.79 (IQR 0.48-0.96) in Group A and B, respectively (p=0.68). Persistence at 24-months was 64% and 61% in Group A and B, respectively (p=0.75). After adjusting for confounders, patients in Group A were not more likely to be compliant (OR 1.06, 95%CI 0.46-2.47, p=0.89) or persistent (HR 0.83, 95%CI 0.27-1.67, p=0.61) than those in Group B. Changes in bone turnover markers or BMD were not associated with measures of adherence.
Conclusion: Adherence to oral bisphosphonate therapy initiated by a FLS remains high over 24 months, independent of follow-up mode or frequency. These results indicate that the main function of a FLS is to overcome barriers to therapy.

OR9

RANKL-induced Myo1b occupies sites of dynamic actin-membrane remodeling and is required for osteoclast formation and function

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The osteoclast (OC) boasts of a highly dynamic cytoskeleton, a necessity that is borne out of cyclical requirements for motility and the generation of a polarized phenotype. Despite the obvious importance of the "membrane-cytoskeleton interface" in establishing OC polarization and function, the precise regulation of the cytoskeleton and the functional significance of its motor protein repertoire remain largely obscure. Utilizing transcriptional profiling, we systematically screened for novel actin-coupling molecules that are robustly up-regulated in OCs during RANKL-driven differentiation. Among the candidates identified, we uncovered myosin 1b (Myo1b), a member of the Class-I family of small actin-based mechano-sensitive motors as a prominent RANKL-responsive gene. Up-regulation of Myo1b expression was confirmed at both mRNA and protein level by quantitative RT-PCR and immunoblotting respectively. Consistent with its involvement in supporting membrane tension and deformation, Myo1b specifically localized to dynamic sites of actin remodeling and membrane ruffling during OC motility and bone resorption. To gauge the potential importance of Myo1b in OC formation and function, we combined Myo1b-targeted RNA interference with the natural compound pentachloropseudilin (PCIP), a specific and allosteric inhibitor of myosin-I ATPase activity. Blockade of Myo1b expression/myosin-I motor function attenuated OC formation and bone resorptive capacity *in vitro,* owing in part to uncoupling between the actin cytoskeleton and the plasma membrane, as well as compromised Akt activity. Collectively, our findings uncover Myo1b as a novel regulator of OC formation and function, most likely serving to bridge the actin-membrane interface at dynamic sites of membrane turnover.

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OR10

Quadiceps weakness is a predictor of fracture risk: a time-dependent analysis

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Lower muscle strength is a risk factor for falling, which is a risk factor for fracture. The present study was aimed at examining the contribution of Quadriceps Strength (QS) at baseline and serial measurements of QS to fragility fracture risk.
The study involved 1337 men and 2191 women aged 60+ years (median: 68) followed for a median of 7 years. At baseline and approximate two-year intervals, QS was measured by a dynamometer. Femoral neck BMD was measured by dual energy X-ray absorptiometry (GE-Lunar). Low-trauma fracture was ascertained from X-ray reports. The magnitude of association between QS and fracture was assessed by time-variant Cox's regression.
During the follow-up period, 216 (16.2%) men and 684 (31.2%) women had sustained a fragility fracture. Each 10 kg lower baseline QS was associated with an 18% increase in the risk of fracture in men (hazard ratio [HR]: 1.18; 95%CI: 1.03 to 1.36) and 14% in women (HR: 1.14; 95%CI: 1.03 to 1.27). These hazard ratios were after adjusting for FNBMD, age and height at baseline, prior fracture, home physical activity, smoking status and history of fall. When time-variant QS was used, each 10kg lower in QS was independently associated with 33% (HR 1.33; 95%CI: 1.16 to 1.53) and 24% (HR 1.24; 95%CI: 1.12 to 1.39) increase in fracture risk in men and women, respectively.
These data indicate that quadriceps weakness is independent determinants of fracture risk. The accuracy of fracture risk prediction could be improved by incorporation of serial measurements of quadriceps strength.

OR11

The Vitamin D Receptor Promotes MCF-7 Cell Growth *in vitro* and *in vivo* via a Ligand Independent Cytoplasmic Function

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Background: Vitamin D inhibits breast cancer growth through activation of the vitamin D receptor (VDR) and via classical steroid hormone nuclear signalling pathways. In contrast, the actions of the unliganded VDR have not been defined.
Methods & Results: Using stable shRNA expression and single cell clonal selection, VDR expression was knocked down by ~85% in MCF-7 cells (MCF-$7^{VDR-/-}$) compared to parental (PA) and non-target (NT) controls. In ligand-free culture, the growth of MCF-$7^{VDR-/-}$ clones was reduced by up to 45-60%, with a 6-fold increase in cell apoptosis compared to controls ($p< 0.001$), suggesting that the VDR has ligand-independent effects in promoting cell growth. When MCF-$7^{VDR-/-}$ and NT cells were xenografted orthotopically into the mammary fat pad of nude mice, VDR knockdown resulted in reduced tumour growth from day 18 to 51 ($p<0.001$). Intra-tibial implantation of MCF-7 NT or PA cells into nude mice resulted in significant osteosclerosis and tumour growth with 88% of mice developing tumours. In contrast, only 25% of mice implanted with MCF-$7^{VDR-/-}$ cells develop tumours, indicating VDR knockdown greatly impact tumour cells capability to form tumours in bone.
Transfection of MCF-$7^{VDR-/-}$ cells with a mutant VDR (mVDR) construct in which VDR nuclear localization signal is mutant, restored MCF-$7^{VDR-/-}$ cell growth to the same level as seen in NT controls. This was associated with cytoplasmic accumulation of mVDR.
Conclusion: In contrast to the known growth-inhibitory effects of the liganded VDR, the unliganded cytoplasmic VDR promotes breast cancer growth in bone and soft tissues.

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OR12

The IFITM5 mutation c.-14C>T results in an elongated transcript expressed in human bone; and causes varying phenotypic severity of osteogenesis imperfecta type V

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Background: The genetic mutation resulting in osteogenesis imperfecta (OI) type V was recently characterised as a single point mutation (c.-14C>T) in the 5' untranslated region (UTR) of *IFITM5*, a gene encoding a transmembrane protein with expression restricted to skeletal tissue. This mutation creates an alternative start codon and has been shown in a eukaryotic cell line to result in a longer variant of IFITM5, but its expression has not previously been demonstrated in bone from a patient with OI type V.
Methods: Sanger sequencing of the *IFITM5* 3' UTR was performed in our cohort of subjects with a clinical diagnosis of OI type V. Clinical data was collated from referring clinicians. RNA was extracted from a bone sample from one patient and Sanger sequenced to determine expression of wild-type and mutant *IFITM5*.
Results: All nine subjects with OI type V were heterozygous for the c.-14C>T *IFITM5* mutation. Clinically, there was heterogeneity in phenotype, particularly in the manifestation of bone fragility amongst subjects. Both wild-type and mutant *IFITM5* mRNA transcripts were present in bone.
Conclusions: The c.-14C>T *IFITM5* mutation does not result in an RNA-null allele but is expressed in bone. Individuals with identical mutations in *IFITM5* have highly variable phenotypic expression, even within the same family.

OR13

Uncoupling protein 1 protects bone mass under cold stress conditions

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Uncoupling protein 1 (UCP1) is found in the mitochondria of brown adipose tissue and is critical to the generation of heat through non-shivering mechanisms. The appreciation of the importance of brown adipose tissue has increased in recent years, as has our understanding of the link between energy and bone homeostasis. This study, for the first time, looks at the relationship between UCP1 and bone mass using a mouse model deficient in UCP1 ($UCP1^{-/-}$).

When 6 to 9-week-old male $UCP1^{-/-}$ mice were kept at thermoneutrality (30°C) for 10 weeks, thereby diminishing the requirement for UCP1-mediated thermogenesis, no differences in cancellous or cortical bone parameters were observed by microtomographic analysis, compared to wildtype. However, under conditions of permanent mild cold stress (22°C), and thus inducing UCP1-mediated thermogenesis, male $UCP1^{-/-}$ mice displayed a significant reduction in cancellous bone mass (21%, $p<0.05$), associated with a reduction in trabecular number and thickness compared to wildtype mice. Furthermore, these $UCP1^{-/-}$ mice exhibited significant reductions in femur length, as well as in cortical periosteal and endosteal perimeters, with no observed differences in body weight. A similar, although reduced, effect was also observed in female mice.

These data demonstrate that UCP1 activity during mild cold stress is protective of bone mass. These results suggest that thermogenesis initiated during cold stress plays a positive role in bone mass, and demonstrates a link between brown adipose tissue function and bone homeostasis.

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SESSION 4: Cancer and Bone

IS7

Anti-tumour effects of bisphosphonates: looking beyond the skeleton

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Bisphosphonate (BP) drugs inhibit bone resorption by rapidly binding bone, internalisation by endocytosis into osteoclasts at high concentrations (at least micromolar), then preventing protein prenylation. By contrast, circulating concentrations of BP are very low (nanomolar), and effects of <1mM BP on prenylation have not been described. BPs also have anti-tumour effects in preclinical models and increased survival in several clinical trials of patients with breast cancer and myeloma, but the cell types capable of internalising BP in tumours are not known, and cells outside the skeleton are considered to be exposed only to very low BP concentrations.
We recently found that long-term exposure to nanomolar concentrations as low as 50nM causes accumulation of unprenylated protein in cultured macrophages. This suggests that prolonged exposure to low, circulating concentrations of BP *in vivo* could affect endocytic cells such as monocytes and macrophages in non-skeletal tissues. We also used fluorescently-labelled BPs to examine the distribution and uptake of BP in 4T1 mammary tumours in live mice. Intravital, 2-photon imaging revealed slow diffusion of BP from leaky vessels and retention of BP in tumour tissue (but not normal mammary tissue), followed by selective, vesicular uptake by F4/80+ tumour-associated macrophages (TAMs). Flow cytometric analysis confirmed cellular uptake by TAMs but little uptake by tumour cells.
Together, these observations provide evidence that, under certain circumstances, BPs can be internalised by endocytic myeloid cells outside the skeleton. The anti-tumour effects of BPs are therefore likely mediated via inhibition of protein prenylation in myeloid cells such as TAMs.

IS8

Bone and Cancer

Professor Andrew C. W. Zannettino
School of Medical Sciences, Faculty of Health Science, University of Adelaide and the Centre for Cancer Biology, SA Pathology

Bone is the most frequent site affected by metastatic cancer. While any type of cancer can spread to bone, metastatic bone disease is most commonly associated with cancers whose primary origin is the breast, prostate or lung. Metastasis of cancer to bone often results in significant skeletal morbidity that manifests as severe bone pain, pathologic fractures, spinal cord compression and life threatening hypercalcemia. Bone metastases are classified as osteolytic, osteoblastic, or mixed, based on their radiographic appearance. Patients can present with osteolytic, osteoblastic or mixed lesions containing both elements. Bone metastases from prostate cancer are predominantly osteoblastic, while bone lesions from breast cancer can be osteoblastic, osteolytic or mixed. In contrast, multiple myeloma, an incurable haematological malignancy characterised by the clonal proliferation of malignant plasma cells within the bone marrow, stimulate the development of purely lytic bone lesions. These lytic lesions are a result of increased osteoclast activity stimulated by myeloma plasma cell-derived factors including MIP-1a, CXCL12 and RANKL. This increase in osteoclast activity is accompanied by suppression of osteoblast differentiation and activity, mediated by plasma cell and marrow stroma-derived factors including DKK-1, sclerostin, sFRP-2 and Activin-A, leading to severely impaired bone formation. This presentation will cover our current understanding of the pathophysiology underlying bone disease in myeloma and the skeletal consequences of current chemotherapeutic agents used to treat myeloma.

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SESSION 4: Cancer and Bone

IS9

FGF23 and Tumour induced osteomalacia

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FGF23 is a humoral factor secreted by the osteocytes and predominantly regulates serum phosphate levels by targeting the renal tubular and modulating phosphate excretion via the expression of the sodium phosphate transporters NaPi-IIa and IIc, and altering intestinal phosphate absorption, through regulation of kidney CYP27B1 and CYP24 expression. Originally, FGF23 was isolated from the tumour of a tumour induced osteomalacia (TIO) patient, and concomitantly identified as the causative gene for autosomal dominant hypophosphataemic rickets/osteomalacia (ADHR).
TIO was believed to be a rare condition. However, after the development of the serum FGF23 assay, the number of reported cases has notably increased.
TIO causes bone pain, debilitating muscle weakness and softened bones due to hypophosphataemia. The causative tumours develop from a single pathological entity, the phosphaturic mesenchymal tumour (mixed connective tissue variant) (PMTMCT) comprised of various cell types and tissues. These tumours usually occur in bone but may be found in connective tissues throughout the body.
While malignant cases are rare, patients can only be cured by the complete resection of the causative tumour. Even though serum FGF23 measurement has facilitated the diagnosis of TIO, in many cases, localisation of the causative tumour is difficult due to the small size and unpredictable location. As conventional imaging methods, such as CT and MRI fail to identify these tumours, octreotide scintigraphy and systemic serum FGF23 sampling were introduced to localize the functional tumours. The sensitivity of these methods is reported to be more than 80%.

IS10

Refining murine models of osteosarcoma

Carl Walkley
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Murine models of human cancer are highly valuable as preclinical testing platforms. The extent to which these models replicate the diversity of the corresponding human cancer is critical to their application. We have previously developed a model osteosarcoma, the most common form of bone cancer, using Cre:lox approaches. Here we describe a new OS model that utilises shRNA technology to bring about lineage restricted p53 knockdown. Our approach allowed for a direct comparison of the *in vivo* effects of targeting the same genetic drivers using different technology: Cre:lox and shRNA. This demonstrated that the effects of Cre:lox and shRNA knock-down are qualitatively different *in vivo* and can yield distinct tumor subtypes. Through use of a different technology for gene perturbation we have revealed a new model of a tumor subtype not previously reported in OS models.

Monday 9 September 2013
SESSION 5A: Clinical Presentations

OR14

Denosumab versus risedronate in women with postmenopausal osteoporosis suboptimally adherent to alendronate: efficacy and safety results from a randomised, open-label study

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Aim: Denosumab (DMAb) has been shown to reduce the risk of new vertebral, nonvertebral and hip fractures.[1] Compared with alendronate, DMAb is also associated with greater gains in bone mineral density (BMD) and decreases in bone turnover markers.[2,3] This 12-month study compared the efficacy and safety of DMAb and risedronate (RIS) in women with postmenopausal osteoporosis (PMO) considered to be suboptimally adherent to alendronate therapy.
Methods: This international, randomised, open-label study compared the efficacy and safety of DMAb 60mg every 6 months with RIS 150mg monthly (one 75mg tablet on 2 consecutive days) in women aged ≥55 years with PMO. The primary endpoint was percent change from baseline in total hip (TH) BMD at month 12 (M12); secondary endpoints included percent change from baseline in femoral neck (FN) and lumbar spine (LS) BMD at M12, median change from baseline in serum CTX (sCTX) at months 1 and 6, and safety.
Results: Subjects were randomised to DMAb (n=435) or RIS (n=435), and baseline characteristics were balanced between the two groups. DMAb significantly increased BMD at M12 vs. RIS: TH 2.0% vs. 0.5%, FN 1.4% vs. 0% and LS 3.4% vs. 1.1% (all p<0.0001). DMAb also significantly decreased sCTX vs. RIS at 1 month (median change from baseline -78% vs. -17%; p<0.0001) and 6 months (-61% vs. -23%; p<0.0001). Overall adverse events (AEs) and serious AEs were similar between groups.
Conclusions: In women with PMO who were suboptimally adherent to alendronate, transitioning to DMAb rather than switching to RIS led to significantly greater increases in BMD at all measured sites, as well as greater reductions in sCTX.
References: 1. Cummings SR, et al. *N Engl J Med* 2009; 361-71. 2. Kendler DL, et al. *J Bone Miner Res* 2010; 25(1): 72-81. 3. Brown JP et al. *J Bone Miner Res* 2009; 24: 153-161.

OR15

Dietary, supplemental and total calcium intake in relation to common carotid artery intima medial thickness and atherosclerosis in older women

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Aim: Calcium is an essential nutrient for skeletal health, however studies have reported calcium supplementation may be associated with adverse cardiovascular health outcomes raising widespread concern about their use. The suggested mechanism is via increased carotid plaque thickness and arterial calcification. We sought to investigate the association of dietary, supplemental and total calcium with these measures.
Method: The participants were from the CAIFOS study; a 5-year RCT of elderly women randomized to 1.2 grams of calcium in 1998 (baseline). An ancillary sub study of 1,103 participants had common carotid artery intima-media thickness (CCA-IMT) and carotid atherosclerosis assessed by B-mode carotid ultrasound assessed at year 3 (2001).
Results: In women randomised to calcium after 3 years of supplementation there was no difference in mean CCA-IMT; 0.778 ± 0.006 vs. 0.783 ± 0.006 mm, P = 0.491 or maximum CCA-IMT; 0.921 ± 0.007 vs. 0.929 ± 0.006 mm, P = 0.404 compared to placebo group. Similarly there was no increased risk of carotid atherosclerosis; OR 0.80 (0.62-1.04), P = 0.095. Increasing dietary calcium intake was inversely associated with mean and maximum CCA-IMT (P = 0.039 and P = 0.037 respectively) while participants in the highest quartiles of dietary and total calcium intake had significantly lower risk of carotid atherosclerosis; OR 0.64 (0.44-0.93), P = 0.021 and 0.63 (0.44-0.92), P = 0.017 respectively.
Conclusions: In conclusion our study does not support the hypothesis that calcium supplementation increase measures of carotid atherosclerosis. High dietary and total calcium intake may reduce this risk.

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SESSION 5A: Clinical Presentations

OR16

Vertebral Modic changes on lumbar spine MRI: a characterisation of bone remodelling and osteocyte morphology

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Aim: MRI identified vertebral Modic changes are associated with disc degeneration and low back pain. The pathophysiological mechanisms underlying the appearance and progression of Modic changes remain elusive. The aim of this study was to investigate whether Modic changes associate with a variation in bone remodelling and osteocyte cell/lacunar density.
Methods: Fourty-one patients (25 men, 16 women; mean age 55±12 years) underwent elective spinal surgery with lumbar vertebrae showing Modic changes on pre-operative MRI. Cases were subdivided: Modic 1 (n=9), Modic 2 (n=25), Modic 3 (n=7). A transpedicular vertebral bone biopsy (25x3mm) from each patient was resin-embedded for histomorphometric quantitation of bone remodelling and osteocyte morphology.
Results: There was less erosion surface in Modic 3 (5.4%) compared with Modic 1 (9.7%) and 2 (8.6%), and reduced osteoid surface in Modic 2 (14.0%) compared with Modic 1 (22.1%) and 3 (18.0%). The osteoid surface/erosion surface ratio was highest for Modic 3, indicative of net increased bone formation. There were no differences between Modic types for osteocyte, empty lacunar, and total lacunar density, and percent empty lacunae/total lacunae. No correlations were found between osteocyte and remodelling parameters.
Conclusion: Lumbar vertebral Modic 1 changes associate with elevated bone turnover, Modic 2 changes with reduced bone formation, and Modic 3 changes are characterised by a stable sclerotic phase, consistent with our recent micro-CT observations (ISSLS 2012:#O235). Osteocyte morphology does not differ between Modic types, suggesting that osteocyte cell network integrity does not play a role in the dysregulated bone remodelling associated with Modic types.

OR17

Cortical bone fragility contributes to fractures in children

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University of Melbourne / Austin Health

About 50% of children sustain fractures during their growth. The highest incidence coincides with puberty, when there is a transient reduction in volumetric vBMD and cortical thickness. We hypothesize that deficits in cortical thickness and increased cortical porosity are present in children with fractures.
We recruited 54 children (52% males) with low-trauma fractures and imaged their distal contralateral radius using high-resolution pQCT. Cortical porosity, degree of mineralization (tissue mineralization density), and transitional zone dimensions (area between compact-appearing cortex and trabecular bone) were determined using StrAx1.0. Fracture cases were compared to 54 age- (11.9±2.9 vs. 11.7±2.8yrs), height- (152.4±16.7 vs. 150.7±15.2cm) weight- (46.6±15.6 vs. 45.8±15.3kg) and maturity-matched controls.
Bone cross-sectional area was similar in cases and controls (224±66 v 208±59mm^2). Cortical vBMD was 5% lower in cases (773±114 vs. 819±135mgHA/cm3, $p<0.05$) due to their 6% higher porosity (53±8 vs. 50±9%, $p<0.05$) and 3% lower tissue mineralization density (61±3 vs. 63±3%, $p<0.0001$). Differences were most evident in pre-pubertal boys (n=26) in whom fracture cases had 26% thinner cortices (0.30±0.07 vs. 0.41±0.12mm, $p<0.01$), and 9% wider transitional zone (2.77±0.19 vs. 2.55±0.30µm, $p<0.05$) than controls. Fracture cases had greater medullary area (107±27 vs. 84±28mm^2, $p<0.05$) with thicker (0.08±0.01 vs. 0.07±0.0mm, $p<0.01$), and fewer (1.8±0.2 vs. 2.1±0.3$^{1/mm}$, $p<0.01$) and more separated trabeculae (0.49±0.08 vs. 0.41±0.06mm, $p<0.01$) than controls.
We infer that cortical material and structural abnormalities contribute to bone fragility during growth predisposing to fractures should a fall occur.

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SESSION 5A: Clinical Presentations

OR18

Longitudinal relationships of antiepileptic drug (AED) therapy with bone mineral and balance measures

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We examined longitudinal bone mineral and balance measures during AED use. 54 same-gender twin or age-matched sibling pairs discordant for AED use had serial DXA (hip, lumbar spine, forearm and whole body) at least 2 years apart. AED use was examined by multiple regression as a predictor of bone change (adjusted for age, weight, height), including relevant covariates (calcium intake, vitamin D supplementation, physical activity, smoking, menopause, osteoporosis history, fractures). Postural sway was examined twice in 26 pairs (Chattecx Balance System) under 7 conditions, with/without distraction, at least 1 year apart. Within-pair differences (AED users v non-users) were compared (paired t-test). No within-pair differences were found in age, body mass index, comorbidities, non-AED medications and lifestyle ($p > 0.05$) Mean (SD) age and median (IQR) years between visits were: 48 (15) years and 2.6 (14) years (bone study); 44 (14) and 3.3 (1.4) years (balance study).
AED use predicted greater bone loss for whole body BMC (-0.26%/year; $p = 0.041$); prolonged (> 20 years) use predicted greater bone loss for forearm (-0.53%/yr; $p = 0.040$) and whole-body BMD (-0.37%/yr; $p = 0.043$). Enzyme-inducing AEDs were associated with increased bone loss for total hip (-1.65%/yr; $p = 0.013$), and whole-body BMD (-0.7%/yr; $p = 0.019$). Postural sway deteriorated more in AED users than non-users for anterior-posterior tilting with concurrent distraction task ($p = 0.016$), and medial-lateral tilting without distraction ($p = 0.027$).
The dual risks of accelerated bone loss and deterioration in balance help to explain the increased fracture risk in patients taking AEDs.

OR19

Maternal vitamin D status during pregnancy and peak bone mass attained at 20 years in offspring

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Aim: Two cohort studies evaluating the association between maternal vitamin D status and bone mass of offspring during late childhood yielded conflicting results. The aim of this study is to investigate the association between maternal vitamin D status and peak bone mass of offspring.
Methods: Study participants were 341 mother and offspring pairs who participated in the Raine study. Mothers' blood samples were collected at 18 weeks of gestation and assessed for serum 25OHD. Offspring had total body bone mineral content (TBBMC), bone area and bone mineral density (TBBMD) measured using DXA at 20 years of age.
Results: The mean serum 25OHD concentration of mothers was 57.2±19.2 nmol/L, with 11 (3.2%) <25 nmol/L, 121 (35.5%) 25-50 nmol/L and 209 (61.3%) ≥50 nmol/L. In regression analyses, maternal 25OHD had significant positive association with TBBMC and TBBMD in offspring, with the multivariate-adjusted mean difference per 10.0 nmol/L of 25OHD being 17.8 g (95% CI 4.4-31.2) for TBBMC and 4.5 mg/cm^2 (95% CI 0.1-8.9) for TBBMD. Compared with the offspring of mothers with serum 25OHD concentrations <50 nmol/L, those of mothers with serum 25OHD ≥50 nmol/L had 2.6% higher TBBMC (2922±16 vs 2849±20 g, P=0.006) and 1.7% higher TBBMD (1071±5 vs 1053±7 mg/cm2, P=0.046) after accounting for season of sample collection, and offspring' sex and body size at birth and 20 years.
Conclusions: Maternal vitamin D deficiency in pregnant women is associated with lower peak bone mass in their children. Maintaining vitamin D sufficiency during pregnancy could confer long-term skeletal benefits in offspring.

Monday 9 September 2013
SESSION 5A: Clinical Presentations

OR20

Late menarche is not associated with decreased bone strength in adulthood: A 7-year longitudinal study

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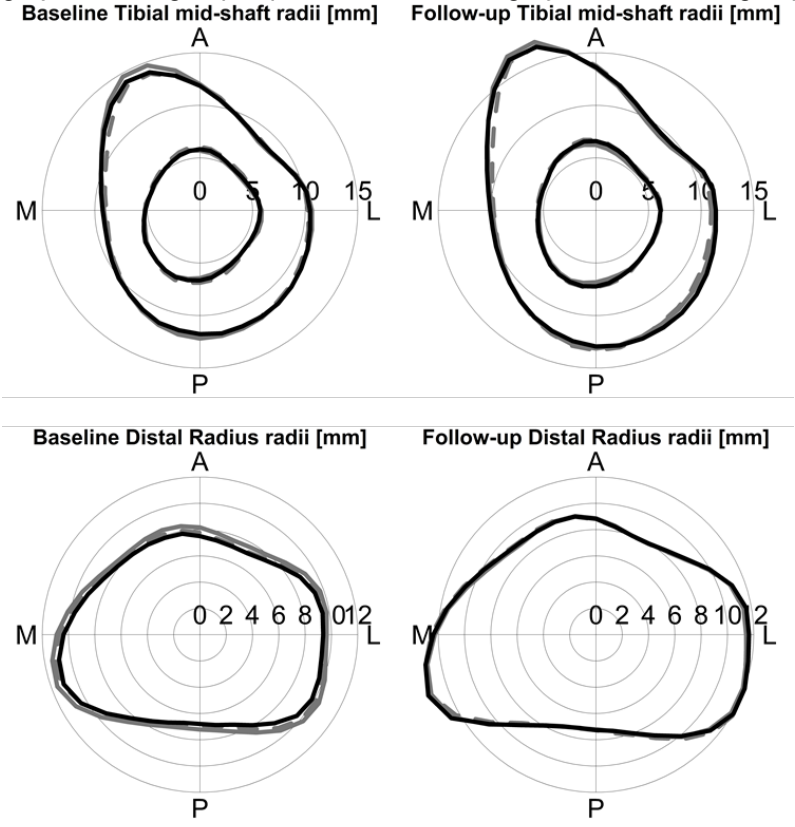
Epidemiological studies have indicated that late onset of menses is associated with higher fracture risk. However, results on bone strength are unequivocal. The purpose of this study was to examine whether girls with later onset of menarche have lower bone strength at early adulthood (the CALEX family study). 7-year prospective data from 94 girls between 10 to 13 years of age at baseline was analysed. 17β-estradiol (E2), testosterone (T), and sex hormone binding globulin (SHBG) were analyzed from serum samples. Bone strength was assessed from femoral neck with DXA (BMD), and from tibial mid-shaft (bending strength [SSI] and pericortical radii for 10˚ radial sectors [r] (FIGURE 1)) and distal radius (compressive strength and r) with pQCT. Age at menarche was defined as first blooding. Girls were grouped into tertiles (earliest, mid, latest) according to their menarcheal age. At baseline, the earliest group was >4% taller, >21% heavier (P<0.001), had >7% higher BMD and SSI (P<0.017), and >4% longer pericortical radii (P<0.040) than the other groups (age-adjusted ANCOVA). The earliest group had higher hormone levels (P<0.001) than the latest group except for SHBG. At the 7-year follow-up the groups did not differ in terms of hormones or any bone trait (P>0.21). However, the earliest group was >10% heavier than the other groups (P<0.031) . Due to catch-up growth girls with later onset of menarche showed no disadvantage in terms of bone strength at early adulthood compared to their counterparts with earlier menarche.

FIGURE 1. Visualization of the baseline and 7-year follow-up bone geometry. Earliest group plotted with solid gray line, mid group is plotted with dashed gray line and latest group is plotted with solid black line.



OR21

Cortical exceeds trabecular bone loss before menopause but net bone loss is modest because periosteal apposition occurs

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Introduction: Bone mineral density decreases before menopause and is held to be due to trabecular, not cortical, bone loss. Yet neither a negative bone balance, nor accelerated remodelling occurs before 45 years of age. We hypothesized that bone loss will first appear after 45 years and will be cortical (as 80% of bone is cortical).
Methods/Design: Images of distal tibia acquired using high-resolution peripheral quantitative computed tomography (Scanco Medical) were analyzed using StrAx1.0 in 212 premenopausal, 42 perimenopausal and 91 postmenopausal women aged 40-61 years, and in 28 women remaining premenopausal during 3.4 years follow-up, in Melbourne, Australia.
Results: In premenopausal women ≥ 45 years (not younger), medullary and total CSA were larger across age ($p < 0.05$) so their ratio, an index of cortical thickness and total vBMD were unchanged. However, for each SD higher age, porosity of the compact cortex and outer transitional zone were 0.28 SD and 0.27 SD higher (both $p \leq 0.001$). Trabecular vBMD was unchanged. Between 40 and 61 years the diminution in bone mass was 75% cortical and 25% trabecular but only 4% preceded menopause and this was cortical. The prospective data in premenopausal women were similar; porosity increased by 0.2-0.3 SD, trabecular and total vBMD decreased by 0.05-0.11 SD ($p < 0.001$), each correlated with remodeling markers.
Conclusion: Intracortical and endocortical remodeling cause cortical bone loss shortly before menopause, but net bone loss is modest because periosteal apposition occurs.

Monday 9 September 2013
SESSION 5B: Biomedical Presentations

OR22

CHKB mutant mice display an osteoporotic phenotype with altered osteoclast and osteoblast activities

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The maintenance of bone homeostasis requires tight coupling between the bone-forming osteoblasts and bone-resorbing osteoclasts. However, the precise molecular mechanism(s) underlying the activities of these specialized cells are still largely unknown. In search of novel molecules involved in bone homeostasis, we systematically screened a number of ENU-induced mutant mouse lines. Here we identify choline kinase beta (CHKB), a kinase involved in the biosynthesis of phosphatidylcholine, as a novel regulator of bone homeostasis. Choline kinase beta mutant mice (flp/flp) exhibit a systemic low bone mass phenotype comparable to osteoporosis. Consistently, osteoclast numbers and activity are elevated in flp/flp mice. 1H Nuclear magnetic resonance spectroscopy revealed reduced levels of choline metabolites (phoshocholine and glycerophosphocholine) in osteoclasts derived from flp/flp mice, leading to a significant reduction in phosphatidylcholine levels. Interestingly, osteoclasts derived from flp/flp mice exhibit reduced sensitivity to excessive levels of extracellular calcium, a feature that allows them to persist at sites of bone resorption. Conversely, supplementation of CDP-choline (Cytidine 5'-diphosphocholine) in vivo and in vitro, a regimen which bypasses CHKB deficiency in the production of phosphatidylcholine, restores osteoclast numbers to physiological levels. In addition to modulating osteoclast formation and function, a loss of CHKB also corresponds with a reduction in bone formation by osteoblasts. Phosphocholine levels were reduced in osteoblasts derived from flp/flp long bones, which is the preferred substrate for the phosphate generating enzyme PHOSPHO1, accounting for reduced mineralizing nodule formation in vitro. Taken together, these data posit CHKB as a new modulator of bone homeostasis.

OR23

Visualisation of tumour cell dormancy and activation in the skeleton by two-photon *intra-vital* imaging

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Cancer cells can exist in a dormant state in bone and be activated to form tumours. However, our understanding of these events is limited due to an inability to identify and define dormant cells. Using myeloma as a model, we developed *intra-vital* imaging to visualize dormant cells in live mice and transcriptome analysis to define phenotypes.
5TGM1eGFP murine myeloma cells were labeled with a membrane dye (DiD), retained by dormant, non-dividing, cells (DiD^{High}), but lost as cells divide (DiD^{Neg}). Cells were injected (i.v.) into C57BLKalwRij mice and visualized after 1, 7, 14, 21, or 28 days, in intact tibia by two-photon, *intra-vital*, microscopy. DiD^{High} and DiD^{Neg} cells were isolated for flow cytometry, whole genome array and $CD138^{+ve}$ cells identified by immunohistochemistry.
Intra-vital microscopy identified individual, dormant, DiD^{High} cells opposed to bone surfaces at all time points including day 28, which was confirmed by flow cytometry ($171\pm31/10^6$ cells). DiD^{Neg}/GFP^{+ve} cells were identified from day 14, increasing in number to day 28. At day 21, DiD^{Neg}/GFP^{+ve} cells were evident and a limited number of distinct DiD^{Neg}/GFP^{+ve} and $CD138^{+ve}$ colonies were visualised (14.8 ± 1.1). Microarray analysis identified a distinct transcriptome profile of DiD^{High} cells compared to DiD^{Neg} cells. Long non-coding RNAs were the most strongly up-regulated transcripts in DiD^{High} cells.
This shows that *intra-vital* microscopy can identify dormant cancer cells and their activation in live mice, demonstrating only a limited number are activated to form colonies. Furthermore, we show that dormant cells have a unique transcriptome profile, which may be critical in retaining dormancy.

Monday 9 September 2013
SESSION 5B: Biomedical Presentations

OR24

Interleukin-6 family cytokines maintain bone material strength through osteocyte gp130 signalling

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Interleukin-6 (IL-6) family cytokines stimulate both bone formation and osteoblast RANKL production via the glycoprotein-130 (gp130) co-receptor. Genetic deletion of osteocyte gp130 (*DMP1.gp130$^{f/f}$* mice) dramatically reduced trabecular bone formation compared to controls, but their increased periosteal diameter, marrow area, and moment of inertia suggested greater resistance to fracture. To determine the influence of osteocyte gp130 on bone material properties, reference point indentation (RPI) and three-point bending tests were carried out on femora from adult male and female *DMP1.gp130$^{f/f}$* mice.
Despite a significant increase in diaphyseal dimensions in *DMP1.gp130$^{f/f}$* mice, ultimate load was not significantly altered compared to *DMP1.gp130$^{w/w}$* controls. Rather, at the material level, *DMP1.gp130$^{f/f}$* mice exhibited significantly lower elastic modulus (males 53% decrease, $p<0.01$; females 33%, $p<0.05$), and male *DMP1.gp130$^{f/f}$* mice exhibited significantly lower yield strength (by 42%, $p<0.05$), and ultimate stress (by 45%, $p<0.05$). This suggests that the increased periosteal diameter of *DMP1.gp130$^{f/f}$* mice is an adaptive response of cortical bone to poor material strength. Surprisingly, tissue mineral density of male *DMP1.gp130$^{f/f}$* femora was not different to controls, nor was there any change in RPI parameters, indicating that the reduced strength is not due to a mineralization defect. However, gene expression analysis of flushed femora revealed a dramatic reduction (62% decrease, $p<0.01$) in collagen type-1 mRNA levels in male *DMP1.gp130$^{f/f}$* mice only, suggesting a deficit in collagen production in these bones.
In conclusion, gp130 signalling in osteocytes is essential for normal bone material strength and collagen production, identifying a new role for the IL-6 family in the skeleton.

OR25

Roquin is a novel regulator of bone homeostasis

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Many studies have indicated that the immune system impacts bone remodelling; however, the underlying mechanism remains to be elucidated. To gain insights into the molecular genetics of bone remodelling, we have employed chemical (ENU) mutagenesis screening to identify novel regulators of bone homeostasis. SanRoque mice, which carry an ENU-induced M199R mutation in the Roquin (*Rc3h1*) gene, exhibit an autoimmune disease consistent to Systematic Lupus Erythematosus, and excessive formation of follicular helper T (T_{FH}) cells in germinal centers. MicroCT and histological analyses revealed that SanRoque mice display an osteoporotic phenotype relative to wildtype littermates. Accordingly, real-time PCR analysis showed elevated levels of RANKL expression in whole bone isolated from SanRoque mice relative to wildtype mice. In addition, flow cytometry analysis revealed a higher percentage of osteoclast progenitors ($CD3e^{-}CD45R/B220^{-}CD11b^{hi}$) in SanRoque mice. Consistently, we observed enhanced osteoclastogenesis of bone marrow macrophages (BMMs) derived from SanRoque mice *in vitro*, accompanied by enhanced RANKL-mediated MAPK signaling. *In vitro* assays show a reduction in bone mineralisation by calvarial osteoblasts derived from SanRoque mice. Furthermore, co-culture experiments revealed that SanRoque calvarial osteoblasts have a reduced ability to support osteoclastogenesis. Consistently, *in vivo* calcein labeling shows a reduction in bone apposition rate in the SanRoque mice. Taken together, our data document that the Roquin gene is an important regulator of bone homeostasis and that SanRoque mice emerge as a useful model to investigate the molecular genetics and mechanisms of bone loss and osteoimmunology.

Monday 9 September 2013
SESSION 5B: Biomedical Presentations

OR26

Neuropeptide Y signalling in osteoblasts controls glucose metabolism in mice

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Recent research suggests that bone plays an active role in regulating glucose homeostasis through both osteocalcin-dependent and osteocalcin-independent mechanisms. This study uses the neuropeptide Y (NPY) system, known to be involved in both energy metabolism and bone mass, to investigate the interaction between bone and pancreas. Specifically, we sought to investigate whether removing osteoblastic NPY receptor signalling in mice would alter glucose homeostasis.

Mice specifically lacking osteoblastic NPY, Y1 receptors (Y13.6Cre) were produced using the 3.6kb 1α (I)-collagen Cre mice. Importantly, these mice display an anabolic bone phenotype characterised by significantly increased bone formation and reduced bone resorption.

Interestingly, despite no changes in body weight, adipose tissue or lean tissue mass, Y13.6Cre mice exhibited significantly increased glucose levels and impaired glucose clearance during a glucose tolerance test associated with decreased insulin levels. In addition, histology revealed a significant decrease in beta cell mass in Y13.6Cre mice compared to control. However, insulin sensitivity as measured by insulin tolerance tests was not affected by osteoblast-specific Y1 deletion. Serum levels of osteocalcin as well as expression levels of specific genes involved in the osteocalcin-mediated control of insulin production were unchanged in Y13.6Cre mice versus control.

This study has identified a novel model in which osteoblastic NPY signalling modulates insulin and glucose homeostasis by a pathway that appears to be independent of osteocalcin signalling. This research has implications for two increasingly common diseases, osteoporosis and type 2 diabetes.

OR27

Mice lacking expression of Oncostatin M Receptor (OSMR) show exacerbated bone loss in response to inflammatory arthritis

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Inflammation-induced bone loss is a cardinal feature of rheumatoid arthritis (RA) The cytokine oncostatin M (OSM) is found at elevated levels in RA synovial fluid and synovial tissues. In synovial fibroblasts and osteoblasts, OSM, acting via its ligand-specific receptor, OSMR, induces expression of the pro-osteoclastic cytokine RANKL and pro-inflammatory cytokine, IL-6. Mice lacking OSMR expression ($OSMR^{-/-}$ mice) have increased bone volume. We hypothesised that $OSMR^{-/-}$ mice would be protected from arthritis-induced bone loss.
$OSMR^{-/-}$ and their wildtype littermates ($OSMR^{+/+}$) were induced with antigen-induced arthritis in one knee only. The development of clinical arthritis and severity of histologic synovial inflammation, cartilage damage and focal bone erosion were similar in $OSMR^{-/-}$ and $OSMR^{+/+}$ mice. Surprisingly, in $OSMR^{-/-}$ mice only, micro-CT analyses revealed a reduction in tibial epiphyseal bone volume in the arthritic-knee compared to the non-arthritic knee ($p<0.01$). Tibial metaphyseal trabecular bone volume was also reduced in both arthritic and non-arthritic limbs in these mice compared to naïve mice. These observations suggest that loss of OSMR expression exacerbates peri-articular and systemic bone loss in inflammatory arthritis. Flow cytometry revealed differences in the cellular composition of the arthritic synovial tissues in $OSMR^{-/-}$ mice: increased immature granulocytes and reduced mature granulocytes and CD4+ T cells when compared to $OSMR^{+/+}$ tissues. Similar observations were made in naive $OSMR^{-/-}$ spleen and bone marrow.
Together our data indicate that OSM signaling via OSMR is not only essential for limiting inflammation-induced bone loss in inflammatory arthritis, but may also function to regulate normal hematopoietic cell development.

Monday 9 September 2013
SESSION 5B: Biomedical Presentations

OR28

Superoxide dismutase 2 deficiency in osteocytes causes bone loss and fragility in mice

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Oxidative stress is thought to be important factor for aging-like symptoms. However, oxidative stress in bone metabolism has not been fully elucidated yet. Superoxide dismutase 2 (*Sod2*) is a mitochondrial antioxidant enzyme that plays a pivotal role in maintenance of redox balance in mitochondria. In osteocytes, however, the physiological function of *Sod2* is still unclarified. Mice lacking *Sod2* in osteocytes were generated by crossbreeding mice harboring a *Sod2* conditional allele with Dmp1-Cre transgenic mice. *Sod2* cKO femur showed significantly reduced BMD (-20%), BV/TV (-29%), cortical thickness (-12%) as well as bone stiffness (-23%) by pQCT, micro-CT, and three-point bending analyses. Histochemical analysis revealed that *Sod2* cKO mice showed significant increased empty lacunae and abnormal canaliculi structure. *Sod2* cKO mice also exhibited reduced MS/BS (-24%), MAR (-32%), and BFR/BS (-41%) in trabecular bone and increased osteoclast number (+35%) and surface (+37%) in trabecular bone. In vitro experiment revealed that *Sod2*-deficient immature osteocytes showed suppressed the formation of mineralized nodule, suggesting decreased bone formation ability. In an osteoclast culture, *Sod2* deletion in osteocytes did not impair differentiation and proliferation of osteoclasts. qPCR analysis revealed that *Rankl* expression and *Rankl* to *Opg* ratio were significantly increased in cKO mice. We confirmed that RANKL protein level was also significantly increased in cKO mice. Our findings indicated that mitochondrial *Sod2* deficiency induced osteocyte loss associated with impaired bone metabolism, suggesting that *Sod2* in osteocytes plays a crucial role for the maintenance of bone mass.

OR29

Osteomacs are closely associated with osteoclasts *in vivo* and regulate osteoclast formation *in vitro* via cardiotrophin-like cytokine factor 1

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Osteomacs are resident macrophages within bone lining tissues that closely associate with osteoblasts. They enhance bone formation by supporting osteoblast function. Using double immunohistochemistry in 3-4 weeks old mouse bone for detection of $F4/80^{+}TRAP^{neg}$ osteomacs and $TRAP^{+}F4/80^{neg}$ osteoclasts, we have observed that osteomacs are also associated with sites of bone resorption and remodelling. At both sites osteomacs are juxtapositioned to osteoclasts and constitute the bulk of cells within the canopy structure encapsulating the basic multicellular unit (BMU). $TRAP^{+}F4/80^{neg}$ immature (mononuclear) osteoclasts are located within the BMU vicinity and are distinct from osteomacs. *In vivo* treatment with RANKL induced osteoclast formation and serum TRAP within 3 days, followed by a delayed induction of osteoblast formation (from day 5). Flow cytometry demonstrated a biphasic significant increase in the percent of $F4/80^{+}$ bone marrow macrophages compared to vehicle control. The $F4/80^{+}$ BM macrophage peaks coincided with elevated bone resorption and elevated osteoblast formation respectively, suggesting macrophage involvement in both biological responses. Conditioned media (CM) from bone marrow-derived macrophages (BMM) greatly enhanced sub-optimal RANKL-stimulated osteoclastogenesis *in vitro*. Proteomic analysis of BMM conditioned media identified cardiotrophin-like cytokine factor 1 (CLCF1) as a candidate macrophage-secreted pro-osteoclastogenic molecule. Real time PCR confirmed that BMM expressed CLCF1 mRNA. Addition of recombinant CLCF1 to a sub-optimal RANKL-treated osteoclast assay was sufficient to recapitulate BMM CM-enhanced osteoclastogenesis. The data implicate osteomacs as stimulators of osteoclast formation potentially via CLCF1. Combined with their ability to enhance osteoblast function, the data indicate that osteomacs regulate both catabolic and anabolic outcomes in bone.

Tuesday 10 September 2013
SESSION 6: Exercise and the Muscle-Tendon-Bone Unit

IS11

Muscle as an Endocrine Organ

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Skeletal muscle is primarily known as an organ of locomotion. However, almost 50 years ago Goldstein[1] proposed the hypothesis that muscle cells possess a "humoral" component that contributes to the maintenaince of glucose homeostasis during exercise. Several years ago, we identified skeletal muscle as a cytokine-producing organ, demonstrating that the metabolic and physiologic effects of exercise may be mediated by muscle derived humoral factors (fore review see[2]). We have demonstrated that interleukin-6 (IL-6) was the prototypical "myokine", up-regulated by muscle contraction and released from contracting skeletal muscle, to play important roles in lipid and glucose metabolism in metabolically active tissues[3-5], while others have demonstrated that the exercise induced incretin response is entirely dependent upon IL-6 release from skeletal muscle[6]. Since the identification of IL-6 as the prototypical myokine, other candidate myokines such as IL-8, IL-15, leukemia inhibitory factor (LIF), brain derived neurotropic factor (BDNF), follistatin like 1 (FST-1) and fibroblast growth factor-21 (FGF-21) have been discovered[7]. Recently, work from the Speigelman laboratory at Harvard University has generated great renewed excitement in the the area of myokine identification. This group identified FNDC5 as a potential mediator of exercise–induced increases in thermogenic genes such as UCP-1 in the adipose tissue[8]. Clearly, the role of skeletal muscle as an endocrine organ is in its infancy, but rapidly expanding.

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IS12

Exercise and bone strength in children

Belinda Beck PhD
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The positive effects of mechanical loading on bone are well–recognized, having been amply demonstrated from animal, observational and experimental studies. The relatively modest responses of older adult bone to exercise however, led to a refocusing of research effort onto children. It is now commonly postulated that osteogenic efficiencies are to be gained from mechanical loading during growth, such that peak bone mass will be optimised, thereby reducing the risk of fractures in old age. While the challenges to obtaining evidence for such hypotheses have been prohibitive (the requirement for vast sample sizes and life-long follow up), a growing body of data suggests positive effects of exercise on bone in childhood can be maintained, at least in the medium term. Exceptions exist and effect sizes are typically small, leading some to argue that even in children exercise benefits are clinically insignificant and unlikely to endure into later life. Such pessimism can be partially countered by observations that the exclusion of trials with poor design from meta-analyses strengthens the effect of exercise on bone, suggesting methodology has been the limiting factor in our search for an exercise effect. Indeed methodologically rigorous paediatric exercise trials are notoriously difficult to accomplish. An inability to blind the treatment group, the mediating influences of puberty and nutrition, and the challenges of running trials within the constraints of school schedules are a few of the inherent obstacles. Nevertheless advances continue to be made. Ongoing challenges include the determination of dose response and the development of truly engaging strategies to translate evidence into practice.

Tuesday 10 September 2013
SESSION 6: Exercise and the Muscle-Tendon-Bone Unit

IS26

Exercise prescription for falls and fracture prevention – Is there an optimal type and dose?

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Exercise is widely recommended to prevent osteoporosis, falls and fractures, but not all forms are equally effective. The beneficial effects of exercise on musculoskeletal health and function are modality and intensity-dependent. Walking alone has little effect on bone and muscle health and may even increase the risk of falls and fractures. Clinical trials and meta-analyses in adults without osteoporosis indicate that multi-modal programs incorporating weight-bearing impact exercise and high-intensity progressive resistance training (PRT) can increase (1-4%) or maintain hip and spine BMD. Traditional PRT can also enhance muscle mass and strength, but has mixed effects on balance and function. However, high-velocity PRT (or power training), which involves rapid concentric movements, can improve muscle function, power and BMD in older adults. For falls prevention, high-challenging balance activities appear most effective, but emerging data indicates that dual-task functional training, which involves exercise combined with additional cognitive and/or motor tasks, can improve balance and gait under dual-task conditions. This is important because many falls occur while walking or performing concurrent attention demanding tasks, such as walking while maintaining a conversation or carrying objects. Whether exercise can prevent fractures remains uncertain, but a recent systemic review and meta-analysis reported that exercise reduces overall fractures and, to a lesser degree, vertebral fractures in the elderly. This presentation will review the latest evidence with regard to the optimal mode and dose of activity that can enhance bone strength as well as improve muscle function and reduce falls risk in the elderly.

IS27

The skeleton and glycaemic control: do we have a link?

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Bone modelling and remodelling require energy which raises the possibility that there is a cross-talk between bone and energy metabolism (Lee et al. 2007). There is evidence, mostly from genetic experiments in mice, to suggest that the skeleton is an endocrine organ participating in energy metabolism and glucose homeostasis via undercarboxylated osteocalcin (ucOC). It has been shown that in the mouse, under physiological conditions, ucOC acts on islet cells to stimulate β-cell proliferation and insulin secretion and stimulates adiponectin secretion from adipocytes. In addition, in mice, ucOC treatment increases mitochondria number, energy expenditure and protects from diet-induced obesity and diabetes. The contribution of ucOC to glycaemic control in humans is still unknown and available evidence is contradictory. There is some correlative evidence that ucOC is related to measures of insulin sensitivity and glycaemic control in several populations. In addition, men and women who are obese as well as those with type 2 diabetes have lower circulating levels of ucOC compared to controls. On the other hand, interventions that reduce circulating levels of ucOC, such as vitamin K supplementation or anti-resorptive drugs used to treat osteoporosis, have no negative effect on glycaemic control. The contradictory evidence can be, in part, due to confounding limiting interpretation of cross sectional studies and lack of longitudinal controlled studies. The purpose of this presentation is to critically review this evidence and to highlight the potential clinical implications if such a link is confirmed in human subjects.

Tuesday 10 September 2013
SESSION 7A: Clinical Presentations

OR30

Low fracture rates maintained with 7 years of Denosumab treatment for postmenopausal osteoporosis: results from the first 4 years of the FREEDOM Extension

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Aims: In the 3-year FREEDOM trial[1], denosumab (DMAb) decreased the risk of new vertebral, nonvertebral and hip fractures versus placebo. The FREEDOM open-label extension is evaluating the long-term effects of DMAb treatment for up to 10 years. Here we report fracture rates from the first 4 years of the extension, representing up to 7 years of DMAb treatment.
Methods: All women in the FREEDOM extension receive 60 mg DMAb subcutaneously every 6 months, with daily calcium and vitamin D. This analysis includes two groups: a 'long-term group' who were in the FREEDOM DMAb group and have now received 4 more years of DMAb, and a 'cross-over group' who were in the FREEDOM placebo group and have now received 4 years of DMAb. In year 7, nonvertebral fractures, clinical vertebral fractures, adverse events (AEs) and serious AEs (SAEs) were collected.
Results: Of eligible participants, 77% enrolled in the extension trial (N=2343 long-term; N=2207 cross-over). The incidence of nonvertebral fractures remained low over years 4–7 of DMAb treatment, with incidences of 1.4%, 1.2%, 1.6% and 1.5%, respectively. Nonvertebral fractures in the cross-over group were consistent with the FREEDOM DMAb group, with rates of 2.5%, 1.9%, 2.2% and 1.0% for years 1–4 of DMAb treatment, respectively. Yearly clinical vertebral fracture rates were also low (long-term: 0.1%, 0.3%, 0.5% and 0.1%; cross-over: 0.0%, <0.1%, 0.3% and 0.2%). Exposure-adjusted AE and SAE rates were similar between the two groups.
Conclusion: DMAb treatment continued to show a favourable risk/benefit profile for up to 7 years and was associated with a low incidence of nonvertebral and clinical vertebral fractures.
Reference: 1. Cummings SR, et al. *N Engl J Med* 2009; 361-71.

OR31

Increased bone loss is associated with post-fracture mortality

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Background The mechanism by which mortality risk increases post-fracture is unknown. Bone loss, a fracture risk factor is also associated with all cause mortality risk, but its role in post-fracture mortality is unclear.
Aim To examine the effect of bone loss on post-fracture mortality.
Methods 462 women and 149 men aged 60+ from Dubbo Osteoporosis Epidemiology Study with incident fractures were followed prospectively (April 1989-December 2012). Femoral neck bone density was measured at baseline and 2-yearly. Bone loss was assessed individually by linear regression. Survival was assessed according to quartile of bone loss.
Results The rate of bone loss was similar for women and men (women: mean -0.75%/year and highest quartile: -1.38%/yr; men: mean -0.74%/yr and highest quartile: -1.34%/year).
Survival was lowest for the highest quartile of bone loss for both women and men (women, p<0.005; men, p=0.05). When analysed by fracture type, the association of bone loss with mortality was observed for vertebral and non-hip non-vertebral fractures (highest quartile vs. lower 3 quartiles of bone loss, p=0.03 and p=0.05 for vertebral non-hip non-vertebral fractures, respectively) but not for hip fractures (p=0.7).
A ≥1%/per year decrease in bone mass was associated with 13% increased mortality risk in women [age-adjusted HR 0.87 (95% CI, 0.80- 0.96)] and 9% in men, albeit not significantly [age-adjusted HR 0.91 (95% CI, 0.80- 1.39)].
Conclusion Increased bone loss was associated with increased mortality risk in both women and men, particularly following vertebral and non-hip non-vertebral fractures. Whether this is associative or causative warrants further investigation.

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SESSION 7A: Clinical Presentations

OR32

Hip cortical porosity predicts non-vertebral fractures in postmenopausal women

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Introduction The current methods for fracture risk assessment based mainly on bone densitometry have limitations. We hypothesized that women with fractures are better distinguished from controls by measurements of microarchitecture, particularly cortical porosity, or the combination of cortical porosity and aBMD, than either risk factors alone.
Methods In a nested case-control design, from the Tromsø Study, Norway, 180 postmenopausal women aged 54-94 years with fractures (hip, humerus and forearm) and 209 age-matched controls, had microarchitecture quantified in computed tomography images of the subtrochanteric femoral site using StrAx 1.0 software, and femoral neck aBMD measured using densitometry. The fracture risk was calculated using logistic regression analyses adjusted for age, height and weight, and fracture prediction by using the area under receiver operator curve (AUC).
Results Women with fractures exhibited 3.3% higher cortical porosity and 0.06 g/cm² lower aBMD than controls (both $p < 0.001$). Porosity predicted fracture independently of aBMD, and each SD higher porosity increased the risk of fracture, with an OR of 2.19 (95% CI 1.69-2.83), and each SD lower aBMD increased the risk with an OR of 2.04 (95% CI 1.59-2.62) both $p < 0.001$. AUC was 0.69 (95% CI 0.63-0.73) for porosity alone, 0.67 (95% CI 0.62-0.72) for aBMD alone and increased to 0.74 (95% CI 0.69-0.79) for the combination of porosity and aBMD. So after inclusion of cortical porosity there was an increase in AUC of 0.07 (95% CI 0.03-0.12), $p = 0.002$.
Conclusion In this general population, cortical porosity improved the diagnostic fracture risk sensitivity.

OR33

Body mass index (BMI) and fracture risk: a bone mineral density (BMD)-dependent and site-specific association

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Aim: The association between body mass index (BMI) and fracture risk is controversial. We sought to test the hypothesis that the effect of BMI on fracture risk is dependent on bone mineral density (BMD) and fracture site.
Methods: The study involved 2,199 women and 1,351 men aged 60+ years whose bone health has been monitored for up to 25 years. Body mass index was derived from baseline weight and height. Femoral neck BMD was measured by dual energy X-ray absorptiometry (GE-LUNAR Corp, Madison, WI). The incidence of low-trauma fracture was ascertained by X-ray reports. The magnitude of association between BMI and fracture risk was assessed by hazard ratio (HR) from the Cox's regression model. **Results:** Individuals with high BMI had lower fracture risk, but the association was mainly observed in hip fracture (HR 0.65; 95% CI, 0.55-0.78 in women; HR 0.59; 95% CI, 0.44-0.79 in men). Further analyses by BMD subgroups revealed that the association between BMI and fracture risk was present in women with T-scores < -2.5 (RR 0.51; 95% CI, 0.29-0.88), but not in those with osteopenic or normal-BMD. After adjusting for BMD, higher BMI was associated with higher risk of fracture at the vertebral (HR 1.21; 95% CI, 1.05-1.39) and upper limbs (HR 1.25; 95% CI, 1.07-1.46); nevertheless, by using a simulation method, we demonstrated that association between BMI and fracture is driven by BMD.
Conclusion: Higher BMI is associated with lower fracture risk, particularly hip fracture, but the association was predominantly seen in women with osteoporotic BMD.

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SESSION 7A: Clinical Presentations

OR34

Associations of accelerometer-determined moderate and vigorous physical activity with sarcopenia two years later in community-dwelling older adults

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Introduction: This study aimed to determine whether higher accelerometer-determined moderate and vigorous PA would reduce odds of sarcopenia two years later in community-dwelling older adults.
Methods: 176 community-dwelling volunteers (mean ± SD: 64 ± 7 years, 52% female) participated at baseline and 2.2 ± 0.1 years later. Baseline PA was assessed by seven-day accelerometer use (Actigraph GT1M). The lowest sex-specific tertiles of dual-energy X-ray absorptiometry-assessed appendicular lean mass (ALM) normalised to height (ALM-H), ALM normalised to weight (ALM-W), and/or lower-limb strength (LLS), were classified as sarcopenic. Multivariable logistic regression examined associations of baseline moderate and vigorous PA with sarcopenia.
Results: Sarcopenic participants classified by ALM-W and LLS completed less moderate and vigorous PA per day compared to non-sarcopenic participants (both $P \leq 0.002$). After adjustment for age, knee and hip pain, and light/sedentary activity, higher baseline moderate and vigorous PA was associated with reduced odds ratios for sarcopenia at follow-up when classified by ALM-W (0.70; 95% CI 0.57 – 0.85 per 10-min/day increase in PA), LLS (0.78; 0.61 – 0.99) and ALM-W and LLS combined (0.68; 0.48 – 0.95), but not when classified by ALM-H alone or combined with LLS (both $P > 0.05$).
Conclusions: Even relatively small increases (10-min/day) in accelerometer-determined moderate and vigorous PA are associated with substantially decreased odds of low muscle mass and strength two years later, with reductions in excess of 30%. Increasing the time of participation in higher intensity PA may be an effective strategy for preventing or reversing sarcopenia in older adults.

OR35

The pathophysiology of subchondral cancellous bone in postmenopausal knee osteoarthritis: diffuse microdamage accumulation and osteocyte cell network deficiency

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Aim: The role of fatigue microdamage in the adaptive response of cartilage and subchondral bone in the pathophysiology of human osteoarthritis (OA) is debatable, given that experimental data are predominantly animal. The prevalence of microdamage in human knee OA is unknown. Thus, the study aim was to investigate the occurrence of microdamage and its relationship to resorption, osteocyte, and microarchitectural morphology in subchondral bone for postmenopausal women with and without knee OA.
Methods: Subchondral cancellous bone samples were cut from medial tibial plateaus obtained from postmenopausal knee OA arthroplasty patients (n=10; aged 70-84 years), and sex-matched cadavers (n=13; aged 70-97 years) with no histological evidence of OA. Bone samples were micro-CT imaged prior to histomorphometric assessment of microdamage, resorption, and osteocyte morphology.
Results: OA subchondral bone was microarchitecturally distinct from non-OA; with increased BV/TV ($p<0.002$), Tb.N ($p<0.001$), Tb.Th ($p<0.03$); decreased Tb.Sp ($p<0.02$), TB.Pf ($p<0.003$), SMI ($p<0.03$). Microcrack and resorption parameters did not differ between OA and non-OA; however, diffuse damage density ($p<0.02$) and ratio of diffuse damage/resorption site density ($p<0.008$) were higher for OA. While osteocyte, empty and total lacunar densities were not different between groups, percent empty lacunae was higher for OA ($p<0.05$). No correlations were found between measured parameters.
Conclusion: Postmenopausal knee OA subchondral cancellous bone is sclerotic and may be functionally compromised at the material level due to accumulated diffuse microdamage and increased numbers of empty osteocyte lacunae. These observations likely associate with the well-reported tissue mineralisation changes[1] and recently reported altered osteocyte phenotype in human OA[2].

[1] Burr and Gallant 2012 *Nat Rev Rheumatol* 8:665-73.
[2] Jaiprakash et al. 2012 *Int J Biol Sci* 8:406-17.

Tuesday 10 September 2013
SESSION 7A: Clinical Presentations

OR36

Multicentric carpotarsal osteolysis may be a disorder of bone modelling and not osteolysis

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Background: Multicentric carpotarsal osteolysis (MCTO) is a rare autosomal dominant skeletal dysplasia characterised by progressive loss of the carpal and tarsal bones and the epiphyses of many long bones in childhood. This has been assumed to be due to pathologic osteoclast-mediated resorption. The causative mutations have been localised to the v-maf musculoaponeurotic fibrosarcoma oncogene ortholog B (*MAFB*) gene. MAFB is known to negatively regulate osteoclastogenesis, and positively regulate macrophage differentiation. We sought to understand the functional consequences of this mutation and extrapolate this to better understand disease pathogenesis.
Methods: Osteoclastogenesis and resorption assays were performed using peripheral blood mononuclear cells isolated from two patients with MCTO. Literature approaches were used to re-examine the MCTO phenotype. MAFB mRNA expression was examined in human fetal bone marrow mesenchymal stem cells induced to differentiate into either chondrocytes or osteoblasts.
Results: Osteoclastogenesis and osteoclast activity was not increased in patients with MCTO compared to controls. Re-examination of the MCTO phenotype and comparison to other phenotypically similar syndromes suggested defects in bone development/modelling. Therefore MAFB expression was assessed during osteoblast and chondrocyte differentiation and demonstrated to be up-regulated during osteoblast differentiation, and down-regulated during chondrocyte differentiation compared to baseline.
Conclusions: Patients with MCTO do not have enhanced osteoclast formation. MAFB is expressed in many cells that participate in bone biology including osteoclasts, osteoblasts chondrocytes and macrophages. Accumulating data suggests that the MCTO phenotype may be mediated by defects in bone formation rather than osteolysis.

OR37

Femoral neck dxa parameters and PQCT tibial cortical density are predictive of survival in dialysis patients

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AIMS: 59 subjects with end stage renal failure (ESRF) on haemodialysis recruited through a single tertiary referral hospital in 2007 were followed prospectively for six years to determine if BMD measured by DXA and/or peripheral quantitative computed tomography (pqCT) were associated with all-cause mortality.
METHODS: Biochemical markers, DXA (Hologic n=14 (24%), Norland n=40 (68%), T-scores calculated by NHANES III), and pqCT parameters (Stratec XCT 3000, Germany) at study entry were examined using stepwise Cox Regression with mortality as the dependent variable. Survival by BMD category was estimated using Kaplan-Meier curves.
RESULTS: At baseline, FN BMD was normal in 22 (41%), osteopenic in 24 (44%) and osteoporotic in 8 (15%). 34 of the original cohort have survived after six years. Data was missing in 5 patients at five years (8%). FN BMD T-score was significantly associated with mortality (HR 1.69 per SD decrease; 95% CI 1.18-2.5). This remained significant after multivariate adjustment for age, gender, dialysis duration, smoking, transplant status, iPTH, serum phosphate, and calcium-phosphate product (HR 2.08; 1.27-3.45). pqCT tibial cortical density was significantly associated with mortality (HR 1.08; 1.02-1.15) for every 10mg/cm^3 decrease (multivariate adjustment HR 1.16; 1.03-1.32). Kaplan-Meier curves for all-cause mortality by FN BMD categories showed significantly reduced survival for osteopenia and osteoporosis compared with normal (p=0.013). Survival at five years for normal, osteopenic and osteoporotic FN BMD was 86%, 57% and 30%, respectively.
CONCLUSIONS: FN BMD and tibial cortical density were predictive of mortality in this dialysis cohort. Treating osteoporosis in this population deserves further study.

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SESSION 7A: Clinical Presentations

OR38

Cross sectional correlations between hip muscles, muscle strength and bone mineral density

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Aims: The objective of this study was to determine the correlations between hip muscle cross-sectional area (CSA), muscle strength and bone mineral density (BMD) in a community-based sample.
Methods: 321 subjects from the Tasmanian Older Adult Cohort (TASOAC) study (mean age 64, 51% male, BMI: 27.8 kg/m^2) with a right hip MRI scan conducted between 2004-2006, were included. Hip muscles were measured on MR images using Osiris X (Geneva) software measuring maximum muscle CSA (cm^2) of gluteus maximus, obturator externus, gamelli, quadratus femoris, piriformis; pectineus, sartorius and iliopsoas. Dual-energy x-ray absorptiometry (DXA) determined total hip, femoral neck and spine BMD and dynamometer-assessed muscle strength was determined by a maximal isometric contraction of the hip extensors and quadriceps.
Results: Muscles that correlated with both femoral neck and total hip BMD were quadratus femoris, pectineus, sartorius and iliopsoas (r=0.20-0.27; *p=0.02 to 0.007* and r=0.13-0.27; *p=0.02 to <0.001 resp.*). Additionally, CSA of obturator externus was correlated with femoral neck BMD (r=0.16*, p=0.01*) and CSA of gamelli with spine BMD (r=0.36*, p<0.001*). Gluteus maximus and piriformis showed no correlation with BMD. Leg strength was more weakly associated with hip BMD (r=0.09-0.15, *p=0.11 to 0.007*). Lastly, CSA of hip muscles (except gluteus maximus, piriformis and sartorius) were positively correlated with leg strength (r=0.20-0.24, *p=0.001)*.
Conclusion: Overall, CSA of hip muscles, and to a lesser extent, muscle strength were positively correlated with hip BMD. This data suggests that both higher muscle bulk and strength may contribute to the maintenance of bone mass in older adults.

OR39

Calcium plus Vitamin D supplementation: a meta-analysis of risk and benefit

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Background and Aim — We sought to examine the effects of calcium and vitamin D (CaD) supplementation on fracture risk and cardiovascular disease (CVD) outcomes.
Methods — We identified 9 primary RCTs on the efficacy of CaD on fracture risk, and 3 post-hoc analyses of RCT on the association between CaD and CVD outcomes. The 9 RCTs on fracture involved 27705 patients on CaD and 25491 patients on placebo. The 3 RCTs with CVD outcomes involved 10081 indivduals on CaD and 9909 on placebo. The data were synthesized by the Bayesian random-effects meta-analysis.
Results — CaD supplements reduced the risk of fragility fracture by 11% (RR 0.89; 95%CI 0.80-0.97), and the reduction was observed in nonvertebral fractures (RR 0.90; 0.79-0.99) and clinical vertebral fracture (RR 0.86; 0.75-0.99), but not in hip fracture (RR 0.88; 0.71-1.06). Meta-regression analysis suggested that the anti-fracture benefit of CaD supplements was more likely observed in individuals with 2% and greater risk of fracture per year. CaD supplements were not significantly associated with any CVD outcome: myocardial infarction (RR 1.18; 0.73-1.74), stroke (RR 1.17; 0.75-1.70), myocardial infarction or stroke (RR 1.14; 0.74-1.64), and death (RR 1.01; 0.67-1.58). The number needed to treat to reduce a fracture was 85, and the number needed to incur a CVD event was 170. Thus, the ratio of benefit over potential risk was 2.
Conclusions — CaD supplements reduce the risk of fracture, but the effect size is likely modest. The association between CaD supplements and CVD outcomes is uncertain.

Risk ratio of fracture and CVD outcomes associated with CaD supplements

Outcome	Relative Risk (RR) and 95% CI	Probability of RR<0.90 (for fracture) or RR>1.10 (for CVD outcomes)
All fractures	0.89 (0.80 – 0.97)	0.48
Vertebral fracture	0.86 (0.75 – 0.99)	0.59
Non-vertebral fractures	0.90 (0.79 – 0.99)	0.46
Hip fracture	0.88 (0.71 – 1.06)	0.47
Myocardial infarction (MI	1.18 (0.73 – 1.74)	0.63
Stroke	1.17 (0.75 – 1.70)	0.62
MI or stroke	1.14 (0.74 – 1.64)	0.59
Death	1.01 (0.67 – 1.58)	0.30

Tuesday 10 September 2013
SESSION 7B: Biomedical Presentations

OR40

Variance in 3D structure of femoral shaft intracortical void volume: a high-resolution micro-CT study

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Cortical porosity reduces bone strength. The 3D characterization of cortical porosity is usually confined to measurements made in small volumes of excised bone tissue that obscures regional variation. We developed a new non-destructive high-resolution micro-CT protocol to scan the entire proximal shaft of the femur allowing quantification of the void volume network in 3D.

Complete 6mm-high transaxial slices of femoral upper shafts obtained from 8 female cadavers (n=3 aged 29-37 years, n=5 aged 72-90 years) were scanned with high-resolution micro-CT (8.65μm/pixel). For analysis, micro-CT volumes were selected in the anterior, medial and lateral regions, each volume 10mm long (circumferential), 5mm high (longitudinal), containing the entire cortex (fig.1). Images were segmented with voids appearing as 3D-interconnected canals (CTAnalyser, Skyscan).

Voids were mainly oriented longitudinally, followed by anterior-posterior and medio-lateral orientations (anisotropy analysis). In the medial cortex, percent void-volume-to-tissue-volume was higher (48%), as were void-surface-to-tissue-volume ratio (33%), and connectivity density (69%), compared to anterior and lateral regions ($p<0.05$ for all, paired t-test).

Percent-void-volume-to-tissue-volume was higher in older subjects by 72% anteriorly ($p<0.05$), 64% medially ($p=0.08$), and 76% laterally ($p<0.05$), as was pore-surface-to-tissue-volume (respectively 62%, $p=0.08$, 46%, $p=0.08$, and 62%, $p<0.05$). Void separation was lower (-39%, $p<0.05$, lateral), connectivity density higher (46%, $p=0.072$, lateral). Void diameter (81-213μm young, 178-355μm old, anterior) was higher though NS ($p=0.10$ anterior). Void number was not higher in older persons.

High-resolution micro-CT provides an accurate quantitative means of studying the 3D-microanatomy of the intracortical canal network, in multiple anatomical locations in health and disease.

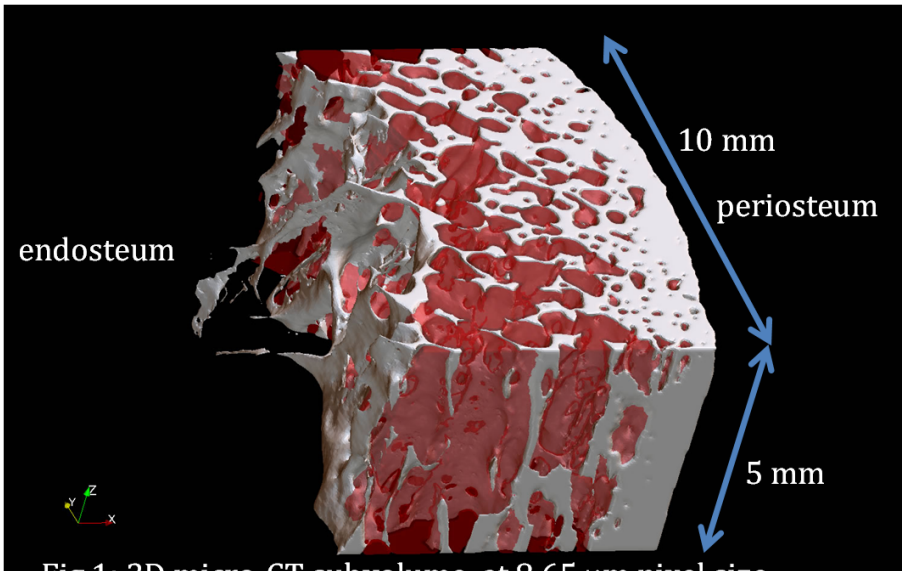

Fig.1: 3D micro-CT subvolume, at 8.65 µm pixel size

OR41

Disulfiram attenuates osteoclast differentiation via suppression of key RANKL-mediated signaling cascades

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Disulfiram (DSF) is a cysteine modifying compound that has been long employed for the treatment of chronic alcoholism. Prolonged administration of DSF has been reported to correlate with improved bone mineral density (BMD) in patients[1] although the underlying cellular mechanism(s) remains unclear. Interestingly, DSF has been recently identified as a novel inhibitor of the acidifying vacuolar-ATPase (v-ATPase) complex[2]. Here we assessed the effects of DSF on osteoclast formation, bone resorptive function and V-ATPase activity *in vitro*. Treatment of bone marrow monocytes (BMMs) with DSF dose-dependently inhibited M-CSF/RANKL-induced osteoclastogenesis (maximal at 100 nM) at early stages of differentiation (1-2 days DSF post-treatment). This inhibition correlated with a decrease in the expression of key osteoclastic marker genes including Ctsk, Acp5, Dcstamp and Atp6v0d2 as well a corresponding reduction in bone resorption *in vitro*. Mechanistically, DSF-mediated suppression of osteoclast differentiation was in part due to the blockade of several key RANKL-signaling pathways including ERK, NFκB and NFATc1 as corroborated by immunoblotting and luciferase-based assays. On the other hand, unlike canonical V-ATPase inhibitors Bafilomycin and Saliphenylhalamide, DSF failed to suppress intracellular acidification and proton transport in osteoclasts using acridine orange quenching and microsome-based H^+ transport assays. Collectively, our findings indicate that DSF is not a V-ATPase inhibitor but rather attenuates osteoclast differentiation via the collective suppression of several key RANKL-mediated signaling cascades.

Reference

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2. Johnson RM, Allen C, Melman SD, Waller A, Young SM, Sklar LA, Parra KJ (2010) Identification of inhibitors of vacuolar proton-translocating ATPase pumps in yeast by high-throughput screening flow cytometry. Analytical biochemistry 398 (2):203-211

Tuesday 10 September 2013
SESSION 7B: Biomedical Presentations

OR42

Intrinsic PTH secretion from the human parathyroid via a prostanoid-dependent autocrine mechanism

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PTH is a key bone modulator that has anti-fracture efficacy in a short-acting form that selectively promotes bone formation. Strategies aimed at manipulating endogenous PTH secretion have been disappointing due, in part, to a failure to properly understand the intrinsic PTH control mechanism. Extracellular Ca^{2+} concentration (Ca^{2+}_o) negatively regulates spontaneous PTH secretion via the calcium-sensing receptor (CaSR). However, the intrinsic mechanism by which the parathyroid spontaneously secretes PTH is unknown and, thus, the mechanism by which the CaSR mediates Ca^{2+}_o-dependent suppression of PTH secretion is also unknown. We have formulated the hypothesis that the parathyroid elaborates its own intrinsic activators that act in an autocrine manner to stimulate PTH secretion. In the present study, we have tested whether prostanoid receptors and prostaglandins are key elements of such a mechanism. Normal human parathyroid cells were prepared by collagenase digestion and perifused with physiological saline solutions as described previously [1]. Inhibition of prostanoid production with COX-1 and/or COX-2 inhibitors reversibly suppressed PTH secretion by around 50-60%. Similarly, inhibition of prostanoid $EP_{2/4}$ receptors with AH6809 reversibly suppressed PTH secretion by around 50-60%. Finally, we detected prostaglandin E_2 (PGE_2) in perifusate samples and found that PGE_2 production was insensitive to changes in Ca^{2+}_o. The results demonstrate that intrinsic PTH secretion is supported by an autocrine mechanism dependent on PGE_2 and prostanoid $EP_{2/4}$ receptors. The results also indicate that the CaSR suppresses PTH secretion by blocking a pro-secretory signaling pathway downstream of the prostanoid receptor.

1. A.D. Conigrave et al. (2004), J. Biol. Chem. 279:38151-9

OR43

Subchondral bone mineral and osteocyte changes in osteoarthritis

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Although subchondral bone sclerosis is a recognised finding in the progression of osteoarthritis (OA), its involvement in OA pathophysiology is poorly defined. Increasing evidence identifies osteocytes as regulators of bone remodelling [1] and osteocyte research represents a paradigm shift for the bone field. Recent studies suggest that dysregulated osteocytic proteins contribute to the pathological changes in the subchondral bone of knee OA patients [2]. Understanding the role of osteocytes in the pathophysiology of OA may provide insights into a biological cure for OA.
Aims: To establish a suitable OA animal model that resembles the clinical situation and allows the investigation of progressive subchondral bone mineral and osteocyte changes. Further, investigate the involvement of osteocyte wnt/β-catenin signaling in OA pathophysiology.
Methods: We induced OA in 12 week old Wistar Kyoto rats by removal of the medial meniscus (n=12). We investigated changes in subchondral bone and osteocytes 8 weeks post-surgery by SEM, microCT, histology, protein expression and compared to sham controls and human knee samples.
Results: Menisectomy rats and human OA demonstrated significantly higher subchondral bone volume and hampered mineral distribution compared to controls. Histological analysis of the menisectomy group demonstrated significantly higher numbers of osteocytes expressing DMP1, E11, AXIN2 and β-catenin; whereas a decreased number of SOST and DKK1 expressing osteocytes were found compared to controls.
Conclusion: Results indicate a regulatory role of osteocyte wnt/β-catenin signaling in OA-subchondral bone remodeling. OA menisectomy experimental rat model resembles human OA pathophysiology and is suitable for studying the progression of OA subchondral bone pathogenesis.

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Tuesday 10 September 2013
SESSION 7B: Biomedical Presentations

OR44

Gender-related differences in the skeletal phenotype of adult global vitamin D receptor (VDR) knockout mice

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Global VDR deletion in a mouse model (VDR-/-) has previously been shown to demonstrate features typical of vitamin D-dependent rickets type II. However, when mice are fed a diet containing high levels of calcium (2%) and phosphorus (1.25%), hypocalcemia and secondary hyperparathyroidism are prevented, restoring bone mineralization in young mice. To investigate the role of VDR in maintaining skeletal health in aged animals, VDR+/- and VDR-/- mice were fed a rescue diet containing 2% calcium and 1.25% phosphorus from weaning until 26 weeks of age. At time of death, femora and tibia were collected for structural, cellular and molecular investigations. Consistent with previously reported data for male VDR-/- mice, metaphyseal BV/TV% was reduced (37%; p=0.05) due to decreased Tb.Th (p=0.03). Cortical bone volume and width were also decreased (23%, p=0.003; 10% p=0.006) when compared to controls. In contrast, female VDR-/- mice metaphyseal BV/TV% was increased 2-fold (p=0.04), compared to control littermates. No significant differences were seen in cortical bone volume or width for female mice. These data indicate significant gender-related differences with regards to the role of VDR in maintenance of bone mineral volume in aged animals. Quantification of osteoblast and osteoclast target genes demonstrate marked decreases in osteocalcin (p=0.01) and TRAP (p=0.02) expression in male VDR-/- mice, while female VDR-/- mice exhibit no changes. Taken together, these data suggest that VDR is required to maintain adequate bone formation and that the extent of this effect is dependent on both age and gender.

OR45

Protease-activated receptor-1: common pathways in bone and muscle repair?

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Protease-activated receptor-1 (PAR-1) is a G-protein-coupled receptor that is activated by thrombin. As osteoblasts and myoblasts express PAR-1 and stimulation of these cells with thrombin elicits similar functional responses, it seems likely that signaling via PAR-1 in response to thrombin is a common regulatory mechanism in bone and muscle tissue. As we have previously shown that PAR-1 plays an important role in the early stages of bone repair, the aim of this study was to identify and describe the effects of PAR-1 on skeletal muscle regeneration.
The right *extensor digitorum longus* muscles of littermate wildtype and PAR-1-null mice were subjected to whole muscle grafting, and contralateral muscles were used as sham operated controls. Mice were euthanased and muscles collected and processed for histological and morphometric analysis, 3, 5, 7, 10 and 14 days after surgery. Histological analysis of grafted muscles 3 and 7 days post-grafting indicated that wildtype muscles were significantly larger and contained a greater number of muscle fibres than PAR-1 null muscles. The area of fibrotic tissue was significantly greater in the PAR-1 null grafts than wildtype grafts 14 days after surgery.
The results suggest that, as with bone repair, normal skeletal muscle regeneration involves signalling via PAR-1. A surprising finding of this study was the ability of PAR-1 to suppress fibrosis of injured muscles. Whilst the mechanism underlying this phenomenon remains unclear, it does highlight how thrombin signalling via PAR-1 may link repair of different tissues following trauma.

Tuesday 10 September 2013
SESSION 7B: Biomedical Presentations

OR46

Osteocyte cell death in subchondral bone in a mouse model of post-traumatic osteoarthritis correlates with the severity of aggrecan loss in overlying cartilage

Brett Tonkin, Natasha Jansz, Evange Romas, Natalie Sims, Nicole Walsh
St Vincent's Institute of Medical Research

The destabilisation of the medial meniscus (DMM) mouse osteoarthritis (OA) model is a model of post-traumatic OA in which the knee joint is destabilised by transecting the medial-meniscotibial ligament leading to increased loading within the medial compartment of the tibia. We sought to define the relationship between changes in tibial subchondral bone and cartilage integrity in DMM-OA.
DMM or sham surgery was performed on right knees of 12-week old male C57Bl/6 mice. In vivo and ex-vivo micro-CT analyses demonstrated a focal increase in medial subchondral bone in DMM-OA tibiae: BV/TV and TMD were increased compared to sham from 4 weeks post-surgery ($p<0.001$). Histologic analyses showed aggrecan loss and cartilage erosion in the medial compartment of DMM-OA tibiae from 4 weeks post-surgery. Interestingly, the medial subchondral bone in DMM-OA tibiae resembled osteonecrotic bone from 4 weeks post-surgery with numerous empty osteocyte lacunae present, indicating osteocyte death. Furthermore, the number of empty osteocyte lacunae at this site negatively correlated with the width of overlying aggrecan-positive (healthy) cartilage ($p=0.01$, Pearson's $r= -0.92$) suggesting a relationship between aggrecan loss and subchondral bone health.
In summary, focal accrual of medial subchondral bone occurs early in DMM-OA tibiae, alongside cartilage damage. Similar to human OA, osteocyte cell death in subchondral bone is a feature of DMM-OA. Mechanical changes or biochemical signals induced by aggrecan loss in articular cartilage may be detrimental to the health of the underlying subchondral bone and osteocyte cell death could contribute to local changes in bone integrity, vascularization or cartilage.

OR47

Stage-specific effects of ephrinB2 in the osteoblast lineage on bone strength

Christina Vrahnas[1], Stephen Tonna[1], Huynh Nguyen[2], Mark Forwood[2], T John Martin[3], Natalie Sims[3]
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We reported that osteoblastic ephrinB2 signalling is required for late stage osteoblast differentiation and bone mineralisation. Since ephrinB2 is also expressed in osteocytes, we sought to examine the role of ephrinB2 signalling in osteocytes *in vivo.*
Bones were collected from 12-week-old female mice with ephrinB2 deleted in osteocytes ($Dmp1Cre.efnB2^{f/f}$) and controls ($Dmp1Cre.efnB2^{w/w}$). Tibiae were analysed by histomorphometry and femora by microCT and 3-point bending tests. $Dmp1Cre.efnB2^{f/f}$ mice showed significantly greater tibial trabecular bone volume (BV/TV by 40%, $p<0.01$), trabecular thickness (by 10%, $p<0.05$) and trabecular number (by 20%, $p<0.01$), while both femoral and tibial trabecular separation were significantly reduced (by 12% and 23% respectively, $p<0.01$, $p<0.05$). Neither osteoblast number nor bone formation rate were significantly altered, however osteoclast surface/bone surface (OcS) and osteoclast size were both ~12% greater in $Dmp1Cre.efnB2^{f/f}$ mice compared to control. The increased OcS and size with increased BV/TV suggests that osteoclast function is impaired by deletion of osteocytic ephrinB2.
$Dmp1Cre.efnB2^{f/f}$ bones were more brittle than controls, showing reduced toughness (33%, $p<0.001$), and lower yield stress (10%, $p<0.05$). Ultimate deformation (20%, $p<0.001$) and energy absorbed to failure (35% less, $p<0.001$) were also reduced. In contrast, osteoblast-specific ephrinB2 null mice displayed greater toughness and ultimate deformation (by 20% and 10%, respectively, $p<0.05$).
These data indicate that the effects of ephrinB2 on bone material properties change during osteoblast differentiation. In addition while ephrinB2 in osteoblasts is required for their ongoing differentiation, its influence in osteocytes is one that supports osteoclast activity.

Tuesday 10 September 2013
SESSION 7B: Biomedical Presentations

OR48

Targeting Class I HDACs to suppress both inflammation and bone loss in arthritis

Melissa Cantley[1], David Fairlie[2], Mark Bartold[1], Victor Marino[1], Praveer Gupta[2], David Haynes[1]
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Aims: Histone Deacetylase (HDAC) 1 is a class I HDAC that is highly expressed in synovial tissues of rheumatoid arthritis (RA) patients. The aim of this study was to determine the importance of the role of HDACs in the disease process by comparing the effects of HDAC inhibitors (HDACi) of class I HDACs (NW-21 and MS-275) with HDAC inhibitors of both class I and II HDACs (1179.4b) in a collagen antibody induced arthritis (CAIA) mouse model.

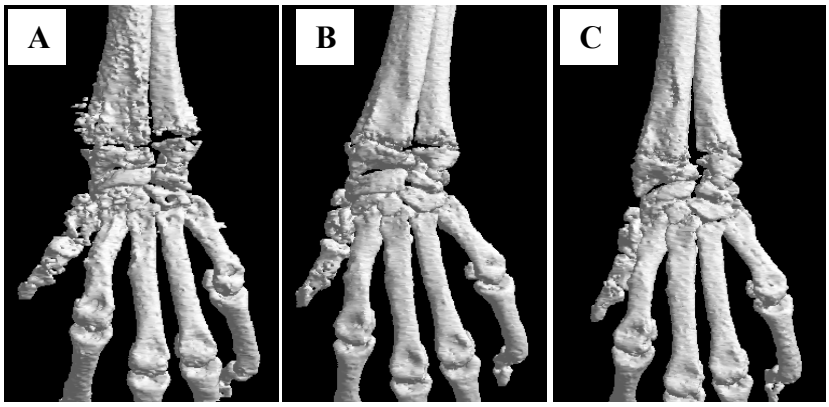

Figure 1: 3D models of A) CAIA only B) CAIA +NW-21 and C) CAIA+MS-275

Methods: CAIA was induced in mice via intravenous injection of a monoclonal antibody against type II collagen (day 0) followed by an intraperitoneal injection of *E.Coli* LPS (day 3). NW-21 at 5mg/kg/day, MS-275 at 10mg/kg/day and 1179.4b at 5mg/kg/day were administrated orally daily starting on day 4. Effects on inflammation and bone were assessed using paw inflammation scoring, histology and live animal micro CT of the joint bone.
Results: HDACi that target class I HDACs (NW-21 and MS-275**)** significantly suppressed inflammation as assessed by paw scoring and histological assessment. There was no significant difference in paw scores between 1179.4b treated and untreated CAIA mice. NW-21 and MS-275 also suppressed bone loss in the radiocarpal joint assessed by micro CT analysis (see figure 1). There was also a reduction in tartrate resistant acid phosphatase expressing osteoclastic cells in joints of NW-21 and MS-275 treated mice.
Conclusion: The results indicate that inhibitors selectively targeting class I HDACs, such as NW-21and MS-275, may be useful for treating RA as they reduce both inflammation and bone loss.

OR49

Inhibition of PDGFRβ increases OPG production from osteoblastic cells - an indirect mechanism by which tyrosine kinase inhibitors inhibit osteoclastogenesis

Mei Lin Tay, Susannah O'Sullivan, Lin Jian-Ming, Usha Bava, Dorit Naot, Jillian Cornish, Andrew Grey
University of Auckland

Imatinib and nilotinib are tyrosine kinase inhibitors (TKIs) used to treat chronic myeloid leukaemia (CML) and gastrointestinal stromal tumors (GIST). Both drugs have been shown to have off-target effects in other tissues including effects on bone cells *in* vitro and on bone metabolism in patients receiving treatment for CML and GIST. *In vitro* both drugs significantly reduce osteoclast development in murine bone marrow cultures, but only imatinib reduces osteoclastogenesis in RAW264.7 cells. In patients, both drugs reduce levels of the bone resorption marker ß-C-terminal telopeptide of type I collagen (ßCTX). Changes in expression and secretion of osteoprotegerin (OPG) from osteoblastic and stromal cells are likely to underlie the indirect effects of TKIs to inhibit osteoclastogenesis, while the mechanism of any direct effect on osteoclastogenesis remains unclear. Inhibition of PDGFRβ has been shown to be the main mechanism by which TKIs affect growth and maturation of osteoblastic cells. In keeping with this, we found that gene silencing of PDGFRB increased OPG expression and production, and this was not the case with gene silencing of PDGFRA or ABL. Furthermore, PDGFBB decreased expression and secretion of OPG in stromal and osteoblastic cells, and abrogated the effects of imatinib and nilotinib to increase these levels. PDGFBB may increase osteoclastogenesis in murine bone marrow culture, but not in RAW264.7 cells. Thus, inhibition of PDGFRβ is a means by which TKIs increase OPG levels and is a mechanism by which they indirectly inhibit osteoclastogenesis.

Tuesday 10 September 2013
Light of the Southern Cross – Icons in Bone Memorial Oration

Brain, Bone, Fat: Unraveling the Role of AP1 Transcription Factors

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Mice in which the expression of ΔFosB, a naturally truncated isoform of FosB that antagonizes AP-1, is driven by the ENO2 promoter in bone, adipose tissues, and the ventral hypothalamus (VHT), develop increased bone mass and energy expenditure , decreased fat mass. These mice also exhibit increased glucose tolerance and insulin sensitivity despite lower levels of leptin. Through crosses with leptin-deficient mice we found that the metabolic phenotype is leptin depenedent but not the bone phenotype.
Interestingly, viral-mediated expression of DFosB in the VHT phenocopies both the bone, energy and glucose metabolism phenotypes, demonstrating the critical role of the VHT and the central link between the regulation of these two metabolic compartments.
To identify the individual AP-1 responsive VHT neuronal circuits that mediate the metabolic, skeletal or both effects we generated Cre-inducible lentiviral vectors that express AP1 antagonists: DFosB, D2DFosB and DNJunD, as well as the AP-1 agonist FosB. The expression of these factors was restricted to specific neuronal types via stereotaxic delivery into the VHT of transgenic mice expressing Cre-recombinase under the control of the neuron-specific promoters, including AgRP-Cre, CART-Cre, NPY-Cre, POMC-Cre and SF1-Cre. Energy expenditure was assessed using calorimetric and monitoring system (CLAMS), whereas bone density was evaluated by micro-CT. Surprisingly, all AP-1 antagonists in either of the neuron-specific expression systems displayed a similar phenotype of high bone mass, lower weight gain and smaller fat pads with smaller adipocytes. Stimulation of AP-1 transcriptional activity via overexpression of full length FosB reversed the metabolic phenotype induced by AP-1 blockade, resulting in contrastingly lower bone mass, energy expenditure and higher fat gain. These findings suggest that bone and energy are at least in part under common neuronal control and these effects are mediated by a central factor common to and/or affecting several hypothalamic neurons
This study demonstrates that: 1) The induction of high energy expenditure, high bone density and improved glucose metabolism by DFosB in the VHT results from its antagonistic activity to AP-1 and 2) Selective inhibition of AP-1 in only one neuron subtype is sufficient to stimulate total body metabolism, decrease fat and increase bone density. These findings suggest that bone and energy are at least in part under common neuronal control and that these effects are mediated by a central factor common to several hypothalamic neurons.

Wednesday 11 September 2013
SESSION 10: Bone Cell Biology

IS15

Osteocyte and PTH

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Osteocytes, the most abundant cells in bone, are ideally positioned to detect and respond to mechanical and hormonal stimuli and to coordinate the function of osteoblasts and osteoclasts. However, evidence supporting the role of osteocytes in specific aspects of skeletal biology has been limited mainly due to the lack of suitable experimental models. Osteocytes express several receptors, including the Parathyroid Hormone (PTH)/PTH-related peptide receptor (PPR). PTH modulates bone turnover and calcium homeostasis by binding and activating the PPR, a G-protein-coupled receptor highly expressed in bone and kidney. Among bone cells, osteocytes express high number of PPRs, however, physiological relevance of receptor signaling in osteocytes remains to be elucidated. Towards this goal, we have generated mice with PPR deletion in osteocytes (Ocy-PPRKO). Skeletal analysis of these mice revealed a significant increase in bone mineral density, and trabecular and cortical bone parameters. Osteoblast activities were reduced in these animals, as demonstrated by decrease Col1α1 mRNA and RANKL expression. Importantly, when subjected to anabolic or catabolic PTH regimen, Ocy-PPRKO animals demonstrated blunted skeletal responses. PTH failed to suppress SOST/Sclerostin or induce RANKL expression in Ocy-PPRKO animals compared to controls. *In vitro* osteoclastogenesis was significantly impaired in Ocy-PPRKO upon PTH administration, indicating that osteocytes control osteoclast formation through a PPR-mediated mechanism. Taken together, these data indicate that PPR signaling in osteocytes is required for bone remodeling and receptor signaling in osteocytes is needed for anabolic and catabolic skeletal responses.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases, part of the National Institutes of Health, under Award Number DK079161 to PDP

IS16

Cell stress, MITF and the control of osteoclast formation

Julian M.W. Quinn
Prince Henry's Institute, Victoria

Osteoclast formation from myelomonocytic progenitors is driven by RANKL, a TNF-related molecule produced in a highly regulated fashion by osteoblasts, osteocytes and lymphocytes. Hormonal regulation of the production of RANKL (and its decoy receptor osteoprotegerin) is critical in controlling osteoclast formation and activity, but there are also numerous factors that act on osteoclast progenitors to influence their responses to RANKL. This is typically evidenced by alterations in activity of those transcription factors, such as NFkB and NFATc1, that respond rapidly to RANKL stimulus.
We have identified a number of cell stress inducing agents that increase osteoclast formation, in particular 17-AAG, a geldanamycin derivative and potential anti-cancer therapeutic. 17-AAG increases both bone loss *in vivo* and osteoclast formation *in vitro* and does so in a manner dependent on transcription factor HSF1, the master controller of cell stress responses. Chemotherapeutic stressors such a doxorubicin and methotrexate similarly increase osteoclast formation. However, these compounds do not affect NFkB and NFATc1, but do increase the levels of transcription factor MITF. The latter is essential for osteoclast formation but is induced in progenitors later than (and is dependent upon) NFATc1 activation. Thus, compounds and pathological processes that cause chronic cell stress may accelerate osteoclast formation and thereby cause bone loss. This work also suggests that MITF regulation may be a significant control point in osteoclast formation.

Wednesday 11 September 2013
SESSION 10: Bone Cell Biology

IS17

Long-chain fatty acids – another player in the fat/bone mass relationship

Jillian Cornish
University of Auckland, New Zealand

Body weight, particularly fat mass, is positively correlated to bone mass. Experiments showing that feeding acutely influences bone turnover, raised the possibility that ingested nutrients have direct bone effects and could function as an additional link between fat and bone. We recently reported that long-chain, saturated fatty acids such as palmitic (C18) and stearic (C16) acids, inhibit osteoclastogenesis *in vitro* in primary murine bone marrow cultures and in RAW264.7 cells. This effect is likely mediated by the fatty acid receptor GPR120, as osteoclastogenesis was also inhibited by the GPR120/40 receptor agonist GW9508.

The potential for therapeutic exploitation of the fatty acids led us to explore two new classes of analogues, modified by ether or by triazole units, and to evaluate their activity in primary murine bone marrow cultures. We found that the triazole analogues were more potent, stable and soluble than palmitic acid.

Long-chain, saturated fatty acids also act on stromal cells and osteoblasts, possibly by signalling through GPR120 and GPR40/41. In primary rat osteoblasts, mitogenesis was stimulated at low concentrations but inhibited at high concentrations, whereas in Kusa4b10 stromal cells, fatty acids stimulated differentiation of both osteoblasts and adipocytes.

This work represents a continuation of a major theme in both our clinical and laboratory research, directed at elucidating the mechanisms by which adipose tissue and intermediary metabolites impact on bone.

IS18

Deciphering the Osteoclastome

Nathan J. Pavlos
Centre for Orthopaedic Research, The University of Western Australia, Crawley, W.A, 6009

Osteoclasts, natures exquisite bone excavators, are multinucleated cells formed by the fusion of mononuclear progenitors of the monocyte-macrophage lineage under the aegis of M-CSF and RANKL. During their RANKL-induced differentiation, osteoclast precursors are bestowed with a unique set of molecular machinery which enables them to fuse, mature and resorb bone. While our understanding of the molecular anatomy of osteoclasts has advanced tremendously over the past decade, we currently lack a holistic and quantitative account of molecules governing osteoclast differentiation, polarisation and function. In recent years, quantitative mass spectrometry has matured as an attractive technology for in-depth characterisation of the protein components of biological systems. By combining chemical labelling (isobaric tag for relative and absolute quantitation: iTRAQ) with high-resolution mass spectrometry (LC-MS/MS) we have quantitatively surveyed the total cellular proteome of the osteoclast (Osteoclastome). Using this global and unbiased approach, we have identified a high confidence list of 1352 proteins, 1193 of which were quantifiable. Among these, 699 were differentially expressed (253/446 up/down-regulated) during the RANKL-mediated progression and fusion of precursor cells into functionally mature osteoclasts. Hierarchical clustering identified several proteins which showed distinct expression patterns throughout osteoclast differentiation, including established osteoclast markers (e.g. TRACP and V-ATPases) and others whose function(s) have yet to be assigned to the osteoclast differentiation/bone-resorption-cycle. Recent progress towards integrating these proteins into a coherent and quantitative map of the osteoclast proteome will be discussed, the elucidation of which may provide a valuable resource for the future identification of therapeutic targets for bone diseases.

Wednesday 11 September 2013
SESSION 11: Vitamins and Bone

IS19

Vitamin D and the Skeleton: The Paradox of a Long Standing Paradigm

Clifford J Rosen
Professor of Medicine Tufts University School of Medicine, USA

Vitamin D is a hormone and nutrient that has been associated with musculoskeletal disorders ranging from distinct forms of rickets to osteoporosis and hip fractures. The principal metabolite of vitamin D is 1,25 dihydroxyvitamin and it modulates calcium balance via the intestine. In vitro vitamin D has important actions on cells in the skeletal remodeling unit, including suppressed mineralization and increased osteoclastogenesis.
This is a paradox and counter to one of the longest standing paradigm in osteoporosis medicine; i.e. that vitamin D supplementation is necessary for optimal treatment of osteoporosis. In addition, the relationship of serum 25OHD levels to fracture risk remain problematic. Finally, the direct actions of vitamin D on muscle are controversial and difficult to distinguish from its effects on calcium absorption and balance. One potential skeletal target that was previously ignored is the osteocyte and its actions on both osteoclastogenesis and osteoblastogenesis. Moreover there is compelling evidence that osteocytes secrete FGF-23 which then directly regulates 1 alpha hydroxylase and thereby impacts both calcium and phosphorus metabolism. This talk will explore some new concepts in vitamin D actions on the skeleton including recent studies on microstructure in vitamin D deficiency and the role of the osteocyte in vitamin D actions.

IS20

What is the optimal 25-Hydroxyvitamin D [25(OH)D] level?

Ian Reid
University of Auckland, New Zealand

Low 25(OH)D has been associated with numerous adverse health outcomes. Some have interpreted this *association* as *causation*, and advocated the maintenance of high levels of 25(OH)D, with a view to reducing frequency of these adverse health outcomes. Meta-regression of randomised trials of vitamin D (with or without calcium) has also suggested that trials that achieve higher levels of 25(OH)D are associated with fracture risk reduction. These considerations have led to the progressive increase in the lower end of the reference range for serum 25(OH)D, contributing to the "epidemic" of vitamin D deficiency.
Another way to determine the optimum 25(OH)D level is to assess the effects of vitamin D supplementation on BMD, in relation to baseline levels of 25(OH)D. To this end, we have performed a systematic review of randomised trials of vitamin D monotherapy, which reported BMD. 23 studies, mean duration 23.5 months, comprising 4082 participants, 92% women, average age 59 years, met the inclusion criteria. Mean baseline serum 25-hydroxyvitamin D was <50 nmol/L in 8 studies (1791 participants). Only one study found benefit at >1 site. Meta-analysis showed a small benefit at the femoral neck (0.8%, 95%CI 0.2, 1.4) with heterogeneity among trials. There was no effect at any other site, including the total hip. There was evidence of a bias toward positive results at the femoral neck and total hip. There was no interaction between baseline 25(OH)D and the change in BMD, suggesting that a 25(OH)D as low as 40 nmol/L might have no negative impact on BMD.

Wednesday 11 September 2013
SESSION 11: Vitamins and Bone

IS21

Photoprotection by vitamin D compounds

Rebecca Mason
University of Sydney, NSW

The production of vitamin D in skin from 7-dehydrocholesterol by UVB is well known. Less well appreciated is that vitamin D is metabolized in skin to the active hormone, 1,25dihydroxyvitamin D (1,25D), as well as other metabolites, and appears to have important actions locally, including protection from UV-induced DNA damage and photocarcinogenesis. Topical application of 1,25D and other analogs reduce several types of UV-induced DNA damage in mice and human subjects. The mechanism of the photoprotective effect is not fully understood, but enhanced expression of p53 in the presence of D compounds, which would facilitate DNA repair, and reduced production of reactive nitrogen species, which would otherwise inhibit repair, may contribute. We have recent evidence that expression of key DNA repair proteins are enhanced with 1,25D. Analog studies as well as experiments with variant vitamin D receptors and knockout of ERp57 protein, indicate that both vitamin D receptor and ERp57 are required for photoprotection, but results are consistent with a non-genomic mechanism. The studies to date support the hypothesis that the vitamin D system in skin contributes to photo-adaptation and suggest that vitamin D compounds may be usefully incorporated into a topical application such as a sunscreen or after sun lotion to reduce DNA damage.

Plenary Poster

PLENARY POSTER PRESENTATIONS

Plenary Poster P1
Knockdown of PTH/PTHrP receptor PTH1R in osteosarcoma cells decreases invasion and increases mineralization *in vivo*
Patricia WM Ho, Megan R Russell, Ankita Goradia, Blessing Crimeen-Irwin, Pece Kocovski, Alvin JM Ng, Alistair M Chalk, Kristi Milley, Janine A Danks, John L Slavin, Ross A Dickins, T John Martin, Carl R Walkley

Plenary Poster P2
Down-regulation of GLI2 prevents metastasis of osteosarcoma via regulation of ribosomal protein S3
Takao Setoguchi, Setsuro Komiya

Plenary Poster P3
OSMR-/- mice show reduced synovial inflammation and cartilage damage, but increased bone loss in response to joint injury
Brett Tonkin, Natasha Jansz, Joshua Johnson, Evange Romas, Natalie Sims, Nicole Walsh

Plenary Poster P4
Assessing not only bone loss but also soft tissue swelling in a murine inflammatory arthritis model: a novel 3D micro-CT method
Egon Perilli, Melissa D Cantley, Victor Marino, Tania N Crotti, Malcolm D Smith, David R Haynes, Anak SSK Dharmapatni

Plenary Poster P5
Androgen action directly via the androgen receptor in osteoblasts is dependent on the stage of osteoblast maturation
Rachel Davey, Patricia Russell, Michele Clarke, Kristine Wiren, Jeffrey Zajac

Plenary Poster P6
Bidirectional regulation of osteoclast formation by ephrinB2/EphB4 signaling in the osteoblast lineage
Stephen Tonna, Patricia W. M Ho, Farzin M Takyar, Carl R Walkley, T John Martin, Natalie A Sims

Plenary Poster P7
Completing the evaluation of the skeletal regulation by the NPY system: the Y5 receptor
Ee Cheng Khor, Yan-Chuan Shi, Ronaldo Enriquez, Herbert Herzog, Paul Baldock

Plenary Poster P8 / Christopher and Margie Nordin Young Investigator Poster Award Finalist
SQSTM1/p62 mutant proteins associated with Paget's disease of bone lead to increased autophagy markers, while attenuating autophagosome maturation
Sarah Rea, Melanie Sultana, Nathan Pavlos, Robert Layfield, Jiake Xu, John Walsh, Thomas Ratajczak

Plenary Poster P9
Spatial control of bone formation using a porous polymer implant
Nicole Yu, Marie Gdalevitch, Aaron Schindeler, Ciara Murphy, Kathy Mikulec, Lauren Peacock, Jane Fitzpatrick, Justin Cooper-White, Andrew Ruys, David Little

Plenary Poster P10
Changes in proximal femur structure with age: A cross-sectional study of 719 Caucasian females aged between 20 and 89 years
Benjamin Khoo, Keenan Brown, Christopher Cann, Richard Prince

Plenary Poster P11
Effects of individualised bone density feedback and educational interventions on osteoporosis knowledge and self-efficacy in young women: a 12-yr prospective study
Feitong Wu, Laura Laslett, Karen Wills, Brian Oldenburg, Graeme Jones, Tania Winzenberg

Plenary Poster P12
Two novel mutations in the osteoprotegerin encoding gene, *TNFRSF11B*, in patients with juvenile Paget's disease
Ally Choi, Dorit Naot, Pelin O Simsek Kiper O, Linda Di Meglie, Tim Cundy

Plenary Poster P13
Parental socioeconomic status and childhood fractures: data from the Geelong Osteoporosis Study Fracture Grid
Natalie K Hyde, Julie A Pasco, Sharon L Brennan

Plenary Poster P14

Selective serotonin reuptake inhibitors (SSRIs) decrease serum markers of bone turnover: Geelong Osteoporosis Study

Lana J Williams, Michael Berk, Jason M Hodge, Mark A Kotowicz, Fiona Collier, Julie A Pasco

Plenary Poster P15

Comparative bone densitometry and anthropometry of the Indian and Nigerian female students, graduated in Ukrainian Medical University

Lubov Stklyanina, Vladyslav Luzin, Helen Nuzna, Vasily Tarasov

Plenary Poster P16

Prevention of aromatase inhibitor-induced bone loss with alendronate in postmenopausal women: The Batman Trial

Anna Lomax, Saw Yee Yap, Karen White, Jane Beith, Ehtesham Abdi, Adam Broad, Sanjeev Sewak, Chooi Lee, Philip Sambrook, Nick Pocock, Margaret Henry, Elaine Yeow, Richard Bell

Plenary Poster P17

Changes in bone mineral density related to incidence of fracture with 6 years of denosumab treatment for postmenopausal osteoporosis

Miller PD, Cummings SR, Reginster JY, Franchimont N, Bianchi G, Bolognese MA, Chapurlat R, Hawkins FG, Kendler DL, Oliveri B, Zanchetta JR, Daizadeh N, Wang A, Wagman R, Papapoulos S

Plenary Poster P18 / Christopher and Margie Nordin Young Investigator Poster Award Finalist

Predictors of refracture in patients managed within a Fracture Liaison Service: A 7-year prospective study

Kirtan Ganda, Markus Seibel

Plenary Poster P1

Knockdown of PTH/PTHrP receptor PTH1R in osteosarcoma cells decreases invasion and increases mineralization *in vivo*

Patricia WM Ho[1], Megan R Russell[1], Ankita Goradia[1], Blessing Crimeen-Irwin[2], Pece Kocovski[1], Alvin JM Ng[1], Alistair M Chalk[1], Kristi Milley[3], Janine A Danks[3], John L Slavin[4], Ross A Dickins[5], T John Martin[1], Carl R Walkley[1]

[1]St Vincent's Institute, Vic, Australia, [2]St Vincent's Institute, [3]Rmit, Vic, Australia, [4]Department of Pathology, St Vincent's Hospital, Vic, Australia, [5]Molecular Medicine Division, Walter and Eliza Hall Institute of Medical Research, Vic, Australia

Osteosarcoma (OS) established in the mouse through Cre:lox based on deletion of *Trp53 (p53)* and *Rb* in osteoblasts are providing a valuable model of human OS. The tumours express the receptor for PTH/PTHrP (PTHR1) linked to adenylyl cyclase activation. After knocking down PTHR1 by >80 % using shRNA we studied *in vivo* tumour growth by explanting OS cells (vector control) in one flank of nude mice and OS (shRNA PTHR1) in the other. After 4 weeks' growth *in vivo* PTH1R knock down resulted in 60% reduction in tumour size, increased mineralization and decreased tumour invasiveness. mRNA for RANKL and CyclinD1 were greatly reduced, while OPG, PHEX and MGP mRNA were significantly increased in PTH1R knockdown tumour. We investigated the possibility that receptor blockade was inhibiting the autocrine /paracrine effects of PTHrP.

OS cell culture conditioned medium had no detectable PTHrP using an N-terminal radioimmunoassay with sensitivity of 2pM, however PTHrP mRNA was readily detectable in both PTH1R knockdown and vector control. Confocal microscopy showed positive staining for PTHrP in nucleus and cytoplasm with far less PTHrP in PTH1R knockdown than in vector control cells. Knocking down PTHrP in cultured OS cells by siRNA or shRNA resulted in decrease of RANKL and increase of OPG mRNA expression. Our results suggest that PTHrP in osteosarcoma is acting in an autocrine / paracrine manner that requires participation of the receptor. *In vivo* study to assess the ability of neutralizing anti-PTHrP monoclonal antibody to recapitulate the effects of receptor knockdown is under way.

Plenary Poster P2

Down-regulation of GLI2 Prevents metastasis of Osteosarcoma via regulation of Ribosomal Protein S3

Takao Setoguchi[1], Setsuro Komiya[2]

[1]The Near-Future Locomotor Organ Medicine Creation Course, Graduate School of Medical and Dental Sciences, Kagoshima University, [2]Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Japan

Aberrant activation of the Hedgehog pathway has been reported in several malignancies. We previously reported that knockdown of SNO or *GLI2* inhibits proliferation of osteosarcoma cells by regulating the cell cycle. In this study, we examined the molecular mechanisms of *GLI2*-mediated migration and invasion of osteosarcoma cells. Immunohistochemical studies showed that GLI2 was over-expressed in osteosarcoma patient specimens. RNAi knockdown of *GLI2* inhibited migration and invasion of osteosarcoma cells. In contrast, the forced expression of constitutively active *GLI2* in mesenchymal stem cells up-regulated this migration and invasion. In addition, xenograft models showed that knockdown of *GLI2* prevented lung metastasis of osteosarcoma. To examine the molecular mechanisms of GLI2-mediated osteosarcoma metastasis, we performed microarray analysis. The gene encoding ribosomal protein S3 (RPS3) was identified as a target of GLI2. Knockdown of RPS3 decreased migration and invasion of osteosarcoma cells, whereas forced expression of RPS3 up-regulated the invasion. Our findings suggest that RPS3 is a novel downstream factor of *GLI2* and is involved in osteosarcoma metastasis. Furthermore, inhibiting GLI2 transcription by RNAi or low molecular weight compound may be an effective therapeutic method for preventing osteosarcoma metastasis.

Plenary Poster P3

OSMR-/- mice show reduced synovial inflammation and cartilage damage, but increased bone loss in response to joint injury

Brett Tonkin[1], Natasha Jansz[1], Joshua Johnson[1], Evange Romas[2], Natalie Sims[1], Nicole Walsh[1]
[1]St Vincent's Institute of Medical Research, [2]Dept of Rheumatology, St Vincent's Hospital

Regardless of the inciting event, mild inflammation within the synovium, progressive loss of articular cartilage, osteophyte formation and accrual of subchondral bone are characteristic features of osteoarthritis (OA). The cytokine oncostatin M (OSM) is found at elevated levels in human OA synovial fluid and is a candidate for contributing to OA pathogenesis. In synovial fibroblasts OSM, signaling via its ligand-speciifc receptor, OSMR, induces expression of pro-inflammatory cytokines such as IL-6, cartilage degrading enzymes and the pro-osteoclastic factor, RANKL. We hypothesized that, mice lacking expression of OSMR ($OSMR^{-/-}$ mice) may be protected from injury-induced osteoarthritic-joint damage.
12-week old male $OSMR^{-/-}$ mice and their wildtype littermates ($OSMR^{+/+}$) underwent surgical transection of the medial meniscotibial ligament (DMM) or sham surgery on the right knee. At 8 weeks post-surgery, micro-CT analyses demonstrated a focal increase in medial subchondral bone (BV/TV) in DMM tibiae compared to sham tibiae in $OSMR^{+/+}$ mice. In contrast, $OSMR^{-/-}$ mice showed reduced BV//TV at this site in DMM tibiae compared to sham tibiae ($p<0.01$). Furthermore, tibial metaphyseal trabecular BV/TV was also reduced in $OSMR^{-/-}$ DMM tibiae compared to sham tibiae ($p<0.01$); this was not observed in $OSMR^{+/+}$ mice. Histologic scoring revealed that $OSMR^{/-}$ mice were protected from DMM-induced articular cartilage damage ($p<0.05$) and show reduced synovial inflammation ($p<0.05$) when compared to $OSMR^{+/+}$ mice.
Together our data indicate that OSM signalling via OSMR likely contributes to synovial inflammation and cartilage destruction in response to joint injury and may also be critical in limiting bone loss under these conditions.

Plenary Poster P4

Assessing not only bone loss but also soft tissue swelling in a murine inflammatory arthritis model: a novel 3D micro-CT method

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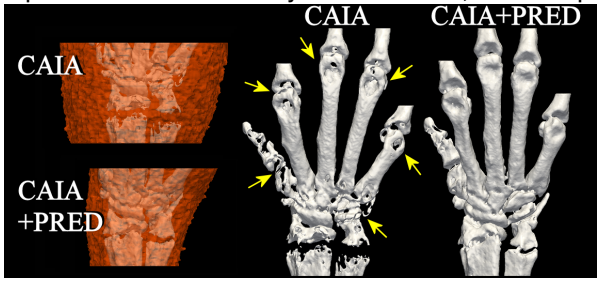
Rheumatoid arthritis (RA) is a destructive inflammatory joint disease. Currently, non-invasive assessments of disease severity in the rodent models of arthritis such as paw-thickness or clinical score grades, evaluate the edema, but neglect the underlying synovial proliferation. To quantify bone erosion, recent studies have utilised micro-CT.
We demonstrate a novel 3D micro-CT analysis method capable of detecting, and measuring, not only paw joint bone erosion, but also soft tissue swelling.
Balb/C mice were divided into two groups: Collagen antibody-induced arthritis (CAIA) (n=10), and CAIA treated with Prednisolone (10mg/kg/day) group (n=12). The latter served for comparison to an established RA-treatment. Clinical paw scores were recorded daily (0=normal, 15=severe). After sacrifice (day 10), front paws were examined by micro-CT (Skyscan 1076) and histology. Micro-CT measurements were paw volume and bone volume done at the radiocarpal joint, and from radiocarpal joint to the proximal phalanx.
Micro-CT showed significantly increased paw volume (+55%, $p<0.01$) and decreased bone volume (-15%, $p<0.05$) in CAIA mice, compared to Prednisolone-treated CAIA mice (Fig.1). Clinical scores were significantly higher in untreated CAIA mice (6.1±3.7 vs. 0.7±0.8, $p<0.001$). Paw volume and bone volume assessed by micro-CT correlated significantly with clinical scores and histological scores ($r>0.5$, $p<0.05$, for all). Consistent with this, CAIA mice exhibited higher histological scores for inflammation ($p<0.01$), cartilage and bone degradation ($p<0.01$), and pannus formation ($p<0.01$) than those treated with Prednisolone.
The novel 3D micro-CT analysis protocol presented is capable to detect not only bone erosion, but also paw swelling, in a murine model of inflammatory arthritis.



Plenary Poster P5

Androgen Action Directly via the Androgen Receptor in Osteoblasts is Dependent on the Stage of Osteoblast Maturation

Rachel Davey[1], Patricia Russell[1], Michele Clarke[1], Kristine Wiren[2], Jeffrey Zajac[1]
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Androgen action via the androgen receptor (AR) is essential for bone growth and accrual during puberty and for bone maintenance post-puberty in males. To determine the relative contribution of these actions of androgens *via* the AR in osteoblasts at different stages of their maturation, we are characterising the skeletal phenotype of two novel mouse lines we have generated in which we have replaced the AR specifically in osteoblasts of global-ARKO mice at either the 1) proliferative (pOBLAR:ARKO) or 2) mineralisation (mOBLAR:ARKO) stage of osteoblast maturation.
Global deletion of the AR in mice (global-ARKOs) results in bones of reduced size, thickness and volume compared to control males. Replacement of the AR in proliferating, but not mineralising osteoblasts of global-ARKOs restored bone size as measured by periosteal circumference at 6 weeks of age in pOBLAR:ARKO and mOBLAR:ARKOs, respectively. Trabecular bone volume was fully restored in pOBLAR:ARKOs to levels observed in WT males, whilst restoration of BV/TV was only partial in mOBLAR:ARKOs. Trabecular number was increased in pOBLAR:ARKO and mOBLAR:ARKOs compared to global-ARKOs (P<0.05), suggesting an inhibitory effect of androgens on bone resorption *via* the AR on osteoblasts. Consistent with this hypothesis, preliminary analyses indicate lower mean values of osteoclast number and cathepsin K mRNA expression in bones of pOBLAR:ARKOs and mOBLAR:ARKOs compared to global-ARKOs.
In conclusion, androgen action *via* the AR to increase bone size during growth is mediated *via* proliferating but not mineralizing osteoblasts, whilst trabecular bone accrual during growth is mediated *via* androgen action in both proliferating and mineralising osteoblasts.

Plenary Poster P6

Bidirectional regulation of osteoclast formation by EphrinB2/EphB4 signaling in the osteoblast lineage

Stephen Tonna, Patricia W. M Ho, Farzin M Takyar, Carl R Walkley, T John Martin, Natalie A Sims
St Vincent's Institute of Medicine

Both ephrinB2 and its receptor EphB4 are capable of intercellular signaling in osteoblasts while only ephrinB2 signals in osteoclasts. We have previously reported that blockade of both ephrinB2/EphB4 with sEphB4 promoted osteoclast formation by increasing osteoblastic RANKL production, while others have reported that ephrinB2 signaling in osteoclasts inhibits osteoclast differentiation. To determine whether increased osteoclast formation is due to reduced ephrinB2 reverse signaling in the osteoclast or EphB4 forward signaling in osteoblasts, we conditionally deleted ephrinB2 from each lineage *in vivo* using CtskCre or Osx1Cre respectively, and knocked down EphB4 from osteoblasts *in vitro* with short hairpin RNAs (shRNAs).
sEphB4 increased osteoclast formation to the same extent from both Ctsk.CreEfnB2f/f and Ctsk.CreEfnB2w/w bone marrow precursors in co-culture with wild-type osteoblasts. This indicates that pro-osteoclastic action of sEphB4 does not result from blocking ephrinB2 reverse signaling in osteoclasts.
In both neonate and adult Osx1Cre.EfnB2f/f mice, osteoclast numbers were significantly lower than in Osx1.Cre.EfnB2w/w controls. Consistent with this, Osx1Cre.EfnB2f/f calvarial osteoblasts expressed 50% less RANKL mRNA than Osx1Cre.EfnB2w/w cells, while OPG mRNA levels were retained. These osteoblasts also demonstrated significantly reduced support of osteoclast formation when co-cultured with wild-type bone marrow, the opposite effect to that observed with sEphB4 treatment. In addition, Kusa4b10 osteoblasts that expressed EphB4 shRNA demonstrated a >70% reduction in EphB4 and had higher mRNA levels of RANKL compared to control.
This data indicates that EphB4 forward signaling within osteoblasts restrains osteoclast formation by limiting osteoblastic RANKL production, while ephrinB2 reverse signaling in osteoblasts promotes RANKL production.

Plenary Poster P7

Completing the evaluation of the skeletal regulation by the NPY system: The Y5 receptor

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It has been established that the Neuropeptide Y (NPY) system is a novel regulatory pathway in bone homeostasis. Through gene knockout mouse studies, NPY receptors Y1, Y2, Y4 and y6 have been previously investigated for their role in bone homeostasis. Y1 and Y2 receptors have been identified as negative regulators of bone formation through central and peripheral networks, while y6 inhibits bone resorption. This first analysis of Y5R knockout mice completes the characterisation of NPY system in bone. Y5R-deficient mice display a reduction in cancellous bone volume(26%) and isolated femur BMD compared to WT. Histomorphometric analysis revealed an increase in osteoclast number and osteoclast surface. In addition, Y5R knockout mice displayed an increase in mineral apposition rate with no change in mineralising surface, demonstrating increased bone turnover favouring bone loss.
The Y5R and Y1R genes are transcribed in opposing DNA strands under a common promoter, which suggests that their gene expression can regulate each other to coordinate NPY signalling in bone homeostasis. However, Y1R deficient mice display a contrasting high bone volume phenotype resulting from direct NPY signalling in the osteoblastic lineage. Thus Y5R may signal through a different/opposing pathway to Y1R.
NPY signalling through the Y5 receptor represents a novel signalling axis in bone, regulating both osteoclastic and osteoblastic lineages. Y5 signalling may represent a counter-balancing pathway to the well-studied direct Y1R signalling in osteoblasts. In this manner, NPY signalling through Y5R may be modulated by local Y1R signalling.

Plenary Poster P8

SQSTM1/p62 mutant proteins associated with Paget's disease of bone lead to increased autophagy markers, while attenuating autophagosome maturation

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Background: Mutations in the SQSTM1/p62 gene are associated with Paget's disease of bone. The SQSTM1/p62 protein is considered to be a signalling hub linking NF-kB signalling, oxidative stress signalling (Keap1/Nrf2), autophagy and apoptosis. We have previously shown that over-expression of certain mutant SQSTM1/p62 proteins leads to hyperactivation of NF-kB and increased osteoclastogenesis, but the underlying mechanisms are largely unclear.
Methods: We have used luciferase assays to determine the effect of 7 mutant p62 proteins on NF-kB activation in non-stimulated cells. We conducted co-immunoprecipitation experiments, where HEK293 cells were transfected with expression plasmids for FLAG-p62 (wild type or mutant) or empty vector. p62 proteins were immuno-precipitated with FLAG antibody and the bound LC3 detected by Western blot analysis. Additionally, we have used a previously validated system to determine the effect of these mutations on autophagosome maturation into protein degrading autophagolysosomes; we transfected a cell line stably expressing mCherry-GFP-LC3 with p62 expression constructs and using confocal microscopy determined the ratio of autophagosomes and autophagolysosomes formed in cells expressing mutant or wild type p62.
Results and Conclusions: Our data shows that SQSTM1/p62 mutant protein expression correlates significantly increased NF-kB activity with increased physical interaction between p62 and the autophagy marker LC3. However, mutant proteins also attenuate autophagosome maturation. By contrast, expression of wild type SQSTM1/p62 promotes autophagosome maturation. Together, our data suggests that SQSTM1/p62 mutant proteins are defective mediators of the final stages of autophagy and this may be important for NF-kB regulation via perturbed degradation of specific protein substrates.

Plenary Poster P9

Spatial control of bone formation using a porous polymer implant

Nicole Yu[1], Marie Gdalevitch[1], Aaron Schindeler[1], Ciara Murphy[1], Kathy Mikulec[1], Lauren Peacock[1], Jane Fitzpatrick[2], Justin Cooper-White[2], Andrew Ruys[3], David Little[1]

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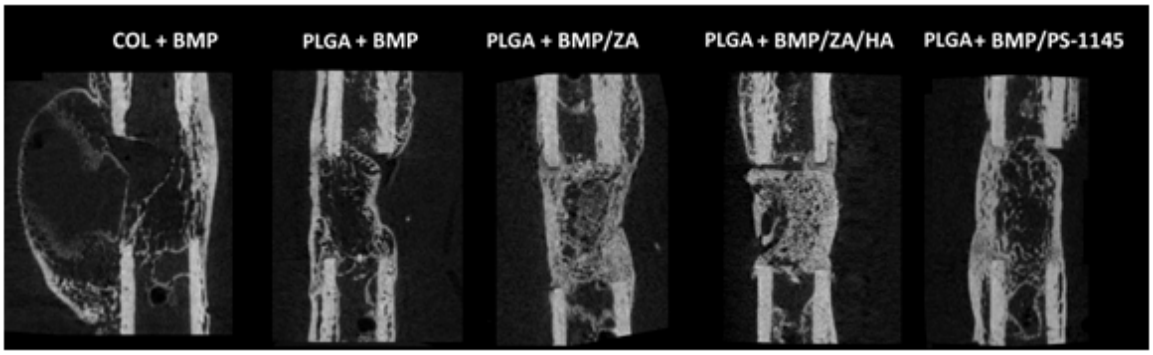
Current clinical delivery of recombinant human bone morphogenetic proteins (rhBMPs) utilises freeze-dried collagen (COL). Despite effective new bone generation, COL + rhBMP can be limited by inflammation and uncontrolled bone formation that can lead to significant complications. This study aimed to produce an alternative rhBMP local delivery system to permit superior and more controllable rhBMP-induced bone formation.

Cylindrical implants (3mm diameter × 5mm height) manufactured from porous poly(lactic-co-glycolic acid) (PLGA) were inserted into rat femoral critical-sized defects (6mm). Implants were encapsulated with anabolic rhBMP-2 ± anti-resorptive agents. Anti-resorptive strategies included zoledronic acid (5μg ZA), ZA pre-adsorbed onto hydroxyapatite microparticles, (5μg ZA/2% HA) or IKK inhibitor (10μg PS-1145). The regenerate region was examined at 6 weeks by 3D microCT and descriptive histology.

3D reconstruction and histological examination revealed rhBMP-2 induced bone was more restricted in the PLGA implants vs. COL scaffolds. 3D bone volume (mm^3) of the regenerate region was higher in PLGA + rhBMP-2/ZA/HA vs. COL + rhBMP-2 (+36.8%, p=0.03). Elution analyses showed HA addition to delay ZA release without altering rhBMP-2 elution. Mechanistically studies using MG-63 human osteoblast-like cells showed cellular invasion and proliferation within PLGA implants (days 3 and 7).

In conclusion, these porous PLGA implants enabled superior spatial control of rhBMP-induced bone formation over clinically-used COL. The PLGA implant has the potential to avoid uncontrollable bone formation-related safety issues and to customise bone shape by implant design modification. Moreover, local treatment with anti-catabolics further augmented rhBMP-induced bone formation.



Plenary Poster P10

Changes in proximal femur structure with age: A cross-sectional study of 719 Caucasian females aged between 20 and 89 years

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Introduction: The use of areal bone mineral density, although useful for fracture prediction, obscures changes in trabecular and cortical mass and volume over the life span.
Objective: A cross-sectional study of changes in three dimensional proximal femur structures in female Caucasians aged 20 to 89 years old.
Methods: 719 QCT scans of the proximal femur of females were obtained and analysed using software that separates cortical and trabecular bone by a thresholding technique applied to each scan. The mineral mass and volume was then computed for the integral, trabecular and cortical segments of the regions analysed and modelled using linear or bilinear models.
Results: Integral volume at the femoral neck (FN), trochanter (TR) and intertrochanter (IT) sites each expanded linearly from 20-89y by between 18 and 37% as did trabecular volume by between 65 and 79%. FN cortical volume and mass decreased linearly by 43 and 45% between 20-89y but at both the TR and IT sites cortical volume and mass were constant until menopause, and decreased linearly thereafter.
Conclusions: At all sites periosteal and endosteal expansion is large and linear from age 20, cortical bone shows contraction with evidence of both menopause and age effects at the TR and IT sites but not the FN which contracts linearly from age 20.

Plenary Poster P11

Effects of individualised bone density feedback and educational interventions on osteoporosis knowledge and self-efficacy in young women: a 12-yr prospective study

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Aims To evaluate the long-term effects of bone density feedback and osteoporosis education on osteoporosis knowledge and self-efficacy.
Methods This is a 12-year follow-up of a previous randomised controlled trial, examining the effects of feedback of bone density-defined fracture risk [high (T-score<0) vs. normal (T-score≥0) risk] and two different education interventions [group-based behavioural education (OPSMC) vs. an osteoporosis leaflet] on osteoporosis knowledge and self-efficacy in women aged 25-44.
Results Of 470 participants at baseline, 74% (N=347) were retained over 12-years. Women lost to follow up were younger (36.3 vs. 38.3 years) and more likely to be smokers (30 vs. 12%) and single (35 vs. 24%). Between group differences in knowledge change at 2 years were not sustained at 12 years regardless of T-score group (1.6 vs. 1.1, p=0.14) or educational intervention (1.4 vs. 1.3, p=0.48). Linear regression showed women in households with an unemployed main financial provider had less increase in knowledge over 12-years (β=-1.64, 95% CI =-3.24 to -0.04). Neither T-score group nor educational intervention predicted change in osteoporosis self-efficacy throughout 12 years. Associations between self-efficacy and number of children and employment status of mother observed at 2 years were no longer apparent after 12 years.
Conclusions Beneficial effects of both the OPSMC and feedback of high fracture risk on osteoporosis knowledge over 2-years were not sustained after 12-years. Neither intervention changed osteoporosis self-efficacy at 2 or 12 years. Thus, more frequent osteoporosis education and bone density feedback is necessary to maintain knowledge, and other interventions to improve self-efficacy need to be explored.

Plenary Poster P12

Two novel mutations in the osteoprotegerin encoding gene, *TNFRSF11B*, in patients with juvenile Paget's disease

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Juvenile Paget's disease (JPD) is a rare genetic bone disorder characterised by progressive skeletal deformities, fractures and deafness. JPD is caused by recessive mutations in *TNFRSF11B,* the gene encoding osteoprotegerin (OPG). So far, about 10 different OPG mutations have been identified in JPD patients. In the current study, the *TNFRSF11B* gene was sequenced in two patients with JPD symptoms in order to determine if they carried OPG mutations.

Case 1 was a 27 year old man with a history of numerous fractures, bone pain and hearing loss. Case 2, a 9 months old male infant, had bone deformity and very high levels of alkaline phosphatase (1692 U/L). DNA from the patients was amplified with primers for intronic regions flanking the five exons of *TNFRSF11B*, and PCR products were sequenced on a 3130xl Genetic Analyzer (Life Technologies).

Patient 1 carried a homozygous missense mutation A>C in exon 2, producing a Threonine to Proline substitution at position 76 (T76P). In patient 2, only exon 1 could be amplified while exons 2 - 5 were missing, indicating a large deletion in the chromosomal region containing *TNFRSF11B*. Mapping the exact boundaries of the deletion is still underway. In the patients described here, the greater genetic aberration produced a more severe phenotype, with diagnosis at a very early age. The two novel mutations provide further evidence for the association of OPG with Juvenile Paget's disease.

Plenary Poster P13

Parental socioeconomic status and childhood fractures: Data from the Geelong Osteoporosis Study Fracture Grid

Natalie K Hyde[1], Julie A Pasco[2], Sharon L Brennan[3]
[1]School of Medicine, Deakin University, [2]School of Medicine, Deakin University; Northwest Academic Centre, the University of Melbourne, [3]School of Medicine, Deakin University; Northwest Academic Centre, the University of Melbourne; Australian Institute for Musculoskeletal Sciences (Aimms), the University of Melbourne

Objective: To examine associations between parental socioeconomic status (SES) and all cause fracture in children aged 10-15 years.
Methods: All cause incident fractures that occurred between 2006-07 were identified using a computerised keyword search of all radiological reports from radiological centres serving the Barwon Statistical Division (BSD), Victoria (n=1,227, 31.1% female). Fracture incidence across SES quintiles was calculated by using all children residing in the BSD aged between 10-15 years as the population at risk (n= 18,773, 47.9% female), assuming equal proportions in each quintile. Parental SES was determined by cross-referencing the patients' residential address at the time of fracture with Australian Bureau of Statistics 2006 Census data. SES was categorised into quintiles based on the BSD reference ranges with quintile 1 being the most disadvantaged group. Subset analyses examined site specific fracture whereby fractures were categorised as; lower limbs (n=40), upper limbs (n=570), fingers and/or hands (n=284) and upper body (n=671).
Results: Of the population at-risk, 1,277 sustained at least one fracture (6.8%) during the 2 year study period. After age-adjustment, no socioeconomic trend in fracture (all sites combined) was observed (Figure). In the subset analyses we observed an inverse association between finger and/or hand fractures and parental SES. No further patterns between site-specific fracture were observed.
Conclusion: In this population of children no associations between parental SES and all-cause fracture were observed, with the exception of an inverse association between lower parental SES and an increase in fractures of the children's hand and/or fingers.

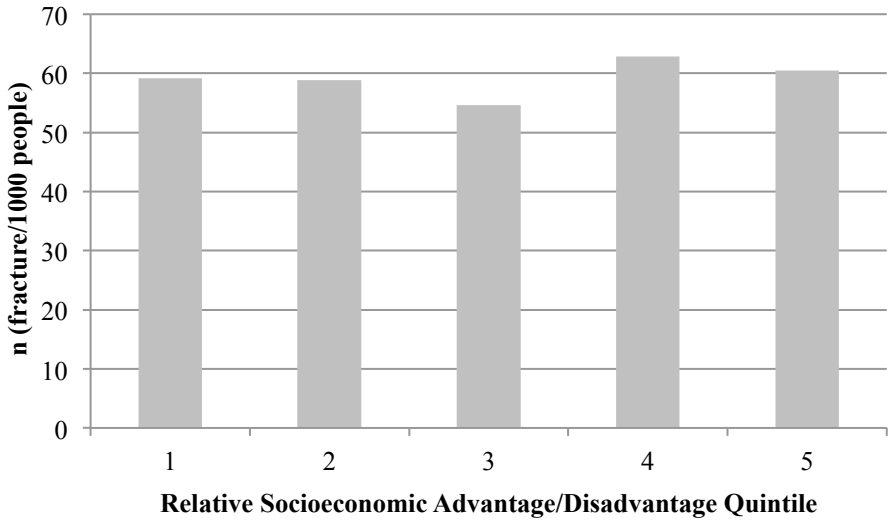

Figure: Parental socioeconomic status (quintile 1 is the most disadvantaged) and incident fracture (all sites combined) in children residing in the Barwon Statistical Division.

Plenary Poster P14

Selective serotonin reuptake inhibitors (SSRIs) decrease serum markers of bone turnover: Geelong Osteoporosis Study

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Aim: The aim of this study was to investigate the association between selective serotonin reuptake inhibitors (SSRIs) and serum markers of bone turnover in a population-based sample of men (n=1139; 20-96yr) participating in the Geelong Osteoporosis Study.
Methods: Serum bone resorption marker, C-telopeptide (CTx) and formation marker, type 1 procollagen amino-terminal-propeptide (P1NP) were measured using the automated Roche Modular Analytics E170 analyser [CTx, detection limit 10ng/L, inter-assay coefficient of variation (CV) <3.8%; P1NP, 5μg/L, <4.9%]. Anthropometric measurements and socio-economic status (SES) were determined and information on medications, depression and lifestyle was obtained via questionnaire. After transformation, CTx and P1NP were included in linear regression models, adjusted for age, weight and dietary calcium.
Results: Thirty-seven (3.3%) men reported using SSRIs. Age was an effect modifier in the association between SSRIs and bone turnover markers. Among younger men (20-60yr; n=557), SSRIs were associated with lower adjusted mean CTx [278.9 (95%CI 213.2-353.4) vs 364.8 (95%CI 349.7-379.4) ng/L, p=0.03] and P1NP [31.4 (95%CI 24.0-39.7) vs 41.0 (95%CI 39.7-43.6) μg/L, p=0.02] compared to non-users. No differences in SSRIs and markers of bone turnover were detected among older men (61-94yr; all p>0.05). These patterns were sustained after further adjustment for activity, alcohol, smoking, SES, depression, medications effecting bone and calcium metabolism and other antidepressants.
Conclusion: Our data suggest that SSRI use is associated with a reduction in serum CTx and P1NP in younger men. The observed decreases are likely to contribute to reduced bone turnover state as seen on bone histomorphometry in men with idiopathic osteoporosis.

Plenary Poster P15

Comparative bone densitometry and anthropometry of the Indian and Nigerian female students, graduated in Ukrainian Medical University

Lubov Stklyanina, Vladyslav Luzin, Helen Nuzna, Vasily Tarasov
Lugansk State Medical University

Objectives: to find out is there are any correlations between the body composition and bone mineral mass exist in young female of the different ethno-geographical groups
Materials and methods: The routine anthropometric procedure (weight, mid-arm and mid-calf circumferences, triceps, biceps, suprailiac and calf skinfolds measurements, measurements of the calcaneal bone mineral density (BMD, g/cm^2) and bone mineral content (BMC, r), estimated on ALOKA-5.0 DXA machine among Indian (n=58) and Nigerian (n=72) female students (18-21 years) were done. Total body fat percentage was calculated by the Durnin J., Womersley J. equation (1974), total body muscular mass by the Kuczmarski R.J, Flegal K.M. equation (2000).
Results: Obtained data reveals that the Indians have the less body weight, but greater total body fat (12.00% while the Nigerians have 11.19%). This parameter strongly correlates ($r_{x/y}$ 0.74-0.81) with the bicipital skinfold and BMD. BMD and BMC in Indians were significally ($p < 0.001$) more than in Nigerians (BMD 0.98±0.02, BMC 77.31±2.16 in Indians; 0.75±0.06 and 53.88±4.94 – in Nigerians). Nigerians expose more muscular bodies: total muscular mass of the shoulder girdle in Nigerians is 19.76 kg, in Indians is only 16.02. Total muscular body mass in Nigerians is more than in Indians up to the 3.49 kg. This parameter in Nigerians strongly positively correlates ($r_{x/y}$ 0.67-0.71) with the BMD and BMC and negatively ($r_{x/y}$ -0.56) correlates with the body fat and skinfolds' thickness.
Conclusions: Muscular body mass and fat percentage determines the BMD and BMC dependently with the racial features of the body composition.

Plenary Poster P16

Prevention of Aromatase inhibitor-induced bone loss with alendronate in postmenopausal women: The Batman Trial

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Postmenopausal women on an Aromatase inhibitor (AI) are at risk of aromatase inhibitor-associated bone loss (AIBL) and fractures.

In 2005 Osteoporosis Australia proposed an algorithm (Figure 1) for bisphosphonate intervention in this setting. Three hundred and three postmenopausal women with early breast cancer (EBC) were enrolled (osteoporotic, n = 25; osteopaenic, n = 146; normal BMD, n = 126). The efficacy of alendronate (70 mg oral weekly) treatment as triggered by the algorithm in preventing bone loss was evaluated. All patients received adjuvant anastrozole, calcium and vitamin D.

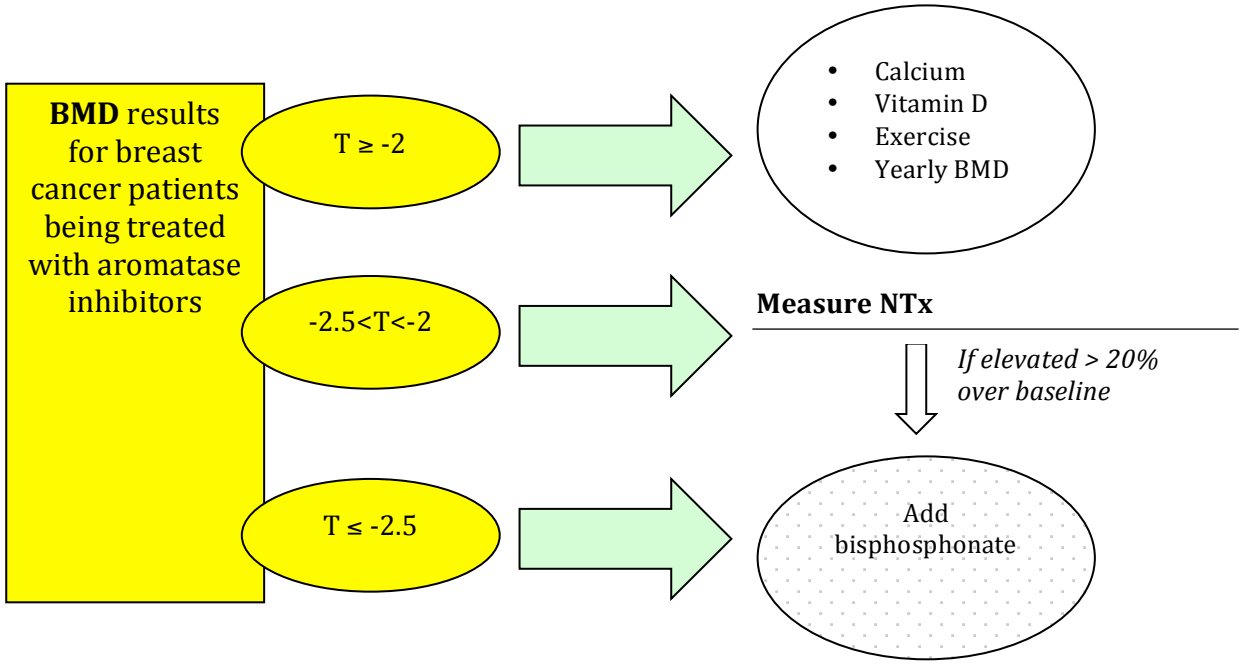
Results: All osteoporotic patients received alendronate at baseline. Eleven out of the 146 (7.5%) osteopaenic patients commenced on alendronate within the first 18 months of participation. Eleven osteopaenic patients commenced on alendronate after the 18 month assessment. One hundred and twenty four out of the 146 (84.9%) patients with osteopaenia and all one hundred and twenty six with normal baseline BMD did not trigger the algorithm.

At three years, the lumbar spine mean BMD increased by 15.6% ($p<0.01$) in the osteoporotic group. BMD in the osteopaenic group with early intervention significantly increased at three years by 6.3%, $p=0.02$ (Figure 2). No significant change was seen in the late intervention group. No change was observed in those with osteopaenia without alendronate.

There was a significant drop in lumbar spine and hip mean BMD of –5.4% and -4.5% respectively, in the normal BMD group, none of whom received alendronate.

Conclusion: In postmenopausal women with endocrine-responsive EBC, BMD improved over time when a bisphosphonate is administered with an AI in osteoporotic patients using an osteoporosis schedule. Subjects with normal baseline BMD experienced the greatest BMD loss, although none became osteoporotic.

Figure 1. Osteoporosis Australia Bone Maintenance Algorithm, using T-score bone mineral density changes and urine Ntx elevation to guide bisphosphonate management.



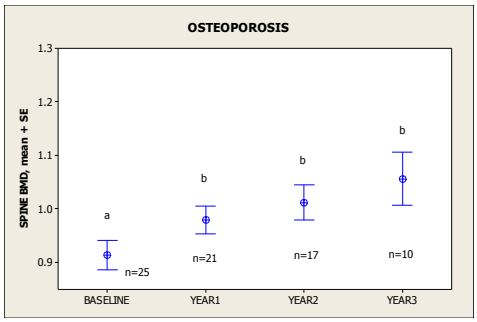


Figure 2. Mean change (g/cm^2) in lumbar spine BMD in Osteoporotic subgroup (n=25)

Plenary Poster P17

Changes in bone mineral density related to incidence of fracture with 6 years of denosumab treatment for postmenopausal osteoporosis

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Aims: In the 3-year FREEDOM trial[1], denosumab (DMAb) was associated with continued increases in bone mineral density (BMD) and reduced fracture risk, with changes in total hip (TH) BMD explaining a considerable proportion of the reduced risk of nonvertebral and new or worsening vertebral fractures[2]. This BMD responder analysis assessed whether this relationship continued with 6 years of DMAb therapy.
Methods: The open-label FREEDOM extension trial is investigating the long-term efficacy and safety of 60mg DMAb every 6 months for up to 10 years of treatment. BMD was measured at the lumbar spine (LS), TH and femoral neck (FN) at the end of the third year in women who had received 6 years of DMAb treatment. The relationship between change in TH BMD from baseline and new or worsening vertebral fracture was examined using a logistic regression model. A comparable approach was employed for nonvertebral fracture using the Cox proportional hazards model.
Results: After 6 years of DMAb treatment, women (N=2343 enrolled) experienced cumulative 6-year BMD gains of 15.2% (LS), 7.5% (TH) and 6.7% (FN). BMD increased for 99% of the women in at least one of these sites, with gains being >3% and >6% in 98% and 95% of these women, respectively. Fracture incidence remained low and was associated with changes in TH BMD (see Figures 1 and 2).
Conclusion: BMD increased at the LS, TH, or FN in almost all women who received 6 years of DMAb. During this time the risk of new or worsening vertebral fracture and nonvertebral fracture decreased with increasing percentage change in TH BMD, providing clinical relevance to the continued BMD gains reported with DMAb over time.

References: 1. Cummings SR, et al. *N Engl J Med* 2009; 361-71. 2. Austin M, et al. J Bone Miner Res 2012; 27 (3): 687-93.

Figure 1.

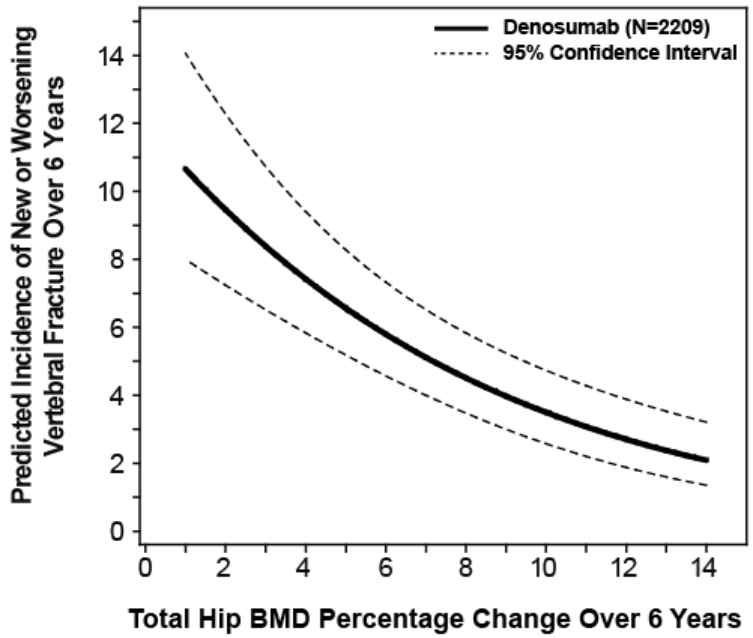
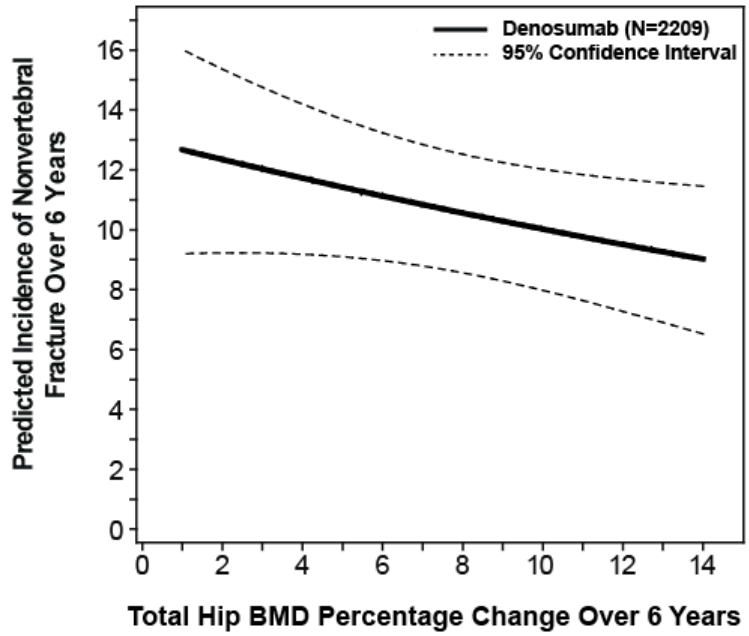


Figure 2.



The predicted fracture incidence was estimated corresponding to the 5th through the 95th percentiles of the observed total hip BMD percentage changes over 6 years. N=number of subjects with an observed BMD value at FREEDOM extension baseline and at ≥1 follow-up visit.

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Predictors of refracture in patients managed within a Fracture Liaison Service: A 7-year prospective study

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Aim: To determine the predictors of re-fracture amongst patients managed long-term for osteoporosis by a fracture liaison service (FLS).
Methods: The analysis included 212 subjects who presented with an incident osteoporotic fracture and were treated within the setting of the Concord FLS for at least 4 years since then. Relevant anthropometric, clinical and technical data were documented at each six-monthly visit. Predictors of re-fracture were identified by logistic regression analysis before and after adjustment for potential confounders.
Results: The mean duration of follow-up was 5.6 (range 4.0-7.5) years. At baseline, mean age (SD) was 72.4 (12.7) years, 79% of subjects were female and 38% had prevalent fractures at the time of the index fracture. Adherence to osteoporosis therapy was high throughout. During the study period, 24% of treated subjects re-fractured. Re-fracture rates in a population of similar composition were reported at 35-46% over 2-6 years.[1,2] After adjusting for confounders female gender (OR 7.3, 95%CI 1.6-33.8, p=0.01), comorbidity (OR 4.1, 95%CI 1.9-9.1, p<0.01), total hip T-score <-1.65 SD (OR 3.9, 95%CI 1.8-8.3, p<0.01), and ≥1 fall within the last year (OR 2.2, 95%CI 1.0-4.8, p=0.04) remained significantly associated with re-fracture. Prevalent fracture status and age were not associated with re-fracture.
Conclusion: Female patients with low hip BMD, a history of recent falls and significant comorbidity are at heightened risk of re-fracture even when treated/ managed by a dedicated FLS. This sub-group of patients requires intensive management including falls reduction strategies.

1. NSW Agency for Clinical Innovation, Sydney, Australia (2012)
2. NSW Re-Fracture Admission Data 2002 – 2008. Greater Metropolitan Clinical Taskforce, Sydney, Australia (2009)

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P20

Osteopontin and the response to muscle injury: requirement for expression by inflammatory or muscle cells

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Osteopontin is a multifunctional glycoprotein that is expressed by several cell types, including osteoblasts and macrophages. We have shown that osteopontin is expressed by myogenic cells, and that acute osteopontin expression is required for inflammation and regeneration following a single severe skeletal muscle injury. This study used whole muscle auto- and allografting of *extensor digitorum longus* muscles of wildtype and osteopontin knockout mice to investigate the relative contributions of osteopontin expressed by host inflammatory cells and that expressed by donor myogenic cells to the response to muscle injury. The right *extensor digitorum longus* muscle of wildtype and osteopontin-null mice was removed by dissecting the distal and proximal tendons of this muscle, and either replaced in the original muscle bed (autografting) or into the muscle bed of a littermate mouse of the alternate genotype (allografting). Grafted muscles were harvested 3, 5, 7, 10 and 14 days post-surgery and subjected to histological and morphometric analysis. In autografted muscle a significant delay in muscle fibre necrosis and regeneration was observed in osteopontin-null mice compared with wildtype mice. In both wildtype and osteopontin-null recipients of allografts, necrosis and regeneration were delayed by comparison with those of wildtype autografts. This result suggests that osteopontin from both donor myogenic cells and host inflammatory cells is important for normal inflammatory and regenerative responses of muscle to a single severe injury. These results highlight the importance of osteopontin from multiple sources in normal muscle repair and suggest osteopontin as a potential target for therapeutic strategies in skeletal muscle.

P21

Neurotrophin-3 (NT-3) attenuates chondrogenesis but enhances osteogenesis during growth plate bony repair

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Injury to the growth plate cartilage often results in undesirable bony repair at the injury site causing bone growth defects in children, for which the underlying mechanisms remain unclear. Neurotrophic factors (NTs) are known to be involved in bone fracture healing; this study investigated potential functions of NTs in the bony repair of injured growth plate. In a rat tibial growth plate injury repair model, up-regulated expression of NGF, BDNF, NT-3, and NT-4 (with NT-3 showing the highest induction) and receptors (TrkA, TrkB and TrkC) by RT-PCR was observed at the injury site at different time points after injury. NTs were found to be immunolocalized in mesenchymal cells, chondrocytes, and osteoblasts at the injury site. In vitro assays with rat bone marrow cells and murine progenitor cells showed that addition of NT-3 protein decreased chondrocyte formation and expression of chondrogenesis-related genes, but enhanced expression of osteogenesis related genes (Runx2, osterix, osteocalcin and BMP-2). Conversely, anti-NT-3 antibody had the opposite effects. In rats with growth plate injury, anti-NT-3 treatment attenuated bone formation at the injury site at day 28 post injury, alleviated the injury-induced down-regulation of cartilage markers, and inhibited expression of osteocalcin. Conversely, NT-3 treatment had a trend of suppressing levels of chondrogenesis markers (Sox9, collagen-2&10) but significantly enhanced those for osteogenesis (Runx2, osterix and osteocalcin). Thus, neurotrophic factor signaling is involved in the bony repair of the injured growth plate; in particular, NT-3 may attenuate chondrogenesis but enhance osteogenesis during growth plate repair.

P22

Adipogenesis occurs at the expense of osteoblast differentiation in primary osteoblasts deficient in proteas activated receptor-2

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The G protein-coupled receptor, protease-activated receptor-2 (PAR_2), is expressed by osteoblasts and required for normal skeletal growth and repair. Prostate cancer (PCa) cells commonly secrete proteolytic activators of PAR_2 (including matriptase) and frequently form osteogenic metastases in bone. The current study investigated the hypothesis that PCa-derived PAR_2 activators modulate osteoblast behaviour in such a way as to support the formation of osteogenic metastases. Primary calvarial osteoblasts derived from wildtype (WT) and PAR_2-null mice were cultured in medium conditioned by the MDA-PCa-2b cell line (MDA-CM); proliferation was assessed by BrdU incorporation, and differentiation was assessed using assays for alkaline phosphatase activity and mineralization, and quantitative PCR analysis of osteoblast-associated genes. MDA-CM stimulated proliferation of osteoblasts independently of PAR_2, but promoted a number of measures of osteoblast differentiation in a PAR_2-dependent manner. Alkaline phosphatase activity and expression of *Runx2* and *Col1* mRNA in 1-day cultures, and mineralization in long-term cultures were all stimulated by MDA-CM in WT but not in PAR_2-null osteoblast cultures. A surprising observation was that long-term cultures of MDA-CM-treated PAR_2-null osteoblasts contained significantly more adipocytes than did matching WT cultures. In agreement with this observation, MDA-CM stimulated expression of the adipogenesis-associated genes encoding peroxisome proliferator-activated receptor-γ (*Pparγ*) and lipoprotein lipase (*Lpl)* in 4-day cultures of PAR_2-null but not WT osteoblasts. Moreover, expression of *Pparγ* mRNA was significantly greater in untreated PAR_2-null cultures than in WT cultures. These results indicate that expression of PAR_2 favours osteoblast differentiation over adipogenesis in mesenchymal cells capable of both osteoblast and adipocyte differentiation.

P23

Clinical manifestation of rickets is enhanced in low calcium/phosphate fed Osteoblast-Specific Vitamin D Receptor (OSVDR) transgenic mice: Evidence for direct negative effects of vitamin D on mineralisation

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The overexpression of Vitamin D Receptor in mature osteoblasts in a transgenic mouse model (OSVDR) has been shown to result in greater cortical and trabecular bone volume and strength due to increase anabolic activities. However, increased vitamin D activity in bone during dietary calcium and phosphate insufficiency mediates bone catabolism. Whether OSVDR mice continue to demonstrate anabolic activities in the presence of a low calcium and phosphate diet and high circulating $1,25(OH)_2D$ levels remains unknown. To address this, female 3 week old OSVDR mice on C57black6 genetic background and WT mice were fed a diet containing either 1%Ca/0.625%Phos (Normal) diet or frankly deficient 0.03%Ca/0.08%Phos (LowCaP) diet for 17 weeks and then killed for analyses. OSVDR mice fed the normal diet demonstrated increased femoral trabecular and cortical bone volume as previously reported. However, when OSVDR mice were fed the LowCaP diet, total bone mineral content was markedly reduced when compared to normal diet fed mice. When compared to WT mice fed LowCaP, femoral length in OSVDR mice was reduced by 8.2% ($P<0.05$), cortical x-sectional area was 9.3% greater ($P<0.05$) and cortical thickness was decreased by 10% ($P<0.05$). The gross morphology of these bones were consistent with the clinical manifestation of rickets. Importantly, these changes occurred despite normocalcemia and normophosphatemia. These findings suggest that although overexpression of VDR in osteoblasts is associated with increased bone volume, during low dietary calcium and phosphate, the enhanced vitamin D levels and activity in mature osteoblasts has direct deleterious effects on mineralization process.

P24

Bone volume increases with loading, but is not altered with unloading, in the homozygous SOST knockout mouse

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Introduction: The Canonical Wnt inhibitor Sclerostin, encoded by the *SOST* gene, is a key regulator of mechanotransduction.
Methods: Unloading and loading were performed on 10 week *Sost*$^{-/-}$ and WT mice.
Unloading: Quadriceps and calf muscles were each injected with 0.5U botulinum toxin (BTX, Allergan).
Loading: 1200 cycles of tibial axial loading, 1200μe on mid-shaft, at 4Hz, 5 days/week.
Treated and control tibiae were µCT scanned (Skyscan 1174) at 2 weeks.
Results: Unloading WT tibiae significantly decreased cortical bone volume (-5%) and thickness (-7%) compared to WT controls ($p<0.01$). A greater bone volume loss (-25%) was seen in the trabecular compartment ($p<0.01$). Furthermore, trabecular thickness and number were decreased by10% and 22% ($p<0.01$). In the *Sost*$^{-/-}$ tibiae, unloading did not change any of these bone parameters.
Cyclic loading of the tibiae increased cortical bone volume in WT (18%) and *Sost*$^{-/-}$ (25%) mice ($p<0.01$). Cortical thickness was also increased in WT (19%) and *Sost*$^{-/-}$ (17%) mice ($p<0.01$). The WT loaded tibiae showed increased trabecular thickening (15%) and metaphyseal cortical bone volume (13%) ($p<0.01$). The *Sost*$^{-/-}$ tibiae did not show trabecular thickening with loading, but greatly increased metaphyseal cortical bone volume (31%) ($p<0.01$), suggestive of metaphyseal corticalisation.
Conclusion: *SOST* knockout inhibited unloading-induced bone loss, but not loading-induced bone gain. Thus Sclerostin may have an important role in bone's response to unloading, but may not be essential for the response to loading.

P25

Associations between lean and fat mass and bone structure: a co-twin study

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Background: We examined the association between total body lean mass (LM) and fat mass (FM) and microstructure in female twin pairs. We hypothesized that lean mass is associated with total cross sectional area (CSA) while fat, not lean, mass is associated with medullary CSA.
Methods: We measured the macro- and microarchitecture of the distal radial and distal tibial metaphysis using high-resolution peripheral quantitative computed tomography (HR-pQCT) in 112 twin pairs aged 44-65 years (mean 53 years); 18% were premenopausal, 20% perimenopausal and 62% postmenopausal. Lean and fat mass were determined by dual-energy X-ray absorptiometry (DXA, GE Prodigy). Associations between within-pair trait differences were tested using linear regression analysis.
Results: Of within-pair differences in lean mass controlling for fat mass, were associated with total, medullary and cortical CSA at the tibia but not radius (r ranging from 0.23-0.44, $P<.05$). Pair differences in lean mass were also associated with pair differences in total vBMD at the distal tibia ($r=0.23$, $P<.05$). After controlling for lean mass, within-pair differences in fat mass were associated with within-pair differences in total vBMD at the tibia only ($r=0.26$, $P=0.02$).
Conclusion: Both FM and LM were associated with total vBMD. LM predictor of total, medullary and cortical CSA at distal tibia. Whether these associations have a genetic basis or are partly the result of environmental factors, such as loading history, remains to be established.

P26

Relationship between body composition and osteoarthritis

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Aim: Obesity is known as a risk factor for osteoarthritis (OA), but it is not known whether fat mass or lean mass is the main determinant. This study sought to determine the association between lean body mass and fat mass and the risk of OA.
Methods: The study involved 578 women aged 45+ years, who had been randomly selected from the general community. The presence of osteoarthritis was ascertained by self-report with verification of medication use. Body composition (e.g. fat mass and lean mass) was assessed by dual-energy X-ray absorptiometry (GE-LUNAR Corp, Madison, WI).
Results: Twenty-one percentage (n=121) of the women reported a diagnosis of OA. Individuals with greater whole body fat mass (FM) had a greater risk of OA. Each eight kg increase in FM was associated with a 38% odds of OA (odds ratio [OR] 1.38; 95% CI, 1.13-1.69), and this remained statistically significant even after adjusting for age, physical activity and femoral neck BMD. Lean mass (LM) was not significantly associated with OA risk. Women with higher fat mass-to-lean mass ratio (FM:LM) also had a greater risk of OA, with OR being 1.30 (95% CI, 1.06-1.59). The risk of OA was increased with fat mass in the abdominal region (OR 1.26; 95% CI, 1.04-1.54), trunk (OR 1.46; 95% CI, 1.20-1.80), right leg (OR=1.41; 95% CI, 1.16-1.72) and left leg (OR 1.44; 95% CI, 1.19-1.76), but not in the arms.
Conclusion: These data suggest that the association between weight and osteoarthritis is mediated through fat mass, not lean mass.

P27

Is Osteoporosis-Related Quality of Life (QoL) Associated With Fracture-Free Mortality: Geelong Osteoporosis Study (GOS)

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Association between QoL and mortality is under-investigated among potential osteoporotic patients. We aimed to investigate associations between QoL and fracture-free mortality.

Age-stratified random samples of 562 women and 784 men aged 50+yr were recruited between 1993-97 and 2001-07 respectively. Women were followed biennially, and men were followed since 2006. Participants' QoL was assessed using the Osteoporosis Targeted QoL questionnaire (OPTQOL) that measures QoL related to osteoporosis from three domains: physical functions (PF), adaptations (social/emotional functioning, vitality/fatigue) and fears. Higher scores indicate higher QoL. We collected QoL data for men beginning at baseline and from women beginning in 2000. Participants' QoL were categorised into quartiles. Gender-specific and overall stepwise cox-regression models were devised with death as the outcome, adjusting for age, smoke history, alcohol consumption, and education. Participants were censored either when they sustained fracture(s), at death, or date of their next interview.

Summary statistics for each domain of QoL are shown in Table 1. Lower QoL in the adaptation domain was associated with higher mortality in models for women (hazard ratio (HR): 0.70, p=0.005, 95%CI: 0.55, 0.90), men (HR: 0.84, p=0.047, 95%CI: 0.70, 1.00), and overall (HR: 0.83 p=0.002, 95%CI: 0.74, 0.93). Lower QoL in PF (HR: 0.68, p=0.03, 95%CI: 0.47, 0.97) was associated with increased mortality in men only.

In conclusion, lower QoL in adaptation domain may be associated with higher mortality in both genders, whereas lower QoL in the PF domain may increase mortality in men.

Table 1: Summary statistics of our sample

Gender	n (No. Deaths)	Follow-up (person-years)	Summary Statistics of QoL (%) Median (IQR%)		
			Physical Functions	Adaptation	Fears
Women	562 (85)	762.50	57.14 (28.57-85.71)	66.66 (50-87.5)	83.33 (50-100)
Men	784 (157)	2301.28	90.48 (71.43-100)	75 (54.17-100)	100 (83.33-100)
Overall	1346 (242)	3063.787	85.71 (47.62-100)	70.83 (50-91.67)	94.44 (72.22-100)

P28

Frequency Distribution of voxels with their varying content of mineralized bone and void volume captures variance in microstructure better than trait means

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High Resolution-peripheral Quantitative Computerised Tomography (HR-pQCT) quantifies microstructure *in vivo* using a fixed threshold. This approach to image analysis erroneously allocates voxels to the cortical or trabecular compartment depending on the threshold attenuation used to define 'cortical' or 'trabecular' bone. We hypothesised that considering the density of individual voxels as a continuum improves discrimination of patients with forearm fracture.

33 women (57±9 years) with forearm fragility fractures and 53 aged-matched controls had a DEXA scan at lumbar spine (LS) and femoral neck (FN) and HR-pQCT at the distal radius. HR-pQCT images were analysed using StrAx1.0 that segments bone from background and cortex into compact-appearing, outer and inner transitional zones. Attenuation of all voxels was compared to fully mineralized bone matrix and soft tissue. Voxels were classified according the mineralized bone matrix/void volume content. Sensitivity and specificity was evaluated using the area under a receiver-operating curve (AUC).

LS (AUC:0.58) and FN (AUC:0.53) aBMD did not discriminate the fracture status. Cortical and trabecular vBMD discriminated cases with AUCs of 0.69 and 0.63, respectively, $p<0.05$. The proportion of voxels containing 50-70%, 70-95% and >95% mineralized bone discriminated cases and controls (AUC from 0.63 to 0.87). The "50-70%" category assessed in outer transitional zone was the best discriminant (AUC:0.87 and $p<0.05$ vs. Ct.vBMD and Tb.vBMD) and conferred an OR (95% CI) for fracture of 11.2 (2.8-52.7).

Fragility is associated with subtle changes in cortical microstructure captured by analysing and expressing the varying composition of individual voxels as a frequency distribution curve

P29

Fracture and utilisation of residential aged care in Australia

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Aim: To examine the association between fracture and residential aged care (RAC) utilisation in older women.
Method: Female participants of the Geelong Osteoporosis Study aged ≥50 years (n=662) at baseline (1993-97) were followed until 30 June 2010 (7670 person-years). Incident fracture (all sites and causes; n=188) was assessed using radiology reports and utilisation of high and low level RAC (n=134) was ascertained using Commonwealth Department of Health and Ageing administrative records from 1 Jan 1998. Using Cox regression with age as the time variable and analysis time split when the first incident fracture occurred, associations were examined with covariates including baseline socio-demographic factors, clinical markers and lifestyle-related exposures. Individuals were censored at the date of service utilisation or death (confirmed using the National Death Index), whichever came first.
Results: In unadjusted analyses, fracture was associated with RAC utilisation (HR 2.35 95% CI 1.65-3.34). The association was strengthened (HR 2.80 95% CI 1.91-4.09) after adjusting for covariates, including socio-economic status, dietary calcium, smoking, femoral neck BMD, appendicular lean mass index, mobility, walking aid use, prior fracture and physical activity.
Conclusion: Women who experienced a fracture during the study period at any site and for any reason had an almost three-fold greater hazard of residential aged care utilisation than those who remained fracture-free. Additional analyses controlling for factors related to fracture and aged care service utilisation are needed to further illuminate this observation.

P30

Generation of highly purified osteocytes by fluorescence activated sorting (FACS)

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Freshly isolated primary calvarial osteocytes express osteocytic gene mRNAs such as sclerostin (*Sost)*, dentin matrix protein (*Dmp1)* and matrix extracellular phosphoglycoprotein (*Mepe)* and abundant calcitonin receptor (*Calcr)*. However, within 24 hours of culturing these cells on plastic, mRNA levels of all these genes is reduced by 85-95% suggesting that interaction with the bone matrix is required for osteocytes to retain their phenotype. To investigate this, a highly purified osteocyte population is required.
Initially, osteocytes were isolated from bones of transgenic mice expressing GFP under the control of the *Dmp1* promoter and purified by fluorescence activated sorting (FACS) for GFP. GFP-positive cells not only expressed mRNA for osteocyte genes but also expressed mRNA for the osteoclast marker gene dendritic cell-specific transmembrane protein (*Dc-stamp)* suggesting the presence of auto-fluorescent osteoclasts. Lineage depletion was used to eliminate these cells. Isolated DMP1-GFP cells were stained for haematopoietic lineage (Lin) surface markers to identify myeloid and lymphoid cells (CD2, CD3e, CD4, CD5, CD8, CD11b, Gr1, CD45, Ter119, B220) and with CD31 to identify endothelial cells. The Lin-negative/CD31-negative population was sorted for GFP expression. The GFP-positive population represented a highly purified osteocyte cell population expressing *Sost*, *Dmp1*, *Mepe, Calcr* but not *Dc-stamp,* confirming the exclusion of osteoclasts. *Dc-stamp* mRNA was only detected in the GFP negative cells. Culture of this highly purified osteocyte population on plastic for 24 hours still led to 85-95% reduction in osteocytic gene expression.
These data demonstrate a method to generate a highly purified osteocyte population, and confirms *Calcr* expression by osteocytes.

P31

Local Synthesis of 1,25-dihydroxyvitamin D (1,25D) Promotes Osteocyte Maturation

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The local synthesis of active vitamin D (1,25D) by the enzyme CYP27B1 within osteoblasts has been previously shown to act in an autocrine manner to regulate cell proliferation and differentiation. Thus, in the current study we focused on the local metabolism of 1,25D within osteocyte-like cells. We utilized the mouse cell lines MC3T3-E1, MLO-A5 and MLO-Y4 which represent increasingly mature stages of the osteoblast/osteocyte lineage, and are capable of further differentiation in culture. Our data confirm that expression of Cyp27b1 mRNA is a feature of the osteoblast lineage as cells transition to an osteocyte phenotype. Furthermore, the addition of 100nM 25D to each cell line induced Cyp24A1 mRNA indicating conversion of 25D to 1,25D. MC3T3-E1 cells, cultured for up to 4 weeks in osteogenic media, expressed low levels of FGF-23 mRNA under basal conditions, but expression was elevated 10-fold in the presence of 100nM 25D. Similarly, chronic 25D supplementation (100nM) increased osteocalcin (Ocn) mRNA by approximately 50% in cultures at Day 21 ($P<0.05$), which declined as cells matured (44% decrease in Ocn mRNA at Day 28) suggesting that the role of locally synthesized 1,25D on activities such as mineralization is dynamic and may depend on the stage of maturation. 25D exposure upregulated Ocn, Col-I and Alk Phos mRNA in MLO-A5 pre-osteocyte cells, but decreased Ocn and Phex in the more mature MLO-Y4 osteocyte cells. Overall, our findings suggest that the local synthesis of 1,25D within osteocytes regulates gene expression in a temporally dependent manner to promote cell maturation.

P32

The effects of recombinant undercarboxylated osteocalcin on insulin-stimulated glucose uptake following *ex vivo* muscle contraction

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Background: Studies in mice suggest that osteocalcin (OC), an osteoblast-specific secreted hormone, in its undercarboxylated form (ucOC), increases insulin secretion and insulin sensitivity. Acute exercise increases skeletal muscle insulin sensitivity as well as circulating levels of ucOC post- exercise. We tested the hypothesis that ucOC plays a role in the increase in muscle glucose uptake post-contraction in mice.
Methods: We used an *ex vivo* muscle contraction model to assess whether recombinant ucOC can improve insulin-stimulated glucose uptake post-contraction. We have compared the differences in EDL muscle glucose uptake from C57BL/6J mice between rest and (a) insulin alone, (b) 2h post-contraction and insulin was added 1.5h post-contraction and (c) 2h post-contraction however, recombinant ucOC was added 45 min post-contraction and insulin was added 1.5h post-contraction. We also examined whether muscle glucose uptake is increased with ucOC alone or 2h post-contraction alone (no insulin). Data reported as mean±SEM.
Results: Insulin increased muscle glucose uptake compared to rest (37.8±13.2%, p=0.024). Contraction prior to insulin treatment increased glucose uptake compared with insulin alone (27.9±10.9%, p=0.037), and ucOC treatment and insulin enhanced muscle glucose uptake post-contraction, compared with contraction plus insulin alone (14.5±6.4%, p=0.039). ucOC alone and contraction alone had no significant effect on muscle glucose uptake 2 hr post-contraction.
Conclusion: ucOC treatment improves insulin-stimulated muscle glucose uptake following *ex vivo* muscle contraction. Given that exercise increases circulating ucOC in humans (from our previous study), this elevation in ucOC may account, at least in part, for the insulin sensitizing effect of exercise.

P33

Vitamin D Receptor in mature osteoblasts and osteocytes is required for normal bone formation and resorption

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The role of vitamin D activity in osteoblasts has been shown to mediate signalling for both bone resorption and formation. Much of this evidence has been gleaned from *in vitro* studies and the mouse model of osteoblast VDR overexpression. It is unclear however, if VDR-mediated activity is required for normal bone remodelling *in vivo*. We genetically inactivated the VDR in the mature osteoblastic lineage (ObVDRKO), by crossing Osteocalcin-Cre mice with floxed-VDR mice. At 6w of age, both male and female ObVDRKO mice demonstrated marked increases (over 20%) in both femoral trabecular and cortical bone when compared to control littermates. At 12w of age, ObVDRKO metaphyseal BV/TV% remained increased albeit only 5% greater in male mice and no differences in cortical bone volume were observed. At 26w of age, ObVDRKO BV/TV% was no longer significantly different from control littermates. In 6w ObVDRKO mice, both RANKL-mediated bone resorption and markers of osteoblast activity and formation were significantly lower than levels in control littermates. At 12w of age, low RANKL expression in ObVDRKO mice was not significantly different from the lower levels in control mice, consistent with no changes in cross-laps levels. However, ObVDRKO metaphyseal Ob.N and BFR remained significantly lower in 12w old mice. Thus, osteoblast/osteocyte deletion of VDR impairs both osteoclast and osteoblast activities. While during growth, the increased bone accrual in ObVDRKO mice is due to reduced bone resorption, the amelioration of the bone phenotype with age appears to be due at least in part to ongoing impaired bone formation.

P34

Biphasic calcium-dependent control of CYP27B1 expression mediated by the calcium-sensing receptor

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Elevated extracellular Ca^{2+} (Ca^{2+}_o) activates 1,25-dihydroxy vitamin D synthesis in the parathyroid and suppresses it in the renal proximal tubule. In both cases, the outcome is a physiologically relevant suppression of Ca^{2+}_o. However, the underlying mechanisms have not been defined and the roles of the calcium-sensing receptor (CaSR) in either or both these processes have been unclear. In the present study, we investigated the impact of various Ca^{2+}_o concentrations on the expression of CYP27B1-luciferase constructs transfected into either control HEK-293 cells or in HEK-293 cells that were stably transfected with the CaSR (HEK-CaSR cells). Although Ca^{2+}_o had no effect of CYP27B1-luciferase expression in control HEK-293 cells up to a concentration of 8 mM, it sensitively stimulated CYP27B1-luciferase expression by 2-3 fold above baseline in HEK-CaSR cells with an EC_{50} for Ca^{2+}_o of around 1.5 mM. Interestingly, at higher concentrations (3-5 mM), however, Ca^{2+}_o paradoxically suppressed CYP27B1-luciferase expression. Furthermore, the calcimimetic (CaSR-selective positive modulator) cinacalcet (1.0 μM) stimulated CYP27B1-luciferase expression by 2-3 fold at baseline Ca^{2+}_o (0.5 mM) and markedly suppressed expression at a higher Ca^{2+}_o level (3.0 mM) demonstrating that both the stimulatory, and inhibitory, effects of Ca^{2+}_o are mediated by the CaSR. The CaSR-mediated, biphasic Ca^{2+}_o-dependent control mechanism we have identified would appear to provide a basis for explaining how elevated Ca^{2+}_o stimulates 1,25-dihydroxyvitamin D synthesis in parathyroid cells and inhibits it in proximal tubule cells.

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Analysis of vitamin D metabolism gene expression in human bone: evidence for autocrine control of bone remodelling

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The metabolism of 25(OH)vitamin D (25D) into active 1,25(OH)2vitamin D (1,25D) by endogenous expression of CYP27B1 in osteoclast lineage cells appears to optimise osteoclast differentiation and inhibits osteoclast activity. The activity of CYP27B1 in osteoblasts promotes proliferation and maturation *in vitro*. To examine relationships between CYP27B1 mRNA expression in bone and its potential function *in vivo*, we examined the expression of vitamin D metabolism genes (CYP27B1, CYP24A1, VDR) in human trabecular bone samples and compared them by linear regression analysis with the expression of osteoclast (TRAP, CA2, CATK, NFATc1), osteoblast (TNAP, COL1A1, OCN, MEPE, BRIL), osteocyte (DMP1, SOST, PHEX, MEPE, FGF23)-related gene markers, genes associated with osteoblast/osteocyte control of osteoclastogenesis (RANKL, M-CSF, OPG, IL-8), and transcription factors (NFATC1, RUNX2, osterix, MSX2, HIF-1A). This revealed multiple significant gene expression relationships between CYP27B1, transcription factors RUNX2, MSX2, NFATc1 and the hypoxia-inducible transcription factor, HIF-1A. Furthermore, CYP27B1 mRNA expression associated mainly with markers of bone resorption (TRAP, CA2, CTR). CYP24A1 expression was associated with VDR, resorption genes RANKL, CTR, and with HIF-1A. VDR mRNA expression was associated with CA2, and the bone mineralisation marker BRIL. Against expectations, there was no association with CYP27b1 expression and 1,25D responsive genes, OCN and RANKL.
The major implication of these relationships in gene expression is that endogenous 1,25D synthesis and the response to 1,25D in human trabecular bone is linked with coordinated functions in both osteoclastic and osteoblastic compartments towards the control of bone remodelling.

P36

EGFL7 expressed in bone microenvironment mediates the migration of endothelial cells via ERK, STAT3 and integrin signaling cascades

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Angiogenesis plays a pivotal role in bone formation, remodeling and fracture healing. The regulation of angiogenesis in the bone microenvironment is highly complex and orchestrated by intercellular communication between bone cells and endothelial cells. Here, we report that EGF-like domain 7 (EGFL7), a member of the epidermal growth factor (EGF) repeat protein superfamily is expressed in both the osteoclast and osteoblast lineages, and promotes endothelial cell activities. Addition of exogenous recombinant EGFL7 potentiates SVEC (simian virus 40-transformed mouse microvascular endothelial cell line) cell migration and tube-like structure formation. We show that exogenous EGFL7 induces phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), signal transducer and activator of transcription 3 (STAT3), and focal adhesion kinase (FAK) in SVEC cells. Inhibition of ERK1/2 and STAT3 signaling impairs EGFL7-induced endothelial cell migration. Bioinformatic analyses indicate that EGFL7 contains a conserved RGD/QGD motif and EGFL7-induced endothelial cell migration is significantly reduced in the presence of RGD peptides. Moreover, EGFL7 gene expression is significantly upregulated during growth plate injury repair. Together, these results demonstrate that EGFL7 expressed by bone cells regulates endothelial cell activities through integrin-mediated signaling. This study highlights the important role that EGFL7 expressed in bone microenvironment plays in the regulation of angiogenesis in bone.

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The role of EGFL6 in bone homeostasis

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Angiogenesis plays a pivotal role in bone development, growth and repair. Angiogenic processes that occur at the interface of cartilage-subchondral bone and in the trabecular bone region within the diaphysis are regulated by angiogenic factors via autocrine/paracrine mechanisms of action. We previously reported that epidermal growth factor-like 6 (EGFL6) is up-regulated during osteoblast differentiation and promotes angiogenesis. Here we investigate the physiological role of EGFL6 in bone homeostasis using $EGFL6^{-/-}$ mice. MicroCT analysis revealed a significantly increased trabecular bone volume in primary spongiosa below the growth plate of $EGFL6^{-/-}$ mice compared with wildtype mice. $EGFL6^{-/-}$ mice showed a decreased CD31 immunostaining in the growth plate region by immunohistochemistry. Histology results of the $EGFL6^{-/-}$ mice demonstrated an increased bone density in the primary spongiosa and a decrease in Safranin O staining of the cartilage. Moreover, osteoclast staining with tartrate-resistant acid phosphatase (TRACP) was decreased in the trabecular bone of $EGFL6^{-/-}$ mice. Interestingly, mineralized bone nodule formation in osteoblasts isolated from $EGFL6^{-/-}$ mice was reduced, indicating that the bone phenotype of $EGFL6^{-/-}$ was predominantly attributed to decreased osteoclast activity. In conclusion, $EGFL6^{-/-}$ mice display greater trabecular bone volume in the primary spongiosa with a decrease in mineralized bone nodule formation *in vitro*, which indicates reduced resorption, as confirmed by decreased TRACP-stained osteoclasts *ex vivo*. Moreover, the increased bone mass in the primary spongiosa of $EGFL6^{-/-}$ mice could be attributed to reduced vascularization and osteoclast formation in the region. Taken together, these results demonstrate that EGFL6 plays an important role in bone homeostasis.

P38

Anabolic action on bone cells by the coiled-coil domain of fasting-induced adipose factor ($FIAF_{CCD}$)

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The impact of adipose tissue on bone is an important research area in skeletal biology, as we and others have shown that fat mass and bone mass are positively correlated. This study aims to further explore the underlying mechanism by looking at the bone cell effects of the adipokine, fasting-induced adipose factor (FIAF) and particularly its naturally truncated product coiled-coil domain ($FIAF_{CCD}$).

Our results show that intact FIAF is inactive on bone cells, but its fragment $FIAF_{CCD}$ potently inhibits osteoclast formation in mouse bone marrow and $RAW_{264.7}$ cell cultures, as well as inhibiting the activity in isolated mature osteoclasts. The inhibitory rates at 500 ng/mL were approximately 90%, 50% and 90% respectively in the above models. It also stimulated osteoblast mitogenesis by ~30% at this concentration.

$FIAF_{CCD}$ greatly reduced the expression of macrophage colony-stimulating factor (M-CSF), nuclear factor of activated T-cells c1 (NFATc1), dendritic cell-specific transmembrane protein (DC-STAMP), and mildly suppressed the expression of connective tissue growth factor (CTGF) in bone marrow cell cultures. However, it did not change RANKL and OPG levels in the ways supporting its osteoclastic effect. The similar effects on NFATc1, DC-STAMP in RAW264.7 cells, and on M-CSF and CTGF in ST2 cells were also observed.

In conclusion, $FIAF_{CCD}$ positively couples fat and bone mass by indirectly or directly acting on osteoclasts. $FIAF_{CCD}$'s action on osteoclasts is independent of RANKL/OPG system, but dependent of M-CSF, NFATc1, DC-STAMP and CTGF pathways.

P39

Osteoclast gp130 signalling stimulates periosteal bone formation

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Interleukin-6 (IL-6) has been reported to stimulate osteoclast activity via the gp130 co-receptor subunit in osteoclasts, but the physiological significance of this pathway remains controversial. To determine the importance of gp130 signalling in the osteoclast, we generated mice with osteoclast-specific gp130 deletion (using CathepsinK-Cre).

Osteoclasts were generated *in vitro* from *CtkCre.gp130$^{w/w}$* and *CtkCre.gp130$^{f/f}$* bone marrow macrophages (BMM) +RANKL/M-CSF and knockdown of gp130 (by 31%, $p<0.0001$) in *CtkCre.gp130$^{f/f}$* cells was confirmed by qPCR. The number of osteoclasts generated was significantly elevated (by 50%) in *CtkCre.gp130$^{f/f}$* cultures compared to *CtkCre.gp130$^{w/w}$*. However, no increase in osteoclast numbers or serum CTX-1 was observed *in vivo*, nor was femoral or vertebral trabecular bone volume altered in *CtkCre.gp130$^{f/f}$* mice compared to *CtkCre.gp130$^{w/w}$* controls at 6, 12 and 26 weeks.

In cortical bone, CtkCre-directed deletion of gp130 dramatically inhibited periosteal bone formation rate (by 68%, $p<0.0001$), reducing both double-labeled surface (by 75%, $p<0.008$) and mineral apposition rate (by 45%, $p<0.05$). By 26 weeks, these mice exhibited smaller femoral width, with reduced periosteal (8%, $p<0.01$) and endocortical perimeter (11%, $p<0.01$). Since IL-6 and IL-11R global knockout mice exhibit a similar reduction in femoral width, we also assessed periosteal bone formation in those strains, and found reductions of a similar extent (by 94% in IL-6, $p<0.01$; by 65% in IL-11R, $p<0.05$) to that observed in the *CtkCre.gp130$^{f/f}$* mice.

These data suggest that gp130 signalling in the osteoclast is not essential for normal bone resorption, but maintains periosteal growth in male mice by stimulating production of osteoclast-derived coupling factors.

P40

Cell death is augmented in ephrinB2-deficient osteoblasts

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The interaction between ephrinB2 and EphB4 in osteoblasts regulates osteoblast differentiation and supports osteoclastogenesis. Mice with an osteoblast-lineage-specific deletion of ephrinB2 have enhanced osteoblast differentiation, but impaired osteoblast activity, but the mechanism by which this occurs is not yet defined. To determine the the role of ephrinB2 in osteoblast function *in vitro*, we infected calvarial cells expressing ephrinB2 *loxP* with a Lenti-viral vector containing both Cre recombinase and the ZsGreen (fluorescent) genes. To quantify cell death, cells were stained with Annexin V (apoptosis) and propidium iodide (necrosis) for anlysis by flow cytometry. Cell morphology was visualised by confocal microscopy following staining for phalloidin.
By confocal microscopy, osteoblast cells lacking ephrinB2 were hypertrophic and fibroblastic in morphology, with elongated phalloidin fibres compared to wildtype controls, consistent with their impaired differentiation to osteoblasts. Flow cytometric analysis revealed that ephrinB2-deficient cells were 6.4 times more apoptotic and 3 times less necrotic than controls. The lack of ephrinB2 also made these cells 2.4 times more susceptible to apoptose following exposure to Staurosporine. In addition, blocking the interaction between ephrinB2 and EphB4 with an EphB4-specific peptide, TNYL-RAW, augmented necrosis of wildtype calvarial primary osteoblasts 2.3 times ($p<0.05$). In contrast, when EphB4 shRNA was infected into a murine stromal cell line to reduce EphB4 expression, cells expressing less EphB4 were neither more apoptotic nor necrotic than vector control. This data suggests that the ephrinB2/EphB4 interaction in the osteoblast lineage maintains cell survival via ephrinB2 reverse signalling.

P41

End-products of a tryptophan degradation pathway have an osteo-anabolic effect on differentiating mesenchymal stem cells

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BACKGROUND: Interferon gamma (IFNg) increases osteoblastogenesis and bone formation *in vitro* and *in vivo*. Indoleamine-2-3-dioxygenase (IDO)1 is an important target of IFNg-induced osteogenesis. Since IDO1 is the critical enzyme in the kynurenine (KYN) tryptophan degradation pathway, we hypothesised that the end products of this pathway would have an osteogenic effect on differentiating mesenchymal stem cells (MSCs).
AIM: To assess the role of the KYN pathway of tryptophan-degradation in osteoblastogenesis.
METHODS and RESULTS: Human MSC were treated with 100ng/ml IFNg for 7 days in osteogenic conditions. After 7 days, supernatants were analysed by HPLC for products of the kynurenine pathway including picolinic (PA), quinolinic (QA) acids and KYN. KYN/tryptophan ratio was increased (~+2.5 fold) in IFNg treated MSC ($p<0.05$) without changing serotonin. The IDOI gene was inhibited using siRNA-IDO1 (33nM) or 1-methyl-D-tryptophan. IDO1 knock-down diminished osteogenic differentiation assessed by RT-PCR (Runx2:~-3 fold; $p<0.01$). Differentiating osteoblasts were treated with PA, QA and KYN. Whereas KYN had no effect on osteogenesis, MSC treated with QA and PA showed increased osteogenesis (~+1.8 fold osteocalcin; +3 fold RunX2; +6 fold osteopontin; $p<0.01$). Alizarin Red staining for mineralization was +2 fold ($p<0.05$) in PA treated MSC. Subsequently, bone phenotyping in IDO1 knock-out (KO) mice showed osteopenia associated with BV/TV ratio ~-35% in KO. MSC derived from IDO1 KO showed increased mineralization when treated with PA and QA ($p<0.05$).
CONCLUSION: This study shows that both PA and QA play a very important role in osteoblastogenesis with a potential osteo-anabolic effect that could be used to treat osteoporosis in the future.

P42

Analysis of bone formation activity utilizing transgenic mice by *in vivo* bioluminescence imaging

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Osteocalcin is a major noncollagenous protein component of bone extracellular matrix, synthesized and secreted exclusively by osteoblastic cells during the late stage of maturation. We introduced a 10 kb human osteocalcin enhancer/promoter (OC)-luciferase (Luc) construct into a hairless mouse line. Examination of tissue RNAs from these transgenic mice showed a predominant restriction of Luc mRNA expression to bone-associated tissues. Immunohistochemical staining of calvaria tissue sections revealed the localization of Luc protein to osteoblasts. Utilizing *in vivo* bioluminescence imaging, supplementation of 1α,25-dihydroxyvitamin D_3 increased Luc activity throughout the skeleton, consistent with *in vitro* transient transfection studies in osteoblast-like cells. Moreover, we observed an abrupt decrease in bioluminescence activity as the mice reached puberty, and a further decrease gradually thereafter. Using a radius skeletal repair model, we observed enhanced bioluminescence at the fracture site in both young (14-22 weeks old) and aged (50-58 weeks old) mice. However, peak bioluminescence was delayed in aged mice compared with young mice, suggesting retarded osteocalcin expression with aging. Our *in vivo* imaging system may contribute to the therapy and prevention of various bone metabolic disorders through its effective monitoring of the bone formation process. Furthermore, our mouse system may offer a feasible detection method for assessing osteogenic activity in the development of functional foods and medicines by non-invasive screening.

P43

Selective serotonin reuptake inhibitors (SSRIs) inhibit human osteoblastogenesis and decrease bone development and mineralization in Zebrafish

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SSRIs are widely used antidepressants and one of the most commonly used medications. Our group was amongst the first to document a link between SSRI use and reduced BMD and have recently demonstrated presence of serotonin receptors and the serotonin transporter in human osteoblasts (OB) and osteoclasts. Studies in animal and human models indicate that SSRIs may directly regulate serotonin signalling in bone cells, however, a direct mechanism of action of SSRIs on bone cells remains unclear. To determine effects of SSRIs on developing bone, we treated human OB *in vitro*, and zebrafish during embryonic development, with Sertraline and Citalopram.

Human OB derived from adipose tissue-derived mesenchymal stem cells, were cultured in the presence of osteogenic media containing dexamethasone, β-glycerophosphate and ascorbate-2-phosphate, with or without SSRIs, for 14d and assessed for gene expression and alkaline phosphatase (ALP) activity. Developing Zebrafish embryos were incubated in the presence of SSRIs and gene expression assessed by *in situ* hybridisation, and bone mineralisation by alizarin red staining.

Sertraline and Citalopram reduced both *alp* gene expression and enzyme activity in human OB. Sertraline also inhibited expression of *runx2* and *col10A1*, and promoted expression of the pro-apoptotic gene *bax*. Expression of *runx2* and *osterix* was unaffected in Zebrafish embryos, whereas expression of *col10a1* and bone mineralisation was significantly decreased.

These data demonstrate that SSRIs inhibit expression of key osteoblastic genes and ALP activity during human osteoblastogenesis, and can reduce bone mineralisation *in vivo*, possibly accounting for the loss of BMD with chronic use.

P44

A novel mouse cortical bone derived mesenchymal stem cell-like cell line

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Mesenchymal stem cells (MSCs) are multi-potent cells that can be differentiated to various cell lineages including osteoblast, adipocyte, chondrocyte and smooth muscle. Cell lines with MSC-like characteristics and that can form mineralising osteoblasts have not been reported previously. Here we report the characterisation of a novel murine cell line with MSC-like properties.
Cells were derived from the culture of 4-week old C57BL/6 mouse long bone cortices plated *ex vivo*. After repeated passage, one such culture spontaneously exhibited transformed properties evidenced by maintenance of proliferative potential up to at least passage 25. Subsequent analysis revealed strong activity of telomerase remaining up to at least passage 7. These cells had uniform morphology and were positive for type 1 collagen and negative for the macrophage marker, F4/80. Further immunophenotyping showed them to be negative for the haemopoietic markers CD11b and CD45, the haemopoietic stem cell marker, CD34, and the endothelial marker CD31. A panel of MSC markers including Sca1, CD44, CD73, CD146, CD166 were highly expressed. The cell line exhibited tumorigenic properties and was able to differentiate towards mineralising osteoblasts, lipid forming adipocytes and collagen type II producing chondrocytes using both *in vitro* culture models and an *in vivo* sub-cutaneous implantation model.
We conclude that this cell line, designated MMSC-13, is a spontaneous osteosarcoma-like population with MSC-like properties having the ability of forming osteoblasts, adipocytes and chondrocytes *in vitro* and *in vivo*. This cell line is a potentially useful tool for studying the differentiation of MSC into mineralising osteoblasts.

P45

Molecular mechanisms underlying accelerated bone remodeling during hyperhomocysteinemia

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RANK ligand (receptor activator of nuclear factor-κB ligand) and osteoprotegerin (OPG) are two significant proteins synthesized by the osteoblast to regulate bone remodeling. The RANK ligand plays a major role in osteoclast formation, function and survival through its interaction with the RANK on the osteoclast and is physiologically regulated by OPG, a decoy receptor which interferes with RANKL-RANK association and inhibits osteoclast activity. Our investigations revealed that homocysteine mediated oxidative events alters the normal synthesis pattern of OPG and RANK ligand by the osteoblast. Homocysteine mediated OPG downregulation occurred through activation of protein phosphatase 2A (PP2A), a negative modulator of the insulin signaling pathway which induced dephosphorylation of redox regulator FOXO1 (Ser253 and Tyr24), FOXO1 nuclear localization, Mn*Sod* expression and p38 MAP kinase downregulation. On the other hand, an increased expression and release of RANK ligand in homocysteine treated osteoblast cultures occurred by a mechanism independent of FOXO1/p38 signaling but involving *c*-Jun/JNK MAP kinase (JNK) signaling pathway. FOXO1 expression and OPG:RANKL ratio were low in bone *milieu* of hyperhomocysteinemic rats. By FOXO1 siRNA knockdown experiments we were able to prove that FOXO1 is integral to both OPG and p38 synthesis but not RANK ligand. Our results shed light on the underlying mechanisms for diminished osteoblast function and bone formation during hyperhomocysteinemia and also points out that modulation of PP2A/ FOXO1/MAP kinase pathway is a potentially useful therapeutic mechanism for curbing increased bone remodeling during disease pathologies like hyperhomocysteinemia

P46

The effects of 17β-estradiol on cellular proliferation and calcification of human mesenchymal stem cell during osteogenesis

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Aim: To study the physiology of estrogen action on osteoprogenitor cells we investigated the effect of estradiol on human adipose tissue-derived (hADSC) and bone marrow-derived stem cells (hBMSC).
Method: hADSC from 18 women and hBMSC from 8 women were exposed to osteogenic media (OSM) for 28 days. Estrogen receptor α blocker ICI 182780 (10^{-8}M) and aromatase inhibitor letrozole (10^{-7}M) and estradiol (10^{-9}M and 10^{-8}M) were used to examine the estradiol effect on cell division and calcification during osteogenesis.
Results: In hADSC, OSM induced a significant increase in endogenous estradiol production, which was reduced by letrozole by 30% ($P<0.05$). In hBMSC, the endogenous estradiol in unstimulated cells was higher than hADSC ($P<0.05$) and not significantly increased by OSM or blocked by letrozole ($P>0.05$). ICI significantly reduced the cellular proliferation by 20% in both cells ($P<0.01$ vs. OSM) and reversed the positive effect of estradiol on calcification in hADSC ($P<0.001$) but not in hBMSC ($P>0.05$). Letrozole reduced early cell division by 29% in both cells ($P<0.05$ vs. OSM) and this effect was reversed by the addition of estradiol at 10-8M ($P<0.05$), which further stimulated calcification in hADSC ($P=0.001$ vs. OSM) but not in hBMSC.
Conclusions: Endogenous estradiol is important for cell division after osteogenic stimulation while higher dose exogenous estradiol enhances calcification. These data support the concept that estradiol has direct effects on osteoprogenitor cell as well as bone resorptive cells.

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Micrornas & cell signaling pathways: Orchestrating osteogenesis and osseointegration

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Aim: Superior osseointegration and *in vitro* osteogenic differentiation properties of micro-roughened titanium implant surfaces make them interesting physiologically relevant models to study the molecular mechanisms involved in bone formation and osteogenesis. Previously, we have demonstrated downregulation of microRNAs potentially targeting the pro-osteogenic TGFβ/BMP & Wnt/Ca^{2+} pathways coupled with upregulation of these pathways on sand-blasted, large-grit, acid etched (SLA) and chemically modified hydrophilic SLA (modSLA) compared to smooth (SMO) titanium surfaces [1, 2]. The present study aimed at exploring the role of two such miRNAs (miR-ST & miR-TSA) in osteogenic differentiation and their influence on the cross-talk between TGFβ/BMP & Wnt/Ca^{2+} pathways.
Methods: Human osteoblast-like SAOS-2 cells were transfected with miRNA-mimics and cultured in osteogenic media. The expression of TGFβ/BMP & Wnt/Ca^{2+} pathways and osteogenic markers were tested using qPCR, Western Blot, ALP activity and Alizarin Red-S staining. Intrinsic expression of miR-ST & miR-TSA under the influence of pro-inflammatory cytokines (osteoinhibitory environment) was tested. Luciferase assays were performed to confirm targets for miR-ST and miR-TSA. Further the cross-talk between the TGFβ/BMP & Wnt/Ca^{2+} pathways was explored using recombinant human BMP2 and KN-93 (Wnt/Ca^{2+} pathway inhibitor).
Results and Conclusion: Overexpression of miRNAs demonstrated lower expression of TGFβ/BMP & Wnt/Ca^{2+} pathways and osteogenic markers. Intrinsic expression of miR-ST & miR-TSA was higher in presence of pro-inflammatory cytokines. Luciferase assays revealed targets in both TGFβ/BMP & Wnt/Ca^{2+} pathways. TGFβ/BMP & Wnt/Ca^{2+} pathways cross-talks were subsequently established. The study concluded an intricate miRNA modulated control of cell signaling pathways that guide osteogenesis in pro-osteogenic environments.

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P48

The roles of Schnurri family in differentiation of osteoblasts and chondrocytes

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Shn1, Shn2, and Shn3 are transcriptional factors, as well as scaffold proteins, of Schnurri family, all of which we found to be highly expressed in articular cartilage, calvaria, and long bone in adult mice, by real-time RT-PCR. Shn3 had been reported to promote proteasomal degradation of Runx2 as a scaffold to suppress osteoblast differentiation, thereby negatively regulates bone mass *in vivo* in mice. We asked if Shn3 induces *de novo* genes as a transcription factor to regulate osteoblast differentiation. An analysis of the expression profile of ST-2 bone stromal cells transfected with Alg2 siRNA by microarray showed Asparagine-linked glycosylation 2 homolog (*Alg2*) to be upregulated. Alg2 inhibited osteoblast differentiation without affecting protein level of Runx2. Instead, it inhibited the transcriptional activity of Runx2 in a dose dependent manner. Most interestingly, Alg2 suppressed BMP signaling, although the mechanism underlying is unclear. In contrast to Shn3, knockdown of Shn1 or Shn2 resulted in inhibited osteoblast differentiation, suggesting their roles are independent of Shn3. Shn1 had been shown to associate with transcriptional repressor MINT to suppress enhancer of a chondrocyte-specific gene *Col2a1*. Indeed, siShn1 enhanced BMP-induced chondrogenic differentiation of C3H10T1/2 and ATDC5 cells. However, knockdown of Shn2 or Shn3 inhibited the chondrogenesis. In summary, Shn2 promoted both osteogenesis and chondrogenesis, while Shn1 had positive and negative roles for differentiation of osteoblast and chondrocytes, respectively, which effects were mutually opposite to those of Shn3. Our results suggest the distinct roles of Schnurri family proteins in bone and cartilage.

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The direct anti-anabolic effect of sclerostin on the mechanical loading response in bovine bone *ex vivo*

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Sclerostin, expressed exclusively by mature osteocytes in bone, is associated with the osteocyte response to mechanical loading/unloading[(1)]. Our recent findings indicate that sclerostin targets pre-osteocytes/osteocytes to regulate bone mineralisation[(2)] and osteoclast activity[(3)], as well as inducing catabolic gene expression in osteocytes themselves and promoting osteocyte-mediated bone resorption[(4)]. The aim of this study was to examine the direct effects of sclerostin on loading-induced bone growth *ex vivo*. Bovine trabecular bone cores were perfused with osteogenic media at 37°C for up to 3 weeks in individual bone culture chambers. The cores were divided into 3 groups; a) mechanically loaded, b) mechanically loaded with continuous perfusion of 50 ng/ml recombinant human sclerostin and c) unloaded controls. Loading was accomplished using a Zetos™ bone loading system. Daily measurements of bone stiffness, media pH and ionic calcium concentrations were made. Gene expression in the bone was examined by real-time RT-PCR. Histomorphometric assessment was performed at the end of the experiment.
Bone cores continued to grow *ex vivo* and the stiffness increased with daily mechanical loading. This increase was blocked by sclerostin. Sclerostin also induced bone acidification and a net release of bone calcium and β-CTX. Sclerostin also completely abrogated loading-induced calcium/calcein uptake and induced an increase in the expression of bone resorption-associated genes. Histological examination revealed a significant increase in the size of the osteocyte lacunae in sclerostin-treated bone cores, suggesting a role for osteocytic osteolysis in this effect. Together, the results suggest that sclerostin directly antagonises mechanical loading-induced bone growth.

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The characterisation of SaOS2 osteosarcoma cells as an *in vitro* osteocyte-like cell model

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The importance of osteocytes in regulating bone homeostasis is becoming increasingly apparent, however the study of these cells has been restricted by their location within the mineralised matrix. Although mouse osteocyte-like cell lines have been established, there is a lack of equivalent human osteocyte cell lines. The SaOS2 human osteosarcoma cell line has been used extensively to study osteoblast behaviour, however the ability of these cells to further differentiate into osteocytes is unknown.
Therefore, we have investigated the differentiation of the SaOS2 cell line by culturing the cells under mineralising conditions over a 28 day time course and examining the temporal expression of osteocyte marker genes and cell morphology.
SaOS2 cells reproducibly synthesised a mineralised matrix over the culture time course, as demonstrated by Alizarin red staining and calcium quantification. This increase in mineral deposition was accompanied by an initial increase in the expression of the early osteocyte marker E11, followed by the mature osteocyte markers SOST, MEPE and DMP1, with the highest expression levels observed at day 21 and 28. The osteoblast marker RUNX2 was initially increased in the early stage cells but decreased with further maturation of the cells. SaOS2 cells cultured in 3D collagen gels acquired a dendritic morphology, characteristic of osteocytes, with multiple interconnecting cell processes.
These results suggest that SaOS2 cells have the capacity to differentiate into mature osteocyte-like cells under mineralising conditions. Thus, these cells can be used to further advance the field of osteocyte research.

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What determines osteocyte density in bone matrix? A computational model

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Introduction. The formation of new bone involves both the deposition of bone matrix and the formation of the osteocyte network. Osteocytes derive from osteoblasts that become trapped in the matrix during the deposition. In turn, osteocytes control osteoblast activity through their interconnected cell processes. In this contribution, a spatiotemporal computational model is proposed to investigate the osteoblast-to-osteocyte transition. **Aims**. The aim of the study is to elucidate the interplays between speed of new bone formation, rate of entrapment, and curvature of the bone substrate in determining the density of osteocytes in the new bone matrix. **Methods**. The computational model is formulated as a cell population model based on the material balance equation. **Results**. We find that newly formed osteocytes are generated at the moving deposition front with a density simply determined by the ratio of the instantaneous rate of entrapment and rate of matrix synthesis. Remarkably, the density of osteocytes generated is independent of the curvature of the bone substrate. The shape of the bone substrate, however, determines the evolution of the moving deposition front. **Conclusions.** The implications of these results are discussed in the context of (i) experimental measurements of osteocyte lacunae determined by synchrotron-based microCT from bone samples from the Melbourne Femur Collection, and (ii) a previous mathematical model of cell development within basic multicellular units [Bone **48** (2011): 918, BMMB In Press (2013)].

P52

Global deletion of the cyp27b1 gene results in impaired osteoclastogenesis and activity in splenocyte cultures generated ex vivo from mouse knockout models

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The metabolism of 25D by osteoclasts resulted in osteoclastogenesis with decreased resorptive activity, consistent with CYP27B1 conferring amelioration of osteoclastic resorption under 25D replete conditions[1,2]. In this study, we examined in more detail the ability of osteoclast precursors to differentiate into osteoclasts in mice lacking *Cyp27b1* expression (global *Cyp27b1*-KO mice). We hypothesised that the loss of CYP27B1 in mice would result in enhanced osteoclastogenesis due to the lack of the ameliorating effect of endogenous 1,25D synthesis.
Isolated splenocytes pooled from 4 mice each of either *Cyp27b1*-KO mice or their WT littermates, between 6-8 weeks of age. Osteoclast formation was measured by staining for the osteoclast marker tartrate resistant acid phosphatase (TRAP). Osteoclastic resorptive activity was measured by plating the cells onto a bone-like substrate. Gene expression was measured by real-time RT-PCR analysis of RNA isolated using Trizol.
TRAP staining indicated significant differences between genotypes in terms of osteoclast formation in response to RANKL/M-CSF. There were lower TRAP-positive multinucleated cell numbers at days 6, 7 and 8 in *Cyp27b1*-KO cultures ($p < 0.05$) compared to WT. There was an apparent difference between genotypes in terms of resorption where the RANKL/M-CSF-treated *Cyp27b1*-KO cells resorbed around 2.5% of total surface area in comparison to WT, which resorbed around 0.5% of the total surface area ($p < 0.03$). The preliminary data so far indicate potentially abnormal osteoclastogenesis due to the absence of CYP27B1 expression. Studies are ongoing to examine larger numbers of animals and relate altered osteoclast functionality to gene expression.

1. Kogawa *et al.* 2010 J Steroid Biochem Mol Biol 121:277-280
2. Kogawa *et al.* 2010 Endocrinology 151:4613-4625

P53

Histological localization of 15N-minodronate by isotope microscopy and its biological effects on bone cells

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Introduction: Monthly administration of minodronate, a third-generation bisphosphonate, has been recently highlighted for osteoporosis treatment in Japan, since minodronate appears to have a sustained effect. We examined the localization of ^{15}N-labeled minodronate in bone by isotope microscopy, thus obtaining insights on the behavior of bone cells after minodronate administration.
Materials and Methods: Eight-week old ICR male mice were injected with ^{15}N-minodronate (1mg/kg) via the external jugular vein. Mice were sacrificed after injection at 3 hrs, 24hrs, 1 week and 1 month, and tibiae were prepared for paraffin- or epoxy resin-embedding. Epoxy resin sections were examined under isotope microscopy for localizing ^{15}N-minodronate. Paraffin sections were used for ALP, TRAP and cathepsin K histochemistry.
Result and Discussion: In the tibial metaphyses, ^{15}N-minodronate was found in areas of both bone resorption and bone formation. Until 24 hrs post-injection, ^{15}N-minodronate was seen on those trabecular surfaces close to the growth plate. One week after injection, ^{15}N-minodronate was found on the superficial layer of the trabeculae, moving to the inner portions of the secondary trabeculae 1 month post-injection. Minodronate, therefore, may have binding affinity to bone surfaces undergoing both resorption and formation. Despite the high dosage of minodronate existent 24 hrs post-injection, TRAP/cathepsin K-positive osteoclasts did not seem to be inhibited, while osteoblasts were morphologically active and ALP-reactive. After 1 week, however, osteoclasts gradually became apoptotic. Altogether, these indicate that minodronate inhibits osteoclastic bone resorption mildly while allowing proper osteoblastic activation, which might sustain its anti-resorptive effects for a longer period.

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Antipsychotics inhibit human osteoclast formation and function.

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Antipsychotics are amongst the most commonly used medications and their use for schizophrenia and related conditions is often life-long. They act by inhibiting dopamine and serotonin signalling. While initially under-estimated, increased fracture risk with antipsychotic use is now a recognised major health problem. The mechanisms underlying this relationship are poorly understood. The aim of this study was to assess a possible direct action of antipsychotics on human osteoclast (OC) formation and function.

Gene expression levels of dopamine and serotonin receptors and respective transporters were assessed in CFU-GM-derived cells (OC precursors) and mature OC by real time PCR. OC formation and resorption in the presence of a number of antipsychotics was assessed in OC precursors treated with RANKL (125ng/mL) and M-CSF(25ng/mL) for 14d.

OC precursors and mature OC (14d RANKL treatment) expressed dopamine receptor 2 (DR2), but not DR1 or DR4. Mature OC also expressed the dopamine transporter. Serotonin receptors 5HTR1B, 2B and the serotonin transporter were expressed in both precursor and mature OC. Antipsychotics all dose-dependently inhibited OC formation and resorption over the range of 1-10μM in the order of potency: chlorpromazine>haloperidol=olanzapine=clozapine=quetiapine>risperidone.
Neither dopamine nor the dopamine agonist, ropinirole, affected these parameters.

These data demonstrate that a broad range of antipsychotics inhibit human OC formation and resorption, but with differing potencies. Given that dopamine agonists had no effect in this system, it is likely that the mechanism of action is independent from dopamine signalling blockade. In vivo bone loss from chronic antipsychotic use likely involves negative impact on bone formation.

P55

Chemotherapy agents induce Microphthalmia Transcription Factor (MITF) in osteoclast progenitors

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Previously we found the anti-cancer therapeutic and HSP90 inhibitor 17-AAG increases tumour growth in bone in an MDA-MB231 inoculation mouse model by increasing osteoclast numbers. Likewise *in vitro* we found 17-AAG increases RANKL-dependent osteoclast formation. We determined that the 17-AAG-mediated increase in osteoclast formation was dependent on heat shock factor 1 (HSF1)-mediated cell stress. This HSF1-mediated stress response increases the protein expression of the critical osteoclast transcription factor MITF. This suggests that other cell stressors might increase MITF levels and osteoclast formation.
HSP90 anti-cancer inhibitors, 17-AAG and the structurally unrelated NVP-AUY922, dose-dependently enhanced osteoclast formation from RANKL-treated bone marrow and RAW264.7 cells. 17-AAG and NVP-AUY922 increased MITF protein expression independently of RANKL. Both compounds cause stress responses in RAW264.7 cells: a HSF1-mediated stress response and activation of the stress MAP Kinase p38. Blocking p38 using SB203580 markedly decreased MITF protein induction by 17-AAG and also basal levels of MITF.
We have also tested the effects of chemotherapeutics, Doxorubicin, MG132 and Bortezomab on RAW264.7 cells. These compounds do not inhibit HSP90 but induced a HSF1-mediated cell stress responses. Moreover, Doxorubicin, MG132 and Bortezomab increase MITF protein expression in a dose dependent manner at both 24 and 48 hours. Both Doxorubicin and MG132 increased RANKL-stimulated RAW264.7 cell osteoclast formation.
These data indicate that cell stress inducing chemotherapeutic drugs increase MITF protein and osteoclast formation suggesting a similar mechanism of action. This suggests treatments which induce cell stress may have bone-damaging effects.

P56

Luman, an ER stress transducer, is involved in osteoclastogenesis through the regulation of DC-STAMP expression

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Osteoclasts differentiate from monocyte-macrophage lineage cells upon stimulation with two essential cytokines, receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Recent studies have clarified the signaling crosstalk during osteoclast differentiation, but the molecular mechanism responsible for osteoclastogenesis still remains to be elucidated.
Luman/CREB3, a transcription factor is a type II transmembrane protein belonging to OASIS family. OASIS family members localize at endoplasmic reticulum membrane under the normal condition. In response to endoplasmic reticulum stress, OASIS family members are subjected to regulated intramembrane proteolysis, and then cleaved N-terminal fragments harboring bZIP domain translocate into the nucleus. We recently demonstrated that some OASIS family members play a key role in development and differentiation of osteoblasts and chondrocytes. It is, however, unknown whether or not OASIS family is involved in osteoclastogenesis.
In this study, we found that the expression of Luman was increased after treatment of bone marrow macrophages (BMMs) with M-CSF and RANKL in a time dependent manner. Immunoblotting showed that Luman protein was cleaved at the membrane domain and activated during osteoclast differentiation. ShRNA-mediated knockdown of Luman in BMMs prevented the formation of multinucleated osteoclasts. Real-time PCR analysis revealed that the knockdown of Luman suppressed the expression of DC-STAMP, an essential molecule for cell-cell fusion during osteoclastogenesis. On the other hand, overexpression of N-terminus of Luman increased the expression of DC-STAMP in BMMs. These results suggest that Luman induces DC-STAMP expression, thereby regulating the cell-cell fusion during osteoclastogenesis.

P57

Interleukin-33 may stimulate functional osteoclast formation in the absence of RANKL

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Formation of active osteoclasts normally depends on RANKL; mice lacking RANKL or RANK lack osteoclasts, and RANKL blockade is an important anti-resorptive therapeutic approach. However, Mun et al (2011, Cell Mol Life Sci 67: 3883) found IL-33 treated human monocytes formed osteoclast-like cells independently of RANKL, although their ability to resorb bone or dentine was not examined. Two other studies showed no such IL-33 response. We therefore attempted to clarify this inconsistency.
We examined osteoclast formation from human cord blood monocytes (CBM) and adult monocytes cultured on dentine substrate. Both of these cell types formed abundant osteoclasts in response to RANKL. No osteoclasts formed from IL-33 treated CBM (from 4 individuals) but, in contrast, IL-33 treated adult monocytes did form osteoclasts, evident from TRAP positive multinucleated cell ($TRAP^+$ MNC) formation and resorption pit excavation. However, this occurred in only a minority of blood samples, 2 out of 11 individuals tested.
IL-33 did not cause $TRAP^+$ MNC formation in mouse bone marrow or RAW264.7 cells, however in the latter we observed small numbers of $TRAP^+$ mononuclear cells, suggesting a weak osteoclastic response. TGFβ enhances osteoclast formation, and indeed treatment of RAW264.7 cells with TGFβ + IL-33 resulted in 8.8 (±1.5) $TRAP^+$ MNC/well compared to 182.9 (±5.2)/well elicited by RANKL. We found IL-33 (like RANKL) elicited NFκB and NFATc1 dependent signals in RAW264.7 cells but did not induce MITF-E mRNA levels. These results indicate IL-33 may, albeit weakly and variably, stimulate osteoclast formation from adult human monocytes and RAW264.7 cells.

P58

Generation and characterisation of the osteoclast - specific Vitamin D receptor knock out mice

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Active vitamin D (1,25D) is necessary for both calcium homeostasis and skeletal health. While much of this activity is associated with the action of 1,25D on the intestine, vitamin D may play a bone-protective role by directly ameliorating the activity of osteoclasts. We have previously demonstrated that 1,25D locally synthesised by osteoclasts or its precursor cells has the ability to directly regulate the process of osteoclastogenesis *in vitro*. We have also reported that splenocytes from mice, in which the *Vdr* gene is globally deleted (VDRKO), form osteoclasts *ex vivo* with increased resorptive ability. VDRKO mice fed a normal calcium diet develop marked hypocalcaemia and hyperparathyroidism but exhibit no apparent change in osteoclast activity, despite high circulating 1,25D and parathyroid hormone (PTH) levels. While these data suggest that vitamin D activity in bone is important for osteoclast activity, direct mechanistic evidence is required *in vivo*. To assess the role of VDR in osteoclasts, an osteoclast-specific VDR knock out model (Ocl-VDRKO) was generated by breeding Cathepsin K-Cre with floxed VDR mice. Male and female Ocl-VDRKO mice were fed a standard diet and humanely killed at 6 and 12 weeks of age for analysis. Preliminary data from 6 week old female Ocl-VDRKOs show a strong trend towards a reduction (15%) in trabecular bone volume (P=0.07). These results support the hypothesis that osteoclastic VDR plays a role in the attenuation of physiological bone resorption. It is yet to be determined whether the VDR in osteoclasts plays a significant role in situations of increased bone resorption.

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The bone has endogenous and cell-autonomous circadian clock

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[Aims] The circadian clock is a mechanism which generates a day within a body, and is composed of clock genes such as *Per1*, *Per2*, *Cry1*, *Bmal1*, and *Clock*. This molecular machinery functions in various peripheral tissues such as liver and heart. Therefore, it is conceivable that bones, with diurnal metabolic oscillation, also have a circadian clock, though the molecular clock mechanism in bone physiology remains largely unclear. In this study, we tried to directly demonstrate a tissue-autonomous bone circadian clock, and unveil its characters.

[Methods] We used PERIOD2^{Luciferase} knock-in mouse which express firefly fused PER2 protein. By using this mouse, we can observe endogenous PER2 expression patterns through luciferase activity. The bone tissues were harvested and cultured in dishes. We performed real-time bioluminescence monitoring by using PMT-base equipment, and also analyzed spatio-temporal pattern by microscope-based high sensitive CCD-camera system. Furthermore, we analyzed the effect of the dexamethasone and forskolin administration to the cultured bone tissues.

[Results] We observed clear circadian rhythm of the bioluminescence from cultured bone tissues. The bioluminescence rhythm persisted over 6 months by medium change. Administration of dexamethasone or forskolin changed circadian clock phase in a time-specific manner.

[Conclusion] Our findings from long-term *ex vivo* culture revealed “tissue-autonomous” circadian rhythm in bone. Dexamethasone and forskolin can phase shift the bone clock. These results suggest that hormones, including glucocorticoids, likely function as internal time-cues for the bone clock.

P60

Neonatal leptin treatment partially reverses developmental programming of bone morphology

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An adverse prenatal environment may induce long term metabolic consequences. Offspring of rats subjected to under-nutrition during pregnancy develop obesity and neonatal leptin treatment has been shown to reverse this developmental programming. In the current study, we have quantified the bone phenotype of the offspring exposed to fetal under-nutrition, followed by neonatal leptin administration. Four groups of offspring from either under-nourished or *ad libitum* fed mothers during pregnancy were injected subcutaneously either with saline or recombinant leptin daily from 3 to 13 days of age. The female offspring were sacrificed at 170 days, the femurs were removed and examined by micro CT (Skyscan 1172). All bone parameters were adjusted to bodyweight.

The offspring from undernourished mothers had highly significant detrimental effects on the trabecular and cortical bone phenotype compared to the offspring from *ad libitum* fed mothers. There were reductions in trabecular bone volume (BV/TV, $p=0.03$) and trabecular number (Tb.N, $p=0.01$) and an increase in trabecular separation (Tb.Sp, $p<0.0001$). Cortical bones were smaller in volume both inside the periosteum ($p<0.0001$) and the endosteum ($p<0.0001$) but not significantly different in cross-sectional thickness. These detrimental effects in the bone morphological indices (BV/TV, Tb.N, Tb.Sp and cortical volumes) were partially reversed by the injection of leptin.

The findings of this study indicate that developmental adaptations during fetal life can be reversed by interventions in the neonatal period, and that alterations in perinatal leptin levels may play a crucial role in reversing the bone loss associated with fetal undernourishment.

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Fluorescent tracking of Vascular and Myogenic Cells during BMP-2 induced Bone formation

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Purpose: Recombinant human bone morphogenetic proteins (rhBMPs) can promote osteogenic differentiation and their implantation can produce ectopic bone and cartilage. The exact location and origin of cell types involved in bone formation is still in debate, with multiple progenitor lineages hypothesised to be involved.
We performed cell tracking studies to investigate the contribution of myogenic and vascular lineage cells to rhBMP-induced bone.
Methods: MyoD-cre:TdTomato and Tie2-cre:TdTomato mice were generated by breeding established Cre and fluorescent reporter mouse lines. A collagen-ceramic scaffold doped with rhBMP-2 was inserted into the hind limb musculature. Samples were harvested at 7d and 14 d, cryo-embedded, and sectioned. Myogenic and vascular lineage cells (TdTomato+) were visualised by confocal microscopy. Cells were co-labelled by immunofluorescence to label current lineage expression markers for myogenic cells (MyoD), chondrocytes (Sox9), osteoblasts (Osterix), and vascular endothelial cells (CD31).
Results: This system provides a unique method for tracking the contribution of different cell lineages to bone formation. TdTomato+ cells were present in the heterotopic bone in the Tie2-cre:TdTomato mouse, indicating contribution to bone formation. It was observed that TdTomato+ Tie2-lineage cells contribute to both the CD31+ and CD31- cell populations, suggesting contribution to vascular and non-vascular components of bone formation. No TdTomato+ cells were present in the bone nodule from the MyoD-cre:TdTomato reporter mouse in contrast with other previously published reporter systems.
Conclusions: Fluorescent lineage tracking mouse models are an effective method for examining the cellular contributors to bone formation

P62

Leucovorin prevents methotrexate chemotherapy-induced bone loss and bone marrow adiposity in rats potentially by modulating Wnt/β-catenin signalling and folate metabolism

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Methotrexate (MTX) chemotherapy, commonly used in treatment of childhood and breast cancers, is associated with bone loss and increased fractures. MTX inhibits dihydrofolate reductase (DHFR), reducing cellular folate pools and disrupting nucleotide methylation for DNA/RNA synthesis. Folate deficiency has been shown to reduce DNA methyltransferase-1 and enhance lipid accumulation in adipocytes. Leucovorin/folinic acid is readily converted to folic acid derivatives without DHFR action and is used clinically to alleviate MTX toxicity in soft tissues. Recently, we observed that MTX treatment causes bone loss and marrow adiposity, which is associated with deregulated Wnt/β-catenin signalling, and that Leucovorin rescue reduces MTX-induced bone loss. However, the mechanisms for Leucovorin bone protective effects remain unclear. In rats receiving 5 daily doses of MTX plus Leucovorin supplementation, Leucovorin rescue significantly prevented the MTX-induced reduction in trabecular bone volume and increased adipocyte-rich marrow. Compared to MTX alone, MTX+Leucovorin treatment up-regulated β-catenin protein and mRNA expression of Wnt10b, LEF and Cyclin D1, yet down-regulated Wnt antagonist sFRP-1 in metaphyseal bone. mRNA expression of folate receptor-1, through which folic acid enters the cell, was also found increased in the rescued group, potentially indicative of improved folic acid scavenging capacity in treated rats. DNA methyltransferase-1 mRNA expression was significantly reduced following MTX alone, but preserved in the Leucovorin supplemented group, suggesting attenuation of MTX-induced deregulation in DNA methylation. These findings suggest that the bone protective effects of Leucovorin during MTX chemotherapy may be direct in countering MTX-induced folate deficiency, as well as an indirect through regulating Wnt/β-catenin signalling.

P63

Expression of novel cartilage genes in growth cartilage and during maturation of cultured ATDC5 cells

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Growth of long bones occurs through the process of endochondral ossification, which depends on proliferation and hypertrophy of chondrocytes in growth cartilage (GC). In a subtractive hybridization study of equine cartilage, we identified a number of genes, the roles of which have not been characterized in GC. We examined the differential expression of a subset of these genes in the zones of GC and during maturation of ATDC5 (murine teratocarcinoma-derived chondrocyte-like) cells, with the aim of identifying genes associated with chondrocyte hypertrophy. Resting, proliferative and hypertrophic zones were microscopically isolated from growth cartilages of foetal horses and ATDC5 cells were cultured to confluence, then treated in medium containing insulin-transferrin-sodium selenite, triiodothyronine and ascorbate-2-phosphate to induce hypertrophy-like maturation. The expression of the genes of interest was examined by quantitative PCR. In horse GC samples, the genes encoding ATPase H+ transporting lysosomal d2 subunit (Atp6v0d2), DEAD box polypeptide-5 (Ddx5), triose phosphate isomerase-1 (Tpi1) and thymosin beta 4 (Tmsb4) were more highly expressed in the hypertrophic zone than in the reserve and proliferative zones, while Foxa3 was more highly expressed in the reserve zone than in the hypertrophic zone. In ATDC5 cells, expression of *Atp6v0d2*, *Ddx5* and *Tpi1* was found to be significantly up-regulated in cultures after four days of treatment than in untreated cultures. Expression of *Tmsb4* and *Foxa3* was down-regulated under these conditions. This study identifies novel hypertrophy-associated genes and indicates that ATDC5 cells represent an appropriate culture system for investigation of their functions during chondrocyte hypertrophy.

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Differential gene expression in growth cartilage and osteochondrosis

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Osteochondrosis (OC) is a developmental orthopaedic condition involving defective endochondral ossification and retention of cartilage in subchondral bone. We have recently shown expression of the hypertrophy-associated genes *MMP13, COL10* and *RUNX2* to be higher in equine OC cartilage than in control cartilage. The aim of this study was to identify genes associated with early OC, and examine whether they are regulated during normal chondrocyte terminal differentiation. From subtractive hybridisation of equine OC lesions and control cartilage, 79 putative differentially expressed genes were identified. In quantitative PCR (qPCR) studies, 9 genes were more highly expressed in OC lesions than in controls. Of these, osteopontin and integrin-binding sialoprotein are known to be upregulated with chondrocyte hypertrophy. An additional 23 genes poorly characterised in cartilage were examined in growth plate cartilage by qPCR. Expression of 13 genes (*ATP6V0D2, BAK1, DDX5, GNB1, PIP5K2A, PP-1B, RAP1B, RP-S7, SRP20, SUB1 homolog, TMSB4, TPI-1, WSB*) was increased and expression of three (*CHM1, FOXA3, SERPINA1*) was decreased in hypertrophic chondrocytes compared to cells in the resting and proliferative zones. The results provide further evidence that chondrocytes in OC lesions express hypertrophy-associated genes. Furthermore, we have identified a number of novel hypertrophy-associated genes; further studies will be required to determine whether they play functional roles in this process.

P65

Disrupted signaling of FGF23/klotho induces not only vascular calcification but also vascular ossification

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FGF23/klotho axis is well-known to regulate serum Pi concentration and its disrupted function induces elevated serum Pi concentration, often resulting in vascular calcification. In this study, in order to clarify histopathology of such vascular calcification, we have histochemically examined the aorta of 7-weeks old klotho-deficient (*kl/kl*) mice.
The tunica media of *kl/kl* aorta revealed broad calcified areas with an intense immunoreactivity of type I collagen, ALP and ENPP1, but a decrease reactivity of α smooth muscle actin. Under transmission electron microscopy (TEM) observations, vascular smooth muscle cells (VSMCs) embedded into or close to the calcified areas possessed developed rough ER and Golgi apparatus, resembling active form of osteoblasts. TEM-EDX demonstrated highly-concentrated Ca and P, but not C in the calcified areas in *kl/kl* tunica media, indicating the presence of calcium phosphates rather than calcium carbonates. Like bone, there were many matrix vesicle-like structures which contained calcium phosphate crystals, as well as numerous calcified globules surrounding the VSMCs. Strikingly, the advanced stage of calcified media of *kl/kl* aorta showed the formation of bone matrix-like structures. FGF23-positive osteocytes and TRAP-reactive osteoclasts were seen inside and on the bone matrix-like structure, respectively. However, αklotho-deficient mice did not show obvious calcification or ossification in tunica media, despite αklotho is necessary for FGF23/klotho function. It seems likely that the disrupted signaling of FGF23/klotho induced not only calcified media but also vascular ossification, probably by means of other factors caused by FGF23/klotho disruption, rather than elevated serum Pi concentration.

P66

Tenocyte viability and function following treatment with the protein component extracted from a hydroxyapatite-based product

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Aims: Hydroxyapatite (HA) products derived from extracellular matrices are often used as the basis for biomaterial scaffolds for bone regenerative therapy. Previously, we have demonstrated the positive osteogenic effects of the protein component of a HA product. The aim of this study was to understand how the same protein component affects tenocyte viability and function.
Methods: EDTA was used to extract 0.1mg/mL protein from a bovine-derived microcrystalline HA product. Primary rat tenocytes were cultured with the protein extract on 2D plastic, 2D collagen-coated surfaces and within a 3D collagen gel. Proliferation, differentiation and matrix production were assessed using alamarBlue® fluorescence, real-time PCR and Sirius red staining, respectively.
Results: Tenocyte viability was dose-dependently decreased in all culture methodologies, with the highest concentration of protein extract (100μl/mL) significantly decreasing viability by approximately 20%. mRNA expression of extracellular matrix proteins biglycan and fibromodulin appeared to increase with higher concentrations of the protein extract in tenocytes cultured on plastic. However, in collagen-coated cultures, genes important in tenocyte differentiation, including biglycan, tenascin-C and collagens I and III, demonstrated a general trend of reduced expression.
Conclusion: Previously, we shown that the protein component of this HA product has ideal properties for bone regenerative therapy, with increased osteoblastogenesis and reduced osteoclastogenesis observed. Here we have demonstrated that the same protein component decreases tenocyte viability and may impair tenocyte function. Due to the proximity of tendon tissue to bone, it is important to consider that treatments designed for bone regeneration may have detrimental effects on neighbouring tissues.

P67

A novel pathway of hedgehog signaling modification by primary cilia in odontoblasts

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Primary cilia are known to be a biosensor monitoring the extracellular environment in order to modulate the cellular activity. We previously have reported a possibility that the primary cilia in odontoblasts control the cell proliferation via Rac1 signaling. On the other hand, dexamethasone (DEX) and b-glycerophsphate (b-GP) have been reported to influence the odontoblast differentiation. In this study, we revealed the molecular mechanisms of DEX and b-GP acting on the odontoblast differentiation via primary cilia.
Administration of either DEX or b-GP upregulated the expression of *Dentin sialophosphoprotein* (*Dspp*) mRNA in odontoblast cell line KN-3 cells revealed by real-time PCR. On the other hand, while b-GP increased the expression of *Gli2*, a hedgehog signaling marker, significantly, DEX induced the decrement of *Gli2* mRNA expression. Moreover, in order to investigate whether these results were directly mediated by the primary cilia, we employed RNAi strategy to knockdown *Ift88* in KN-3 cells. As a result, *Dspp* mRNA levels were decreased even if either DEX or b-GP were administered. However, *Gli2* mRNA levels in the presence of either DEX or b-GP showed an inversed trend when compared with the intact ciliated cells. These data collectively demonstrate that DEX and b-GP regulate the odontoblast differentiation through primary cilia. Our findings further imply the interaction of DEX and b-GP on the hedgehog signaling by primary cilia.

P68

Assessment of the rate of decalcification and histological outcome of microwave-assisted and traditional methods of bone decalcification

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Background: Decalcification (Decal) of bone tissue is required for histological analysis, but can be time-consuming and variable. We hypothesise that microwave radiation will accelerate decalcification without adversely affecting histological analysis. The aim was to compare the rate and histological outcome of decalcification using traditional methods and microwave radiation at 37°C (KOS Microwave Tissue Processor).
Method: Rat long bones and bovine cortical bone samples were decalcified under different conditions using 14% EDTA. Bovine bone was Decal in EDTA at 4°C, and 25°C under constant rotation; and 37°C using the (n = 5 per group). Rat bones were decalcified in EDTA at 4°C and using the KOS, and a control group included at 37°C alone. Wet weight was taken at regular intervals and the EDTA solution was sampled for Ca and PO4 using ICP-MS and Atomic Absorption spectroscopy. Decal samples were embedded in paraffin for histological analysis using 0.2% toluidine blue.
Results: KOS Decal was approximately 4.5 times faster than cold and room temperature, but only 0.6 times faster than Decal at 37°C. Ca and wet weight were useful indicators of the end point of decalcification, but PO4 concentrations were not. Histological analysis indicated variation in stain intensity between traditional and KOS Decal.
Conclusion: Microwave radiation has a profound effect on the rate of decalcification of bone samples, decreasing the time taken from weeks to days. Histological analysis was altered by this method, but monitoring pH in the Decal solution improved staining.

P69

***In vivo* monitoring of bone and fat in mice using the Quantum Fx scanner**

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Monitoring of bone mass and fat mass *in vivo* is usually performed using dual energy X-ray absorptiometry (DXA), providing quantification of fat and lean mass and the determination of BMD and BMC. DXA is 2-dimensional and therefore, volumetric bone data, as well as differentiation of visceral and subcutaneous fat is not possible.
Currently, we have been piloting the use of an *in vivo* 3-dimensional microCT, the Quantum Fx. This technology scans down to 10mm, and generates high-quality CT images which can be segmented and volumes rendered based on tissue density. The Quantum Fx has several advantages: short scan time and low X-ray doses, thus scans can be performed more frequently and applied to longitudinal studies.
Like existing microCT, the Quantum Fx produces consistent scans of skeletal tissue; however we sought to examine its ability to track changes in adipose tissue. We have analysed a set of mice over time to determine its ability to monitor changes in fat volume. The total fat volumes generated from the scan correlated with DXA data from the same mice. By further segmentation of the fat into abdominal subcutaneous and visceral fat, we were able to track the deposition of fat over time. In addition to the *in vivo* capabilities of the Quantum Fx, excised bones can be scanned at high resolution for cortical and cancellous analysis.
Based upon our initial observations, the Quantum Fx is capable of simultaneous monitoring of both bone and adipose tissue in longitudinal studies.

P70

In vitro tenocyte mechanobiological studies mimicking *in vivo*

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Aims: The musculoskeletal system experiences severe mechanical strain often causing trauma; this has led to increased studies evaluating mechanical strain on musculoskeletal cells. Most studies use devices where levels of strain applied do not correspond to those experienced by the cell. Therefore, we utilised a novel cell stretching device, where strain is evenly distributed across the culture surface, with the aim of mimicking an in vivo study of tendon collagen synthesis.
Methods: In an attempt to mimic an in vivo experiment looking at collagen synthesis following 1hr of knee extension [1], primary rat tenocytes were cultured on collagen type I coated silicone and exposed to 4% strain for 1h. Sirius red staining and real-time PCR were performed over 72h to measure collagen protein synthesis and gene expression.
Results: Collagen type III expression increases between 0 and 48 hours, and then decreases between 48 and 72 hours. Collagen type I expression continues to increase until 72 hours post-stretching. These results differ slightly from Miller's study, which measured a peak at 24 hours after exercise, but displays a similar trend.
Conclusion: This study demonstrates that our novel cell stretching device is a useful tool in mechanobiological studies, and while the in vitro results differed slightly from those observed in vivo, we believe this device is capable of mimicking the in vivo environment.

1. Miller, B.F., et al, *Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise.* J Physiol, 2005. **567**: p.1021-33.

P71

The features of ultrastructure biomineral of dentin lower incisors in intact rats of different ages

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Using X-ray diffraction to study the features of ultrastructure biomineral of dentin lower incisors in intact rats of different ages.
The study was conducted on 90 intact immature male rats (with a average weight 35-40 g), mature (130-140 g) and elderly age (310-320 g). Periods of observation were 7, 15, 30, 90 and 180 days. Gravimetrically prepared dentin of incisor and its powder was investigated on the apparatus DRON-2,0 with goniometric attachment GUR-5. Size of crystallites and coefficient of microtexture were investigated on obtained diffractograms.
In immature intact rats, size of crystallites increased from 7 to 180 per day from 26,47±0,21 to 28,54±0,25 nanometers and coefficient of microtexture – from 0,48±0,01 to 0,55±0,01 standard units. In mature rats, the size of crystallites also increased from 28,81±0,38 to 32,44±0,36 and coefficient of microtexture from 7 to 90 day – from 0,56±0,01 to 0,57±0,01, and then to 180 days in some decreased to 0,56±0,01. In elderly rats, size of crystallites increased from 34,16±0,49 to 36,99±0,31 and coefficient of microtexture, however, was reduced from 7 to 180 per day with 0,57±0,01 to 0,50±0,23.
Thus, the most active processes of growth and the formation of the unit cells biomineral of dentin lower incisor and the degree of order of the crystal lattice occurred in intact immature rats, which slowed in mature rats. In elderly rats showed signs of destabilization of the crystal lattice of biomineral dentin incisor and increase the degree of its amorphous, which could probably be linked to development of age-related generalizied periodontitis.

P72

Lateral-projection areal bone mineral density (aBMD) predicts vertebral failure load better than posterior-anterior aBMD

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Areal Bone Mineral Density (aBMD) of the lumbar spine measured by Dual Energy X-ray absorptiometry (DEXA) provides suboptimal estimates of individualized vertebral fracture risk. Bone distribution, microstructure and strength vary within the vertebra. Thus, vertebral subregional aBMD measurements from lateral-projection DEXA might be more informative about vertebral fragility than standard posterior-anterior (PA) projection DEXA. Therefore we measured vertebral subregional aBMD on lateral DEXA and assessed its ability to predict vertebral mechanical failure load, compared to whole vertebral PA DEXA.

Eight embalmed and 4 fresh-frozen human cadaver spines were scanned by DEXA in PA and lateral projections; subregional aBMD was measured from lateral images (L2, L3), dividing the vertebral area into three equal subregions (anterior, middle, posterior). The vertebrae were then excised and mechanically tested in compression to determine failure load. Whole-vertebral aBMD in lateral projection (R^2=0.70, $p<0.05$, embalmed; R^2=0.72, $p<0.05$, frozen) was a better predictor of failure load than in PA-projection (R^2=0.36, $p<0.05$ embalmed, R^2=0.30, $p=0.15$ frozen).

aBMD differed significantly between subregions for both embalmed and fresh-frozen spines (repeated-measures-ANOVA, $p<0.001$), with posterior subregions exhibiting the highest aBMD (paired t-test, Bonferroni correction, $p<0.01$ for both groups). aBMD from the anterior subregion was a better predictor of failure load (R^2=0.66, $p<0.05$, embalmed; R^2=0.81, $p<0.05$, frozen) compared to other subregions.

DEXA is the clinical tool most widely used to inform clinical decisions regarding fracture risk. This ex vivo study highlights the capability of DEXA-derived lateral subregional aBMD to predict vertebral strength, and supports further exploration of the clinical application of lateral-projection DEXA analysis.

P73

Utility of reference-point indentation for characterization of brittle murine bones

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Reference-point indentation (RPI) is a recently-developed cyclic micro-indentation technique designed to assess bone material properties in a minimally-destructive manner. We applied RPI to assess the oim mouse model of osteogenesis imperfecta. Contralateral femurs were collected from 12wk wt, heterozygote (oim/+) and homozygote (oim/oim) mice for mechanical characterization by 3pt-bending or RPI analysis. Initial RPI testing was conducted at the midshaft with a BioDent (Active Life Scientific) instrument using BP3 probes and a maximum force of 2N. As larger test forces increase the magnitude of measured parameters and can amplify differences, test forces up to 7N were applied to wt and oim/+ bones.
3pt-bending tests confirmed the expected mechanical deficits in oim/oim bone, with an intermediate phenotype in oim/+, both contributed to by weaker structural and material properties. The 2N RPI test protocol did not reveal any significant differences between groups, yet a higher test force (4N) routinely resulted in complete failure of the cortex in oim/oim femurs. Higher test forces (5N, and then 7N) were subsequently applied to oim/+ and wt bones. Increased IDI (Indentation Distance Increase) in oim/+ bone was revealed in the 7N test (8.3±1.0 vs 6.6±0.8 μm, $p<0.05$), consistent with previous reports of an inverse correlation between IDI and toughness for large rodent (rat) bones.
We conclude that RPI can quantitatively distinguish between bone from wt mice and those with a mild brittle bone phenotype (oim/+), but structural failure remains a confounding factor that limits the utility of RPI in the assessment of very fragile mouse bone.

P74

Integrating micro CT indices, CT imaging and computational modelling to assess the mechanical performance of fluoride treated bone

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In this study we present a novel non-destructive framework for evaluating the influence of low-dose fluoride treatment on the bone strength of 23 patient biopsies. Computational finite element (FE) models of each biopsy were subjected to a range of non-destructive loads including compression, shear and torsion. The modelling framework was validated against a 3D printed biopsy simulant with known material properties subjected to compression using an Instron machine. The primary outcomes from this study were that mechanical strength was not significantly correlated to low-dose (less than 10 mg/day) of fluoride levels (one-way ANOVA, P-values > 0.62). However, when bulk bone material properties were derived from DXA bone mineral density (BMD) from each patient's proximal femur a non-significant linear decline in mechanical strength with increase in fluoride was predicted, consistent with previous studies. When the same material property was used for all bones (to evaluate bone architecture influence) then mechanical strength was consistent with the variation of micro CT derived percentage bone volume (BV/TV). The secondary outcomes from this study were that in compression, BV/TV was observed to be a strong surrogate measure for mechanical strength (R^2=0.83), while bone surface density (R^2=0.6), trabecular thickness (R^2=0.5) and intersection surface (R^2=0.6) also explained the variation of mechanical strength well. However, trabecular separation and trabecular number were mildly correlated with mechanical strength (R^2 of 0.31 and 0.35, respectively). This framework may be used to inform clinical trials about anabolic treatment effects and highlight which micro CT indices are good surrogate measures for mechanical strength.

P75

Atypical femoral fractures are associated with physiological patterns of bone tensile deformations

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Atypical femoral fractures (AFF) are predominantly transverse stress fractures from the lateral aspect of the subtrochanteric and diaphyseal region for which long-term bisphosphonate treatment and tibio-femoral geometry have been suggested as important risk factors. We examined the physiological loading environment to test the hypothesis that typical AFFs onset location is associated with high cyclic tensile strains occurring during typical daily activities. The study was based on a model from a donor femur (female, 81-years-old) and motion data from a volunteer (female, 25-years-old), who matched the donor anthropometry (body weight, height, and body intra-segmental lengths). Motion data included skin marker trajectories and ground reaction forces during walking, chair up and down, stair ascent and descent, step up, and squatting. The model was obtained by coupling the donor's lower-limb musculoskeletal model validated against published measurements of the hip reaction force and the finite-element model of the donor's right femur validated against measurements of cortical strains. All the activities induced the peak tensile strain in the lateral femoral diaphysis corresponding with the most common AFF location of onset. The tensile strain magnitude, however, was activity-dependent, with walking and stair descent inducing the highest tensile strain magnitude (0.2-0.5%). We conclude AFFs are associated with the physiological pattern of bone tensile deformation, while daily activities are likely to be AFF risk factors by causing different tensile strain intensities over a number of cycles. In this regard, walking is a critical activity because it induces high tensile strain levels over a significant number of daily cycles.

P76

Inter- and intra-individual variation in human osteon geometry across age

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Variations in the bone remodelling process determine differences in osteon cross section and in the anterior quadrant of the human mid-shaft femur these have been shown to be associated with an individual's age and weight. The current study aimed to extend the measurements to include data from the medial and lateral cortices. If differences could be detected between the medial (compression) and lateral (tension) sides this could help to elucidate the previously observed effect of weight.
The study materials were digital images of microradiographs of whole cross-sections of the femoral mid-shaft from 86 individuals. A total of 18,767 osteons were measured manually within the anterior, medial and lateral regions using imageJ software.
Age was significantly related to osteon size for all geometric variables in all areas ($p<0.05$). This relation was negative for area and diameter (decreasing with age) and positive for circularity (increasing circularity with age) for all regions. Partial eta-squared values revealed that age accounted for the largest proportion of variance in the medial (area 8.0%, diameter 4.7% and circularity 12.9% and 11.8%, 9.5% and 14.2% respectively in the lateral).
Comparison between anterior and lateral showed a significant difference for the effect of weight on both area ($p=0.044$) and diameter ($p=0.023$) with smaller osteons in the lateral region then in the anterior. No significant difference was seen between lateral and medial regions which may eliminate direction of loading as a factor in the relationship between osteon geometry and weight.

P77

Validation of ultrasound transit time spectroscopy of solid volume fraction in bone: Marrow replica models by comparison of geometric calculation, computer simulation, and experimental studies

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Objective: The acceptance of broadband ultrasound attenuation (BUA) for the assessment of osteoporosis suffers from a limited understanding of ultrasound wave propagation through cancellous bone. Langton has proposed a concept of parallel sonic rays [1]; the transit time of each ray defined by the proportion of bone and marrow propagated. A Transit Time Spectrum depicts the proportion of sonic rays having a particular transit time, effectively describing lateral inhomogeneity of transit times over the surface of the receive ultrasound transducer. We tested the hypothesis that solid volume fraction (SVF) may be derived from Ultrasound Transit Time Spectroscopy (UTTS).
Materials & Methods: A range of acrylic:water models replicating bone:marrow was manufactured, varying in thickness of acrylic in one dimension normal to the direction of propagation, thereby exhibiting a range of transit time lateral inhomogeneities. 1 MHz broadband ultrasound transmission signals were recorded. Transit time spectra were derived a) via computer simulation of Langton's concept, and b) via digital deconvolution of the experimental data. The solid volume fraction (SVR) of each model was calculated using geometrical data ($SVF_{calculated}$) and compared to SVR values a) predicted by computer simulation ($SVF_{predicted}$), and b) derived experimentally ($SVF_{experimental}$).
Results: Comparison of $SVF_{calculated}$ with $SVF_{predicted}$ and $SVF_{experimental}$ yielded agreements (R^2) of 99.9% and 97.33% respectively, as shown in Figure 1.
Conclusion: The high agreement of both predicted and experimental SVF values with those geometrically calculated supports the Langton theory. Future work will consider more complex replica structures.

[1] C. M. Langton, "The 25th anniversary of BUA for the assessment of osteoporosis: time for a new paradigm?," *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine*, vol. 225, no. H2, pp. 113–125, Feb. 2011.

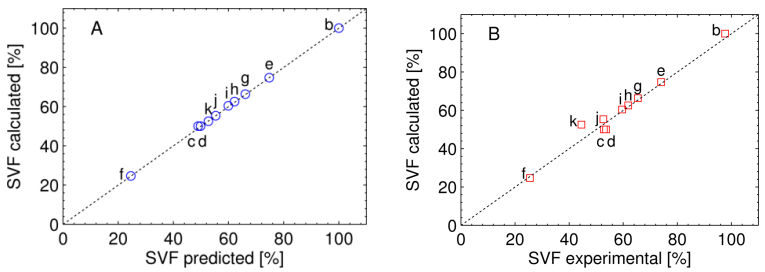

Figure 1: Calculated SVF values versus those A) predicted using computer simulation of Langton's theory and **B)** derived experimentally.

P78

The specific surface of cortical bone and its influence on the temporal evolution of cortical porosity during age-related bone loss

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Our aim was to investigate the relationship between specific surface and porosity as measured in 3D by high resolution micro-CT. This relationship had previously been studied by Martin [1]. However Martin's data was based on 2D histological measurements and with a limited number of data points.
Images from femoral cortical bone samples of the Melbourne Femur Collection were obtained using synchrotron radiation micro-CT (SPring8, Japan). Sixteen individuals were analysed in order to find porosity values ranging from 1% to 70%. In an attempt to understand the evolution of the specific surface as porosity increased a series of geometrical models were developed with increasingly complex assumptions about the manner in which pores were spaced and merged.
The specific surface values obtained using micro-CT were noticeably higher than those obtained by Martin. To test the possibility that this was due to geometric effects the same analysis was applied to 2D samples but the range of values remained similar to the originals.

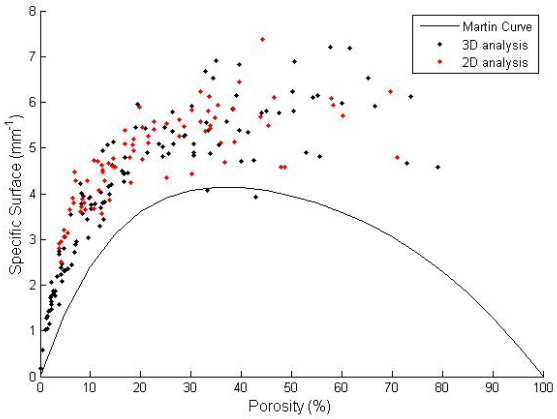

Figure 1: Specific Surface vs Porosity

Micro-CT provides a technology to accurately estimate the bone specific surface as a function of porosity. We believe that the differences between our curves Martin's are due to the use of 3D data from well-documented samples thus overcoming the limitations of histology and provenance of Martin's study.

[1] Martin RB., Porosity and specific surface of bone, *Crit Rev Biomed Eng*; **10**; 179 – 222; 1984.

P79

Biophysical properties of rats humerus after implantation into the tibia of biogenic hydroxyapatite, saturated with zinc

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Aim: to study the biophysical properties of the humerus in rats implanted into the tibia of biogenic hydroxyapatite, saturated with zinc at various concentrations.
Materials and methods. The study was conducted on 168 white mature male rats, divided into 4 groups: 1st group - animals in which the proximal part of tibial shaft implanted blocks of hydroxyapatite without zinc saturation (this group served as a control for the following groups), 2nd-4th groups of rats, in which the proximal part of tibial shaft implanted blocks of hydroxyapatite saturated with zinc at concentration 0.20%, 0.50% and 1.00% respectively. Periods of observation were 7, 15, 30, 60, 90 and 180 days. The animals were taken from the experiment by decapitation under ether anesthesia, the humerus were isolated and identified some of its biophysical properties (tensile strength and minimal work of destruction) on an universal testing machine P-0,5.
Results. The tensile strength and minimum work of destruction was significantly more than in 1st group at 7th day by 25.08% and 25.74% in 2nd group ($p<0.05$). The minimum work of destruction increased from 7 to 180 days by 22.37%, 5.60%, 17.53%, 11.69%, 19.14%, 7.31% in 3rd group, and - by 30.14%, 8.41%, 26.23%, 12.30%, 22.23%, 9.34% in 4th group ($p<0.05$).
Conclusion. Thus, saturation of implanted into the tibia biogenic hydroxyapatite with zinc accompanied by an increase of the amplitude and duration of the smoothing effect of implantation on the strength of rats` humerus. The optimum concentration of zinc, according to our data, is 1.00%.

P80

Racial differences in cortical and trabecular microstructure arise during puberty

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To examine the underlying structural and biomechanical basis of the lower fracture risk in Chinese than Caucasians, distal radius images acquired using high-resolution pQCT (XTreme CT, Scanco) were quantified for 81 healthy Chinese and 103 Caucasian women aged 7 to 46 years using Strax 1.0 (Zebaze et al 2013). The proportion of cortex that is void (porosity) was quantified as the average of void spaces in each voxel. The 1st CT slice commenced at the 4% of the radius length to adjust for effects of bone length on morphology.

There was no detectable structural difference between races before puberty. After menarche, Chinese had a similar cortical area but smaller medullary area relative to Caucasians (105.6 vs.124.4 mm^2 $p<0.05$) and lower porosity of the compact-appearing cortex, outer and inner transitional zones (36.5 vs. 40.4%; 45.8 vs. 49.1%; 74.2 vs. 75.9% respectively, $p = 0.1$). Chinese pre-menopausal women had a similar cortical area but a smaller medullary area (111.1 vs. 138.4 mm^2, $p<0.001$), and lower porosity of the corresponding cortical regions (21.0 vs. 23.1%; 31.3 vs. 33.0%; 67.8 vs. 69.3% respectively, all $p<0.01$).

Modelling and remodelling assemble a more robust skeleton in Chinese than Caucasian women. The smaller bone has a relatively thicker and less porous cortex perhaps attributed to excavation of a smaller medullary canal and fewer osteons. These morphological features may contribute to racial differences in fracture risk.

P81

Up-regulation of 11beta-hydroxysteroid dehydrogenase 2 decreases the sensitivity of cancer cells to glucocorticoids

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Aims: Glucocorticoids are widely used to treat haematological malignancies since they induce apoptosis, inhibit proliferation, and sensitise cells to chemotherapy drugs, but many solid cancer cells demonstrate glucocorticoid resistance. We hypothesize that glucocorticoid resistance is due to expression of 'pre-receptor' isozymes, 11b-hydroxysteroid dehydrogenase (11b-HSD) 1 and 2, which activate and inactivate glucocorticoids, respectively. This study aims to elucidate the role of 11b-HSD2 in cancer cell glucocorticoid resistance.
Methods: Osteosarcoma (MG-63) and breast cancer (MDA-MB-231 and MCF-7) cells were treated with 10^{-7}M and 10^{-6}M cortisol for 8 hours and mRNA expression for glucocorticoid receptor (GR), 11b-HSD1, 11b-HSD2 and the downstream target gene, glucocorticoid-induced leucine-zipper (GILZ) were determined by qRT-PCR.
Results: MG-63 and MDA-MB-231 expressed similar basal mRNA levels for GR and 11b-HSD1, while MCF-7 had 3- and 8-fold lower expression compared to those two cell lines, respectively. While 11b-HSD2 mRNA is not detected in normal bone cells, its expression was detected in all three cancer cell lines. However, breast cancer cells expressed 11b-HSD2 at higher levels (4-fold in MCF-7, 3-fold in MDA-MB-231) when compared to MG-63. GILZ expression was determined after cortisol treatment. Cortisol induced GILZ expression by 11.7-fold at 10^{-7}M, but showed no further induction at 10^{-6}M. In contrast, MDA-MB-231 cells only showed a 2.8-fold induction at 10^{-7}M (no further induction at 10^{-6}M). In MCF-7 cells, GILZ expression was unchanged at both concentrations.
Conclusions: Our results suggest that breast cancer cells are resistant to glucocorticoids and this appears to be due to higher expression of 11b-HSD2, decreasing intracellular glucocorticoid availability.

P82

Molecular mechanisms of bone invasion by oral squamous cell carcinoma (OSCC)

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This three-part study aims to investigate signalling pathways involved in the crosstalk between OSCC cells, osteoblasts and osteoclasts. Firstly, conditioned medium (CM) was collected from OSCC cells (SCC15 and SCC25), and from osteoblasts of hFOB, then used for indirect co-culture: OSCC cells were treated with CM from hFOB and vice versa. Zymogenic activities of both MMP-2/-9 were increased in OSCC cells with CM from hFOB. The RANKL/OPG ratio, zymogen and protein expression of MMP-9 were increased in hFOB with CM from OSCC cells. Secondly, to determine which component of CM caused gene expression changes, OSCC cells of SCC25, HN5, and Tca8113 were artificially induced to display epithelial-mesenchymal transition (EMT) by using TGF-β1(5 ng/mL). Expressions of EMT markers Twist1 and N-cad were up-regulated; Snail1 and E-cad down-regulated. CM of OSCC cells pre-treated with TGF-β1 prolonged the survival of mature osteoclasts up to 4 days. Thirdly, a plasmid with the inhibitor of MCP-1 (7ND vector) was transfected into SCC25 cells, and stabilized SCC25 cells with 0.6 μg of 7ND vector (SCC25-7ND) were generated. 10% CM of SCC25-7ND cells efficiently inhibited the formation of osteoclasts. OSCC cells were further injected onto the surface of calvaria of nude mice. Well-differentiated SCC was formed in both groups of SCC25 and SCC25-7ND cell injections, tumour cells invading bone, osteoclasts locating in typical resorption lacunae. TRAP staining showed significant differences between two groups for cell numbers of osteoclasts. These studies prove that understanding and interfering with these pathways may provide therapeutic approaches.

P83

Assessment of structural and remodeling indices in periarticular bone tissues in the osteoarthritic femoral head: influence of age and gender

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Aims: To assess the influence of age and gender on both structural and remodeling indices of the periarticular bone in osteoarthritic femoral heads.

Methods: 110 human femoral heads were collected from patients with severe OA undergoing hip arthroplasty. A bone cylinder was extracted from the primary compressive stress region in each femoral head. All samples comprised three different regions: subchondral bone plate, subchondral trabecular bone and deeper trabecular bone. Each cylindrical bone specimen was scanned by micro-CT, for the analysis of bone structure parameters. After scanning, the specimens were embedded in methyl methacrylate, and sectioned on a microtome. The sections were then stained by Goldner Trichrome for the measurements of remodeling indices.

Results: In the subchondral bone plate and subchondral trabecular bone, no significant differences between males and females were found for structural and remodeling indices. However, in the deeper trabecular bone, there were significantly higher Tb.Th and lower Tb.N in females. Accordingly, some resorption parameters were significantly less in females. In all the three different regions, none of the examined structural or remodeling indices was found significantly dependent on age, neither for males nor females.

Conclusion: The structural and remodeling indices in the subchondral bone plate and trabecular bone of osteoarthritic femoral heads are completely independent of gender. However, deeper trabecular bone is still dependent on gender, where thicker and more trabeculae were found in females. OA changes the age dependence of both the structural and remodeling indices, in all the three different regions of periarticular bone.

P84

Impaired muscle function in a mouse model of osteoarthritis

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In knee osteoarthritis (OA) loss of muscle mass, strength and function is common and this contributes to impaired quality of life through deterioration of functional capacity. We examined the changes in muscle function during the onset and progression of OA in a mouse surgical model of OA (destabilisation of the medial meniscus, DMM-OA) to better understand the relationship between OA development and muscle function.
Male C57BL/6 mice underwent DMM or sham surgery on the right knee only. Tibialis anterior (TA) muscle function was assessed in situ at 1, 4 and 8 weeks post surgery. At 1 week post surgery, there was no difference in either the tetanic or twitch force parameters between the DMM-OA and sham TA muscle, but improvement in both groups was observed with time. However the tetanic parameters absolute force and specific force were both reduced in DMM-OA muscle compared to sham muscle at 4 weeks and 8 weeks post surgery ($p<0.05$). At 8 weeks post-surgery the twitch force was reduced ($p<0.05$) and the time taken to relax to 50% of peak twitch force (1/2RT) was also increased ($p<0.001$) in DMM-OA muscle compared to sham. The changes in muscle function parameters did not correlate with the degree of synovitis within the joint, but did correlate with measures of cartilage damage.
Together our data suggests that similar to human OA, muscle function in DMM-OA deteriorates with worsening OA joint damage. Further investigation of these changes may yield insight into the mechanisms mediating muscle degeneration in human knee OA.

P85

Post-traumatic osteoarthritis and lower limb muscle functions

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Anterior cruciate ligament (ACL) injury is a culprit to post-traumatic osteoarthritis (PTOA) among young generations. At the onset of ACL injury, ligaments are not the only components that are influenced by high impact load. In fact, other components of the knee joint including meniscus, cartilage, subchondral bone and lower limb muscles may be affected. Majority of studies investigating the onset of PTOA during high-risk movements lack the role of muscle forces that may load or unload the knee joint. For instance, quadriceps and hamstring activations increase the joint reaction forces. This increased joint force may potentially lead to cartilage or subchondral bone damages. To find the role of individual muscles that can load or unload the knee joint during landing, we simulated a single-leg landing task of 8 athletes using 92 muscle-tendon units and 23 DOF in OpenSim from two heights: 30 and 60cm. The results suggest that ankle plantaroflexor's role is vital in reducing the knee joint force. Gastrocnemius muscle forces (<1 BW) were negligible when compared to soleus force (3-9BW) for both landing heights at peak ground reaction force. Soleus does not span the knee joint and it can provide stability to the ankle and knee joint without loading the knee joint. Soleus and hamstrings prevent excessive tibial rotation. Large soleus force in this study is equal to less hamstring muscle force required (i.e. potentially less joint reaction force) to stabilize the knee joint during landing. These findings may also have implications for training purposes following ACL reconstruction to prevent PTOA.

P86

Rheumatoid arthritis and incident fracture in women: A prospective study

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We examined fracture incidence in women with clinically diagnosed rheumatoid arthritis (RA). Women aged ≥35years, resident in the Barwon Statistical Division (BSD), south-eastern Australia, and with a diagnosis of RA from the sole rheumatology practice serving the region between 1994-2001 were included as cases (n=1,008). The control population (n=172,422) comprised the entire female BSD population aged ≥35years, excluding women already identified as having RA. Incident (first) fractures 1994-2001 were identified from the prospective Geelong Osteoporosis Study Fracture Grid. Rate ratios (RR) were calculated to compare the age-adjusted incident rate of fracture between the RA and non-RA populations, and used a chi-square test to compare proportions of fractures between women with and without RA, and a two-sided Mann-Whitney U-test to examine age-differences.
Among 1,008 women with RA, 19 (1.9%) sustained a fracture, compared to 1,981 fractures sustained by the 172,422 women without RA (1.2%). Fracture rates were greater among women with RA (unadjusted RR 1.65, 95%CI 1.13-2.42), and similar after age-adjustment. Women with RA sustained vertebral fractures at twice the expected frequency (26.0%vs.13.4%), whereas hip fractures were underrepresented in the RA population (6.0%vs.16.3%) (p<0.001). No associations were observed between RA and incident fracture at sites adjacent to joints most commonly affected by RA (p=0.22).
Our results may underestimate the risk, as a proportion of the RA population may be receiving treatment with anti-fracture therapies. Given that women with RA have a greater risk of fracture compared to women without, it may be appropriate to target these patients for anti-fracture agents.

P87

The xIAP inhibitor Embelin suppresses inflammation and bone loss in Collagen Antibody Induced Arthritis (CAIA) mice

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AIMS: Inhibitor of Apoptotic protein (IAP) is an endogenous inhibitor of caspases known to inhibit apoptosis in many cell types. Previously we have reported the increased expression of two IAP family members, xIAP and survivin, in synovial tissues of patients with Rheumatoid Arthritis (RA). This study aimed to investigate the effect of xIAP inhibitor Embelin on inflammation and bone loss in an experimental model of inflammatory arthritis in mice.
METHODS: Balb/C mice were divided into 4 groups of 6 animals: collagen-antibody induced arthritis (CAIA) group, CAIA treated with prednisolone (10mg/kg/day), CAIA treated with low-dose Embelin (30mg/kg/day) and CAIA treated with high-dose Embelin (50mg/kg/day). Paw scores were recorded daily and at completion mice were sacrificed and paws were collected for micro-CT analysis of bone volume (BV) and histological analysis (scores for inflammation, pannus formation, cartilage and bone degradation).
RESULTS: Both low and high-dose Embelin inhibited inflammation of the paws, as assessed by paw scoring (Figure 1a). Low-dose Embelin mice had lower paw score significantly (p<0.05) throughout the disease course and also demonstrated lower score for inflammation (p<0.05), cartilage and bone degradation (p=0.057), and pannus formation (p=0.167) as compared to CAIA untreated mice. Mice treated with high-dose Embelin demonstrated higher BV than the untreated CAIA and the low-dose Embelin group (Figure 1b).
CONCLUSIONS: This study demonstrates the potential of inhibiting xIAP to reduce inflammation and joint erosion using a murine model of inflammatory arthritis. Different doses of Embelin may produce different effects on inflammation and related bone loss, which requires further investigation.

Fig 1a

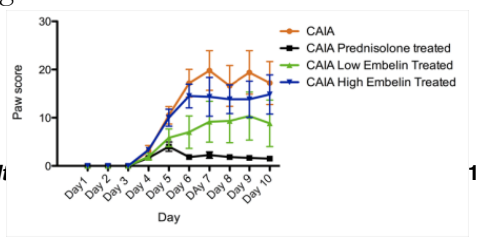
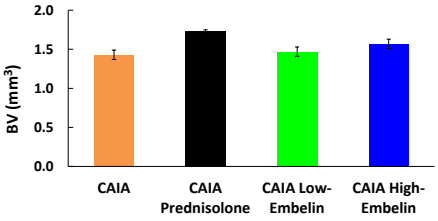


Fig 1b



P88

Subchondral bone injury precedes cartilage degeneration when articular surfaces undergo fatigue loading

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Background and aim: Joint injuries due to fatigue loading commonly occur in athletes and may have serious consequences including fracture, subchondral bone (SCB) attrition and subsequent osteoarthritis. As fatigued bone can be replaced by remodelling but articular cartilage turnover is more limited, it would be advantageous if bone was more prone to injury than cartilage. We investigated the articular surface response to fatigue using a common injury of the metatarsophalangeal joint surface in racehorses and hypothesised that SCB injury precedes cartilage injury.
Methods: Metatarsal bones from 45 racing Thoroughbred cadavers were examined grossly and joint surface injury graded 0-3. Histopathology was performed using resin-embedded and cryo-sections. Microscopic hyaline cartilage injury indicators (erosion score, cartilage layers thickness, chondrocyte density, cartilage matrix and cell morphologic changes) and SCB injury indicators (empty osteocyte lacunar density, SCB plate thickness and microfracture score) were recorded. Statistical analysis was by Spearman correlations.
Results: Macroscopically joint surface injury was accompanied by minimal or no cartilage injury, except attrition in grade 3 injuries. Microscopic hyaline cartilage injury indicators were not correlated with joint surface injury (r_s<0.21, P>0.07) or microscopic SCB injury indicators (r_s<0.34, P>0.07). All SCB injury indicators were associated with joint surface injury score (r_s >0.39, P<0.009).
Conclusions: Fatigue loading of the distal metatarsal articular surface in racehorses primarily damages the SCB. Articular cartilage at this site appears to be more resilient than bone which is advantageous given the lower potential for healing of cartilage.

P89

The relationship between serum and synovial tissue levels of osteoclast associated receptor (OSCAR) in early Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is characterized by synovitis and increased osteoclast numbers and activity, leading to bone erosions and joint destruction. OSCAR, a member of the leukocyte receptor complex, is involved in co-stimulating osteoclastogenesis. Increased levels of membrane bound OSCAR is associated with macrophages and the vasculature in synovial tissues from active RA compared to osteoarthritic and healthy controls (Crotti ART 2012). Conversely soluble (s)OSCAR is lower in the serum of active RA patients compared with healthy individuals (Herman 2008). We investigated whether successful treatment of RA results in increased secretion/cleavage of cell-associated OSCAR resulting in increased sOSCAR levels in the serum and decreased levels in the synovial tissue.
Methods: sOSCAR was measured in serum from 10 active RA patients from initial diagnosis (before combination DMARD therapy), 6 and 12 months by enzyme-linked immunosorbent assay (ELISA). sOSCAR levels were compared at initial diagnosis and at 12 months using a paired t-test. Associations between sOSCAR and erosion scores and disease activity score (DAS28) over time were compared. OSCAR associated with pre-osteoclasts and vasculature was detected by immunohistochemmistry in synovial tissues from the same patients.
Results: sOSCAR significantly (p = 0.04) increased after 12 months treatment from baseline with a mean increase of 37ng/ml. In 6 of 8 patients DAS28 decreased whilst sOSCAR increased. Endothelial associated OSCAR decreased in synovial tissue from 6 of 10 patients, with no detection in 3 patients.
Conclusion: Elevation of sOSCAR may be an early marker that can predict reduced joint damage and successful treatment.

P90

An injectable formulation for co-delivery of BMP, bisphosphonate, and hydroxyapatite microparticles for bone tissue engineering

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We present a novel injectable delivery system for bone tissue engineering that abrogates the need for open surgery and reduces infection risk. The carrier system is a sugar-based ester, sucrose acetate isobutyrate (SAIB), which undergoes a rapid phase transition to semi-solid following injection.
SAIB was found to be an effective delivery system, generating significantly more bone than porous collagen with the same rhBMP dose ($P<0.05$). We compared a range of additives for synergy with rhBMP-2 in an ectopic bone formation model. Agents were introduced with SAIB:ethanol (85:15)/rhBMP-2 (5μg) by direct injection into the hind limb musculature of C57BL6 mice. Inhibitors against PPARγ (BADGE), MEK (PD0325901) and JNK (SP600125) led to increases in bone volume, but none greater than +100%. Anti-resorptives blocking IKK (PS1145), Cathepsin K (AFG495), and the bisphosphonate Zoledronic Acid (ZA) all increased bone volume, with ZA increasing bone by +400% ($P<0.05$). Addition of 2% hydroxyapatite (HA) microparticles with ZA led to a +900% increase in bone volume. Alexa647-labelled bisphosphonate was used in an *in vivo* biodistribution study, and fluorescence in bone nodules in the rhBMP+ZA+HA but not the rhBMP+ZA group. Furthermore, *in vitro* studies showed that addition of HA microparticles to tissue culture media was cytoprotective, preventing the adverse effects of high dose ZA on cultured osteoblasts.
These new data now demonstrate that bone formation can be more considerably increased by the combination of a bisphosphonate and hydroxyapatite microparticles delivered locally.

P91

$PTH_{(1-34)}$ treatment for improved healing of bisphosphonate associated stress fractures

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Osteoporosis is often treated bisphosphonates (BP), a class of anti-resorptive drugs with high affinity to bone. Chronic BP treatment has been associated with stress fractures and atypical femoral fractures. The latter can have a grave prognosis. While bisphosphonate holidays have been suggested to mitigate the risks of long term BP treatment, we hypothesized that treatment with Teriparatide ($PTH_{(1-34)}$) could increase bone turnover and decrease BP load.

To test this, closed femoral fractures were created in the proximal femur of Wistar rats. Rats were dosed with a dual treatment of functional bisphosphonate (Zoledronic Acid/ZA) admixed with fluorescently tagged (AlexaFluor 647) Pamidronate (AlexaPam 647). The experimental groups were ZA/AlexaPam 647 (0.02mg/kg, 6 doses bi-weekly), ZA/AlexaPam (0.02mg/kg, 6 doses bi-weekly followed by daily 25μg/kg $PTH_{(1-34)}$), and saline controls. Rats were sacrificed at 6 weeks for x-ray, microCT and histological analysis. Increased levels of BPs distributed to the fracture side in comparison to the un-fractured side. When treated with PTH, the BPs were redistributed away from their primary binding site and a decrease in ZA levels within the fractured femurs was observed (Fig. 1). As such, an improvement in fracture healing was observed within the PTH treatment group. These *in vivo* investigations provide quantitative data to support $PTH_{(1-34)}$ as a possible treatment to abort the process of atypical fractures related to bisphosphonate use.

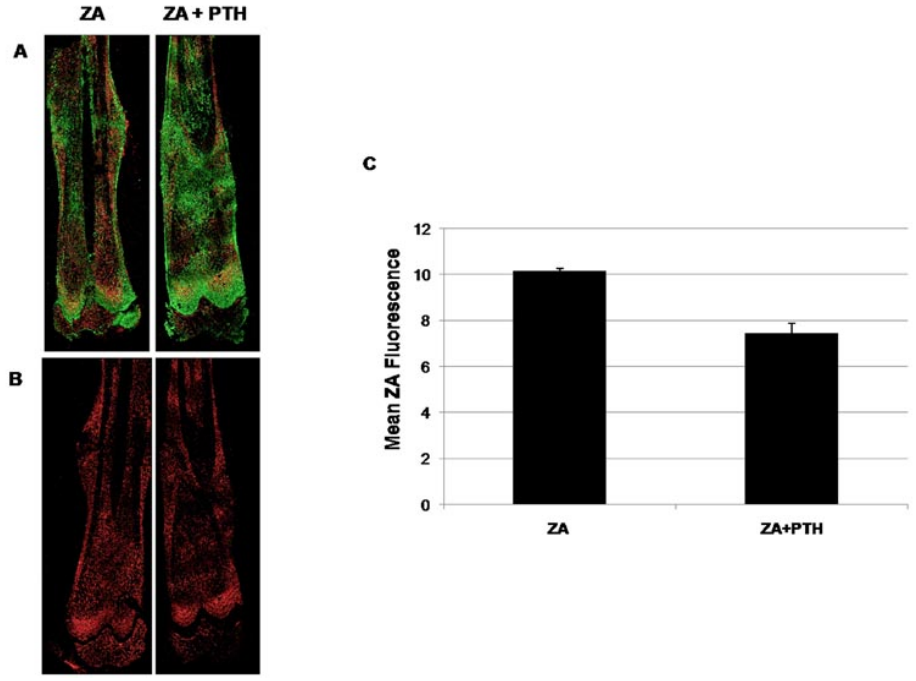

Figure 1: A & B) Both calcein (green) and ZA (red) distribution within the fractured femurs of ZA and ZA+PTH treated groups. **C)** Quantification of ZA fluorescence

P92

The effect of parthenolide treatment on a murine calvarial model of peri-prosthetic osteolysis as assessed by live-animal micro-computed tomography

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Introduction: Particle induced bone loss by osteoclasts is a common cause of loosening around implants. The study aims to determine whether an NF-κβ inhibitor, Parthenolide (PAR), reduces bone resorption in a murine calvarial model of polyethylene (PE) particle-induced osteolysis.
Methods: 24 mice were scanned 7 days prior to the administration of particles using the live-animal micro-computed tomography (μCT) scanner (Skyscan 1076 High Resolution) to determine baseline bone volume (BV). At day 0, 30 mL (2×10^8 particles/mL) PE were implanted onto the periosteum of the mice calvariae. Mice were subcutaneously injected with 1mg/kg PAR at days 0, 4, 7 and 10. Following sacrifice at day 14, bone resorption was assessed by μCT and CTX-1 serum ELISA (RatLaps, Nordic). Serum levels of Osteoclast Associated Receptor (OSCAR) were measured by commercial ELISA (Cusabio). A rectangular region of interest (ROI) was selected to determine BV change overtime. Statistical significance ($p<0.05$) was determined by student *t*-tests.
Results: μCT analysis found all mice increased BV overtime. PE particles significantly induced bone loss compared to the control. This was consistent with three-dimensional stereo imaging at day 14 showing the presence of pits on the calvariae and a significant increase in the CTX-1 level in serum. Serum OSCAR levels were also significantly increased in animals given PE particles. PAR treatment did not significantly increase BV in the PE treated groups ($p>0.05$).
Conclusion: The NF-κβ inhibitor PAR at 1mg/kg did not significantly inhibit bone loss in a calvarial murine model of peri-prosthetic osteolysis.

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The effect of programmable mechanical stimulation on tendon repair in a bioreactor system

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Introduction: Mechanical stimulation has been identified as an essential factor for maintaining tendon homeostasis. Previous study in our lab elucidate that subjected to loading deprivation culture, tendon developed progressive tendon degeneration, and only a narrow range of mechanical stimulation is able to create anabolic effect on tendon homeostasis. Therefore, in the present study we hypothesize that PMS is able to rescue the tendon degeneration caused by loading deprivation culture in bioreactor system.

Methods: Full-length Achilles tendons were dissected from the hindlimbs of New Zealand white rabbits and subjected to different biomechanical environment as shown as Figure 1. To evaluate the tendon, histological assessment, type III collagen immunohistochemistry, TUNEL assay, QPCR and biomechanical testing were preformed.

Results: Tendons in loading deprivation group developed progressive tendon degeneration, increased apoptosis and weaken mechanical properties with extended culture period, however, the morphology, cell viability and mechanical properties appeared normal in rescue group (Figure 2). Moreover, rescue effect, including upregulation of COL1A1 and TGFb, was triggered by mechanical stimulation, and catabolic effect was suppressed by downregulation of MMPs and upregulation of TIMPs (Figure 3).

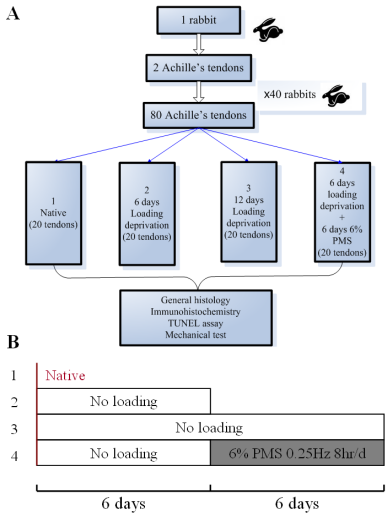
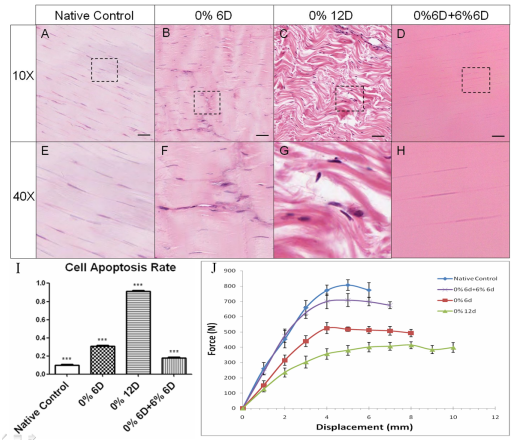


Figure 1. Tissue distribution and PMS regime

Discussion: Immobilization is a common postoperative treatment of bone fracture and tendon injury. Although tendon degeneration in Achilles tendon was reported after long term immobilization in both human and rat, the mechanism still remained unclear. Moreover, no guideline of clinical rehabilitation for Achilles tendon weakness after a period of immobilization was found. In this study, we found 6% mechanical stimulation is able to regain the tendon normality, which explores the possibility that precise PMS is able to reverse early-stage tendon degradation.



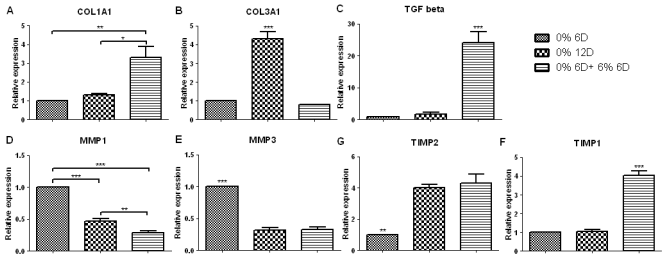


Figure 2. (A-H) H&E staining, (I) Cell apoptosis rate (J) Biomechanical testing

Figure 3. Comparison of gene expression

P94

Can bone quality markers predict nonunion?

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Background: The ability to predict susceptibility to nonunion would enable measures to be taken at an earlier stage, such as performance of more rigid fixation before surgery. Although low-intensity pulsed ultrasound (LIPUS) has been used for nonunion, bone union is not always achieved even with this method. While blood homocysteine (Hcy) and urinary pentosidine (Pen) levels have been reported as useful predictive markers of fracture, relationships to nonunion have not been elucidated.
Objective: To retrospectively investigate bone quality markers, specifically uncarboxylated osteocalcin (ucOC) (reference value, <4.50 ng/ml), blood Hcy (reference range, 3.7-13.5 nmol/ml), and urinary Pen (reference range, 0.019-0.070 μg/mg･Cr) in patients with nonunion and to clarify relationships to nonunion.
Subjects: Subjects comprised 13 patients (9 men, 4 women; mean age, 56.7 years; range, 29-84 years) who underwent additional treatment due to lack of porosis with LIPUS alone among the 37 patients who provided consent for measurement of bone quality markers among the 169 patients who underwent LIPUS for nonunion after osteosynthesis.
Results: Elevated levels of bone quality markers were seen in all 13 patients, with elevated ucOC in 4 patients (mean, 7.11 ng/ml), Hcy in 5 (mean, 16.4 nmol/ml), and Pen in 9 (mean, 0.091 μg/mg･Cr)(including cases of overlap). Additional pharmacotherapy (e.g., PTH therapy) was given to all 13 patients, and bone union tended to progress in many patients. In addition, further surgery was performed for 5 patients and combination with electric stimulation therapy was given for 1 patient.
Discussion: Management of nonunion involves many factors. However, causes of nonunion are often unclear. Bone quality markers may serve as predictors of nonunion.

P95

Tenofovir therapy decreases bone mineral density in hepatitis B: implications for clinical management

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Introduction: The bone disease associated with chronic liver disease is common and poorly characterised in patients with chronic viral hepatitis. Tenofovir is an established treatment for chronic hepatitis B (CHB); however, an association with increased bone loss and kidney toxicity in HIV has been reported.
Aim: To investigate bone mineral density (BMD) and renal tubular function in CHB patients treated with tenofovir compared to untreated CHB patients through a cross-sectional study.
Method: BMD was measured by dual-energy X-ray absorptiometry (DXA) at the hip and lumbar spine (LS). Testosterone, oestradiol, calcium, phosphate, PTH, 25(OH)VitD and bone turnover markers were measured. Urine testing for phosphate, amino acids, B2-microglobulin and glucose was performed. Multiple logistic regression was used to adjust for cirrhosis status, age, gender and weight.
Results: 10 untreated controls and 22 patients treated with tenofovir (treatment duration 2.6±1.4 years) were enrolled. Cirrhosis was present in 16% of patients. LS Z score was lower in the tenofovir treated group in the univariate analysis (-0.2±1.3 vs -1.14±1.18, P=0.02) and remained significant in the multivariate analysis (P=0.03). Increasing duration of tenofovir use was correlated with increased urinary excretion of phosphate (r=0.57, P=0.03).
Conclusion: Tenofovir is significantly associated with reduced BMD at the LS. Increased duration of tenofovir use is associated with increased urine phosphate loss through which accelerated bone loss may occur. Routine monitoring of BMD and urine phosphate is required in CHB patients on tenofovir to reduce the risk of accelerated bone loss.

P96

The association between gonadal status, body composition and bone mineral density in transfusion-dependent thalassemia

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Introduction: Patients with transfusion-dependent thalassemia are known to have diminished BMD and a high incidence of hypogonadism
Aim: To investigate the relationship between skeletal muscle mass (SMM), fat mass and BMD in subjects with transfusion-dependent thalassemia based on their gonadal status.
Method: A retrospective cross-sectional cohort study of 186 young adults with transfusion-dependent thalassemia was analysed. Body composition and BMD (lumbar spine, femoral neck and total body) were measured using Dual energy X-ray absorptiometry (DXA) along with anthropometric measures. The association between SMM, fat and BMD was investigated through uni-, multi- and step-wise regression analyses after adjusting for multicollinearity.
Results: 43.5% were males and 56.5% were females with a median age of 36.5 and 35.4 years, respectively. Hypogonadism was reported in 44.4% of males and 44.7% of females. SMM and BMD were positively correlated and were strongest in eugonadal males ($0.36 \leq R^2 \leq 0.59$) but the association was attenuated in hypogonadal males. SMM ($0.27 \leq R^2 \leq 0.69$) and total fat mass ($0.26 \leq R^2 \leq 0.55$) were positively correlated with BMD in hypogonadal females but the correlation was less pronounced in eugonadal females. Leg lean tissue mass and arm lean tissue mass in males and females, respectively, were most highly correlated to BMD in the stepwise regression analysis.
Conclusion: Hypogonadism attenuates the strength of the muscle-bone relationship in males but strengthens the positive correlation of SMM and fat mass in female subjects. This study supports the notion that exercise is important for maintaining BMD and the need to optimize treatment of hypogonadism in patients with transfusion-dependent thalassemia.

P97

Is transfusion-dependent thalassemia associated with an increased risk of antiresorptive agent-induced Osteonecrosis of the Jaw (ONJ)?

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Background: Antiresorptive agent-induced ONJ is a potentially debilitating condition that can affect individuals with benign and malignant bone disease. Risk factors include malignancy, duration of bisphosphonate exposure, dental extraction and advanced age. Bisphosphonates are commonly used in the treatment of low bone mineral density in transfusion-dependent thalassamia and these patients have increased orofacial and dental issues.
Aims: To screen for possible cases of ONJ in transfusion-dependent thalassemia at a single tertiary referral centre.
Methods: A retrospective cohort study of 140 adults with transfusion-dependent thalassemia treated at Monash Medical Centre between 2008-2013 was performed. ONJ was defined as the presence of exposed bone in the maxillofacial region that did not heal within 8 weeks after identification by a health care provider. Patient age, smoking status, bisphosphonate exposure, dental, malignancy and splenectomy history were documented from the examination of medical records.
Results: 26 patients had exposure to bisphosphonates (26/140;18.6%) with an average duration of 6 years. Two patients aged 39 and 61 (2/26;7.7%) were identified to have exposed mandibular/maxillary bone of at least 8 weeks duration; both had oral bisphosphonate treatment (6 and 11 year exposure), dental caries and extraction at the site >1 year earlier. Bisphosphonates were ceased and the patients were treated with conservative measures.
Conclusion: Patients with transfusion-dependent thalassemia treated with bisphosphonates may be at increased risk of ONJ as a consequence of thalassemia-associated changes to bone and coexisting dental and periodontal disease. The establishment of an international registry is warranted to prospectively study this important complication.

P98

Prior use of bone-related medication in adults with recent low trauma fracture

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As part of AusICUROS, an international osteoporosis cost and outcomes study, we identified the proportion of adults aged 50+years taking bone-related medication (bone-meds) at the time of sustaining a low-trauma fracture. Bone-meds included calcium and/or vitamin D supplements (CaD) and pharmaceuticals (Table). Bone-meds were recorded in 500 participants with recent fracture using patient and hospital data at 4 Australian-teaching hospitals from 2009-2012. Logistic regression was used to predict odds ratios.
Participant profile was: age 70 years (IQR: 59 to 78 years) 76% female; 17% had a previous fracture in past 5-years. 55% were not taking any bone-meds, 12% took calcium alone, 12% vitamin D alone and 15% took both. Only 14% were taking pharmaceuticals, mainly bisphosphonates (9%). Of those self-reporting previous fracture(s), only 23% were taking pharmaceuticals although they were more likely to do so compared with those without previous fracture (OR 1.7 [1.1, 2.7]; p=0.027).
Our findings confirm that few older adults are taking effective anti-fracture therapy at the time of their fracture. Compared with those with no fracture history, those with previous fracture are almost twice as likely to be taking effective therapy. Despite this, 77% of these adults are not receiving adequate secondary fracture-prevention therapy.
These results highlight the need to increase awareness of fracture-prevention therapy, particularly in persons with prevalent fractures. Although the use of CaD has increased[1] and suggests some awareness of bone health, the proportion of women with prior fracture taking pharmaceuticals is low and appears not to have increased in the last 10-years[1].

	No bone meds	Calcium[2]	Vitamin D[3]	Both calcium & vitamin D	Bisphosphonates	Other pharmaceuticals[4]
No previous fracture	**57% (237)**	**12% (49)**	**12% (48)**	**14% (58)**	**7% (29)**	**5% (22)**
Previous fracture	**44% (38)**	**10% (9)**	**16% (14)**	**22% (19)**	**20% (17)**	**3% (3)**
All	**55%**	**12%**	**12%**	**15%**	**9%**	**5%**

Table footnote: Number of participants in parentheses: [2]Includes only calcium taken without vitamin D; [3]Includes only vitamin D taken without calcium; [4]Includes HRT, selective estrogen receptor modulators, strontium ranelate and teriparatide.

[1]Eisman et al, JBMR, 2004

P99

Predictors of bisphosphonate uptake in Australian adults following a low trauma fracture

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Access to appropriate medication following a low-trauma fracture is critical to preventing further fracture(s) in adults but treatment rates are frequently low. We identified predictors of commencing bisphosphonates following recent fracture in adults aged 50+ years.
AusICUROS is part of an international osteoporosis cost and outcomes study. Bisphosphonates commenced as a consequence of the fracture were recorded from patient and hospital data collected 4-months post-fracture in 789 participants treated at 8 Australian teaching hospitals from 2009-2012. Factors predicting the commencement of bisphosphonates (Y/N) were identified using logistic regression. Predictors tested were age, fracture site (hip, wrist, vertebral, other), income (low, medium, high), education (4 levels), fracture in past 5-years (Y/N) and gender.
Participant profile was: 69 years (IQR: 59 to 78 years) 76% female; 18% had a previous fracture and few (14% of 500 with known pre-fracture medications) were already on bone-related medication(s). Fracture sites at study entry were: hip 22% (171); wrist 36% (288); vertebral 10% (80); other 32% (257). Only 9% (74) commenced bisphosphonates. Odds ratios of predictors were: age 1.04 [1.01, 1.06] and fracture-site: hip 2.2 [1.1, 4.5], vertebral 4.3 [2.0, 9.3].
Our findings demonstrate that post-fracture medical treatment is suboptimal. Less than 10% commenced bisphosphonates, the most commonly used first-line anti-osteoporosis medication for refracture prevention. Importantly, those who were younger or who had a wrist fracture were not more likely to commence bisphosphonates. Such gaps in the management of osteoporosis need to be urgently addressed by a systems-based approach to capture low trauma fractures.

P100

Non-osteoporotic low-trauma fractures and the burden of re-fracture and mortality

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Background: Half of all low-trauma fractures occur in people with non-osteoporotic BMD, however their re-fracture and mortality risk is unclear.
Aim: To determine the risk of re-fracture and mortality according to BMD.
Methods: Women and men 60+ from the Dubbo Osteoporosis Epidemiology Study with incident fractures were followed for re-fracture and mortality (April 1989-Dec 2012). Femoral neck BMD was measured close to initial fracture. Re-fracture rates were compared to initial fracture rate and mortality rates were compared to age-adjusted population mortality rates.
Results: There were 528 fractures in women (49% non-osteoporotic) and 186 in men (67% non-osteoporotic). Re-fracture risk was highest for those with osteoporosis [women: 3.24 (2.72- 3.86) and men: 3.83 (2.81-5.21)], but was still elevated for those with osteopenia [women: 2.11 (1.70- 2.63) and men: 3.38 (2.23- 5.11)] and normal BMD [women: 1.99 (1.19- 3.31) and men 2.11 (1.19- 3.76)]. Increased risk was observed in the non-osteoporotic group for all fracture types.
In women, mortality risk was increased for all levels of BMD [osteoporosis 1.71 (1.45- 2.02)], osteopenia 1.32 (1.05- 1.66) and normal BMD: 2.16 (1.34-3.48)]. Hip fractures dominated the high mortality in osteopenic women and non-hip non-vertebral fractures in women with normal BMD. In men mortality rates were increased in those with osteoporosis [2.67 (95% CI, 1.98- 3.60)] and osteopenia [2.21 (1.67- 2.92)], but not normal BMD. Overall ~ 70% of re-fractures and 22-40% of deaths were attributable to non-osteoporotic fractures.
Conclusion: Non-osteoporotic low-trauma fractures are associated with substantial risk of re-fracture and mortality for both genders.

P101

Osteoporosis management among Australians at high risk of osteoporotic fractures: an overview from the 45&Up Study cohort

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Aim: To evaluate osteoporosis management among Australians at high risk of osteoporotic fractures.
Methods: This is a cross-sectional study of participants in the 45&Up Study which recruited about one in 10 men and women aged over 45 in NSW during 2005 to 2009 (n=267,141). We excluded persons who were aged <50 years, had missing body mass index (BMI), or were on anti-resorptive therapies for non-osteoporosis diseases. Fracture Risk Assessment Paper Charts (Australia), FRAX, was used to calculate the 10-year fracture probability for the 213,375 participants. Persons with a risk ≥3% for hip fracture or ≥20% for major osteoporotic fractures were considered high risk. Participants were regarded as on treatment if an osteoporosis treatment (anti-resorptive agent or teriparatide) was recorded in the PBS dataset in the time period from 45 days before to 45 days after the recruitment date. Logistic regression was used to identify variables significantly associated with treatment.
Results: Of the 50,244 high-risk individuals with a median age of 79.3 (interquartile range: 74.6 to 82.6) years, 28,501 (56.7%) were females and only 6,797 (13.5%) were on osteoporosis treatment at recruitment. The treatment rate was much higher in women (19.5%=5,562/28,501) than in men (5.7%=1,235/21,743). Other variables significantly associated with treatment were fracture in previous five years, oral glucocorticoids use for >3 months, severe rheumatoid arthritis, history of fractured hip among parents, lower BMI and older age, as well as non-excessive drinking.
Conclusion: In Australia, osteoporosis management was suboptimal even among very high fracture risk individuals especially among men.

P102

Risedronate slows or partly reverses microarchitecture deterioration depending on whether remodelling is perturbed or in steady state

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Remodelling is slow, balanced and in steady state in young adulthood. After menopause, steady state is perturbed; more and deeper resorption sites is not matched instantaneously by bone formation. The fewer sites initiated before menopause refill produce a net decrease in vBMD. Later, remodelling is rapid and in steady state; larger numbers of resorption sites created in early menopause refill and the same high number of resorption sites appear; so the decline in BMD decelerates. We hypothesized that risedronate (RIS) slows structural deterioration in the former and partly reverses it when administered later.

RIS 35 mg/week or placebo were given to postmenopausal women (Group 1, n=161, range: 44-55 yrs, Group 2, n=163, range: 55-76) in a DBPC. High resolution-peripheral computed tomography was used to measure distal radius and tibia microstructure quantified using StrAx 1.0.

In group 1, controls had a 4.2±1.6% increase in cortical porosity and a 3.6±1.4% decrease in trabecular vBMD at the radius (all p<0.02). RIS prevented these changes. In group 2, controls had no changes in morphology. RIS decreased porosity by 0.9±0.4% (p=0.047). At the tibia, in group 1, porosity increased in controls by 3.4±0.9% (p=0.001) but but 2.4 fold less so in treated women. In group 2, trait changes were not significant. RIS decreased porosity 0.7±0.3% (p=0.016) and increased trabecular vBMD by 0.4±0.2% (p=0.013).

Risedronate slows appearance of porosity in cortical bone following menopause and partly reverses it later; changes likely to preserve bone strength.

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Relationships between serum sclerostin levels and bone metabolism-related indices as well as bone fragility

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Background: Sclerostin is produced in osteocytes and inhibits bone formation by blocking Wnt-β-catenin signals. Anti-sclerostin antibodies have also been shown to have an increasing effect on bone mineral density (BMD) in humans. This cross-sectional study was performed to examine the relationships between serum levels of sclerostin and bone metabolic markers, BMD, muscle strength, as well as fractures. Methods: We enrolled 190 postmenopausal women who were undergoing examination for osteoporosis. Serum levels of Ca, P, Cr PTH, 25-hydroxy vitamin D {25(OH)D}, PINP, CTX and sclerostin were measured. The BMD of the lumbar spine (L2-4) and femoral neck was measured using DXA, the presence or absence of morphological vertebral fracture was determined, and the presence or absence of existing non-vertebral fracture was determined through physician interviews. Results: Mean values of age and BMI were 63.4±7.5 years and 22.9±3.1 kg/m^2, respectively. Sixty-six subjects had fragility fractures. Sclerostin levels were not correlated with age, 25(OH)D, PTH or bone metabolic markers, but showed significant positive correlations with BMI, Cr, grip strength and BMD. Sclerostin levels were significantly higher in the fragility fracture group (fracture group, 1.38±0.40 ng/dl; non-fracture group, 1.23±0.37; p<0.05). In logistic regression analysis, sclerostin level was identified as a significant risk factor for fragility fracture (odds ratio, 1.55; 95%CI, 1.02-2.36; p=0.04) even after correcting for age, BMI, grip strength, Cr, PTH, 25(OH)D, CTX, and L2-4 BMD. Conclusion: High sclerostin levels were shown to represent a risk factor for fragility fractures independent of motor function, 25(OH)D, renal function, bone metabolic markers, and BMD.

P104

Distributions and reference intervals for serum osteocalcin, N-propeptide of Type 1 collagen and C-terminal cross-linking telopeptides of Type 1 collagen in older men. The Health In Men Study.

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Aims: Bone turnover markers (BTMs) have been advocated for stratifying fracture risk and monitoring response to osteoporosis therapy. We characterised the distribution of BTMs and describe reference intervals (RI) for healthy older men.
Participants and Methods: We studied serum specimens collected between 0800h-1030h from 4,020 community-dwelling men aged 70-89 years resident in Perth, Western Australia. Osteocalcin (OC), type 1 collagen N-propeptide (P1NP) and type 1 collagen C-terminal telopeptide (CTX) were assayed using an automated analyser (Roche E170, Roche Diagnostics, Australia). Current results are from 366 men reporting excellent or very good health on a 5-point questionnaire, with no history of smoking, diabetes, cardiovascular disease, cancer, depression, dementia, Paget's disease, osteoporosis, glucocorticoid use, warfarin or bisphosphonate therapy. Reference intervals were calculated using recommended methodology.
Results: Distributions of BTMs were skewed to the right. In multivariate analyses, logOC, logP1NP and logCTX were all significantly associated with age (P <0.001, P =0.03 and P = 0.003, respectively); log OC and log P1NP were associated with serum 25-hydroxyvitamin D deficiency (P=0.04 and P=0.02, respectively). No BTM was associated with low eGFR or presence of overweight. RIs, based on the 2.5th and 97.5th percentiles, were: OC 10.0-40.9 μg/L, P1NP 18-117 μg/L and CTX 105-690 ng/L.
Conclusions: Calculated RIs for BTMs are higher in healthy older men compared with their younger counterparts. Further research is required to establish whether these RIs can predict incident fracture more accurately in a similarly aged group with poorer health.

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Diabetes-related foot complications and their treatment are associated with bone deficits

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Plain X-rays often suggest osteoporosis in patients with diabetes-related foot complications (DFC). We investigated the prevalence of osteoporosis in individuals with DFC and whether bone deficits worsen during their treatment. Consecutive patients presenting to the Royal Melbourne Hospital Diabetic Foot Unit with diabetes, a DFC for no more than 6 weeks, and requiring pressure off-loading treatment, were recruited. Consenting patients completed an osteoporosis risk factor questionnaire, had blood taken, DXA of the lumbar spine and both hips, and pQCT of both tibias. Densitometry was repeated 6 months later. Preliminary data in 26 participants (81% male; mean age +/- SD = 61 +/-14 years) revealed: mean 25-hydroxyvitamin D level 51 +/- 21 nmol/L and osteoporosis or osteopenia on initial DXA scan in 59%. The mean total hip Z-score, tibial cortical density and leg muscle cross-sectional area ipsilateral to the DFC declined from baseline to follow-up (0.205 vs 0.097; P=0.04; 1088 mg/ccm vs 643 mg/ccm; P<0.001; and 8325 mm^2 vs 7701 mm^2; P=0.015; respectively). The mean stress-strain index and tibial trabecular density ipsilateral to the DFC increased (1776 mm^3 vs 1850 mm^3; P=0.014; and 240 mg/ccm vs 275 mg/ccm; P<0.001; respectively). The mean total hip Z-score of the contralateral side was unchanged (0.309 vs 0.267, respectively; P=0.352), while lumbar spine Z-score increased (1.407 vs 1.655, respectively; P<0.001). DFC are associated with low BMD at presentation and bone loss at the ipsilateral hip and tibial cortex, indicating high fracture risk in these patients. Increasing BMD at some sites requires further investigation.

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P106

Vertebral Fractures are under-recognised and rarely actioned by hospital clinical teams

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Under-recognition and lack of treatment of osteoporotic fracture is common in Australian teaching hospitals [1]. We aimed to determine whether patients with vertebral fracture(s) reported on lateral chest radiograph were appropriately managed for osteoporosis at a large teaching hospital.

We performed a retrospective audit of men and women aged ≥50 years with lateral chest radiograph in 2011 reported by a radiologist to show vertebral fracture(s). The hospital radiology database was searched for key terms in the report free text. Charts were reviewed to determine if the fracture or report were documented by the caring team, if osteoporosis and/or its management were documented at any time during admission; and whether information of the fracture or osteoporosis was conveyed to the GP at discharge. Data were assessed using descriptive statistics.

13,529 lateral chest radiographs were performed in this population during 2011. 204 radiographs from 187 individuals reported vertebral fracture(s). 130 medical records have been audited. Fracture was noted in 13%. And the report noted in 11.5%. Osteoporosis or its management were noted on admission in 37%; for these patients further clinical decision making may have been unnecessary. For the remaining 63%, fracture and/or osteoporosis information was conveyed to the GP in only 6%. When the indication for imaging was back pain, the fracture was noted and managed appropriately in 2/3 cases.

This audit highlights a deficiency in current hospital practice. Vertebral fractures are rarely recognized and radiologist reports appear to be ignored rather than prompting appropriate management by caring clinical teams.

1. Teede et al. Fracture prevention strategies in patients presenting to Australian hospitals with minimal-trauma fractures: a major treatment gap. Internal Medicine Journal 37 (2007) 674–679

P107

Effects of Denosumab in men with low bone mineral density (BMD), men with prostate cancer receiving androgen deprivation therapy and women with Postmenopausal Osteoporosis (PMO)

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Aim: Denosumab (DMAb) 60 mg every 6 months (Q6M) increased BMD and decreased bone turnover markers (BTMs) and risk of fractures in women with PMO in the FREEDOM[1] trial, as well as in men with prostate cancer on hormone ablation therapy with low bone mass or a history of fragility fracture in the HALT[2] trial. The 12-month ADAMO[3] trial also demonstrated DMAb's efficacy and safety in men with low BMD. This analysis was conducted to evaluate the consistency of the effects of DMAb across these populations.
Methods: Efficacy result comparisons used data from the first 12 months of each study. The analysis set for each BMD endpoint and serum CTX (sCTX) included all randomised subjects who had both a baseline measurement and at least one post-baseline evaluation at or before 12 months.
Results: Baseline values among the 3 studies showed considerable overlap. Men treated with DMAb in ADAMO showed gains from baseline in LS BMD of 5.7% at 12 months, compared with 4.3% and 5.5% among subjects in HALT and FREEDOM, respectively. Each was significantly greater than placebo ($p<0.0001$). Median sCTX levels were reduced by 81% at day 15 in ADAMO, compared with 90% at month 1 in HALT and FREEDOM. The 12-month safety profiles in each trial were also similar.
Conclusions: The effects of DMAb were consistent across these populations, with similar LS BMD increases at 12 months. In HALT and FREEDOM increases in BMD with DMAb 60mg Q6M were associated with decreases in the risk of fracture, suggesting that the BMD increases observed in ADAMO are clinically meaningful. No new safety risks associated with DMAb treatment were identified in ADAMO compared with HALT and FREEDOM.

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P108

Mild to moderate chronic kidney disease is associated with vertebral fracture independent of Albuminuria in patients with Type 2 diabetes

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Aims of the study: Type 2 diabetes mellitus (T2DM) patients have an increased risk of vertebral fractures (VFs) compared to non-T2DM controls, independent of BMD. Several studies demonstrate that renal dysfunction is associated with an increased fracture risk. The aim of this study is to clarify that renal dysfunction is associated with bone fragility in T2DM patients.
Methods: We divided 367 Japanese T2DM men over the aged of 50 (DMm) and 326 postmenopausal T2DM women (DMw), all of whose creatinine levels were within normal range, into 3 groups based on their calculated Ccr (using the Cockcroft-Gault equation): ≧90 (stage 1: S1), 89–60 (S2), and 59–30 ml/min (S3).
Results: The prevalence VFs rate in S1, S2 and S3 were significantly elevated among both males (30, 39, 58%, $P<0.01$) and females group (24, 37, 41%, $P<0.01$). The VF risk relative to S1 was 1.8 (95%CI 1.1-3.0, $P<0.05$) for S2 and 4.8 (2.3–9.8, $P<0.01$) for S3 in men and 2.9 (1.2–6.8, $P<0.01$) for S3 in women, after adjustments were made for BMI, HbA1c, duration of T2DM, the uAlb, and the spine BMD Z score.
Conclusion: CKD stages 2 and 3 were risk factors for VFs in T2DM patients. These associations were independent of both uAlb and BMD, suggesting that proteinuria may not serve as an index for VF risk and that the progression of CKD may cause bone fragility due to deterioration in bone quality among T2DM patients.

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The Orthogeriatrics Model: Testing the effectiveness of an integrated model of care in older patients with hip fracture

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Background: Older adults with hip fracture have a 5- to 8-fold increased risk for all-cause mortality and much higher risk of institutionalization. Therefore, standardized and evidence-based interventions are highly needed. In this study, we aimed to assess the effectiveness of an integrated model of orthogeriatrics care.
Methods: Two geriatricians run the program with the assistance of a multidisciplinary team. Assessment includes surgical risk, physical examination, nutrition, cognition, and blood tests. A comprehensive intervention plan was designed following the guidelines from the NSW Agency of Clinical Innovation. Two populations of NOF fracture patients admitted prior (2006) and after (Jan. 2011-June 2012) the implementation of the program were compared.
Results: Two groups of NOF fracture patients were compared: n=270 in 2006, and n=150 in 2011-12. Mean age (83±7) and gender (73% female) were similar in both groups. The orthogeriatrics group showed a significantly shorter delay in surgery (from 59.7% to 21.6%, $p<0.01$), lower in-hospital mortality (5.4% vs. 1.9%, $p<0.05$) and lower rate of di novo admission to nursing homes (24% vs.17%, $p<0.05$), with a higher number of patients receiving rehabilitation at discharge in the orthogeriatrics group (50% vs. 61.5%, $p<0.05$).
Conclusion: Our data demonstrates the effectiveness of an evidence-based standardized orthogeriatrics program run by geriatricians together with a multidisciplinary team.

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GPs' perceptions of the usefulness of absolute fracture risk assessment in their practice: barriers, facilitators and other issues for implementation.

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Aims: The role of absolute fracture risk (AFR) assessment in guiding osteoporosis prevention and management is emerging. However, to ensure its successful implementation, it is important to understand how AFR assessment tools (AFRAT) might be used by GPs. This study aims to investigate GPs' perceptions of AFR assessment.
Methods: We performed semi-structured interviews exploring GPs' views on using AFR in clinical practice, preferences for AFRAT format and potential barriers to and enablers of using AFRAT. We recruited GPs using purposive sampling from the General Practice South Database. Recorded interviews were transcribed in full and analysed independently by two people using an iterative interpretive technique.
Results: We interviewed 25 GPs. Few GPs were aware of the availability of AFRAT. Many felt that using AFRAT would reinforce their decision to initiate osteoporosis therapy. Nearly all GPs identified the main potential advantage of using AFRAT as assisting with patient education, for example by making risk reduction comparisons through hypothetical patient lifestyle changes or reinforcing treatment decisions. Most GPs (2/3) preferred a computer-based tool; the remainder preferred a printed version. An easily accessed, simple tool that provides visual representation of risk and accompanying patient handouts was identified as being useful.
Conclusion: GPs are receptive to using AFR to guide management and for patient education. A variety of formats may be needed, with supporting materials, for implementation into practice to be successful and for maximum benefits for patient care to be gained. These issues need to be considered in the design and dissemination of AFRAT.

P111

An uncommon and difficult case of osteoporosis

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Introduction: Pregnancy and lactation associated osteoporosis is a rare condition resulting in minimal trauma fractures, predominantly vertebral, in late pregnancy or early post-partum period. Cessation of breast-feeding with adequate calcium and Vitamin D replacement generally suffices for its treatment, however management can occasionally be very challenging as demonstrated in our case.
Case report: We present the case of a 25-year old lady who was 6-months post-partum and referred to our Endocrine Unit for evaluation of persisting severe low back pain that she had initially developed in the immediate post-partum period. Imaging revealed fractures involving lumbar vertebrae, inferior pubic ramus and several ribs. DEXA scan demonstrated osteoporosis with a Z-score of -3.48 in the lumbar spine. No significant risk factors for osteoporosis were noted and biochemical testing ruled out other common causes of osteoporosis. A diagnosis of 'lactation-associated osteoporosis' was made and the patient was advised to stop breast-feeding. Calcium and Vitamin D supplements were initiated and she was commenced on risendronate. She had ongoing issues with severe pain over the next 6 months which prompted further imaging and demonstration of new fractures of mid and lower thoracic vertebra. Risedronate was replaced with Strontium ranelate which improved her Z-scores. Whether this translates into reduction in fracture-risk is, however, uncertain. We intend to do bone biopsy to rule out malignancy, if she were to fracture again. Alternative treatment option includes teriparatide.
Discussion: Discussion for this case includes detailed literature review of pregnancy and lactation associated osteoporosis addressing pathophysiology, risk factors, diagnosis and treatment options.

P112

Odanacatib: Cathepsin K Inhibitor as a Therapeutic Agent in Osteoporosis

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Garvan Institute, St Vincents, UNSW UNDA

Osteoporosis development is associated with excessive bone resorption; a major target of effective osteoporosis therapies. Cathepsin K, a osteoclastic cysteine protease and a key enzyme in bone matrix degradation, is targeted by a selective cathepsin K inhibitor, odanacatib.
In Merck's animal and phase I human studies, odanacatib inhibited bone resorption with modest effects on formation, possibly due to odanacatib not affecting osteoclast metabolic activity or survival.
In animal studies and phase II human studies, continuing increases in bone density of hip and spine were observed. Weekly dosing, with or without food, is possible due to a 2-3 day plasma half-life. It doesn't accumulate in bone and, upon cessation, its effect wears off relatively rapidly with bone density gains being lost over 6-12 months. By contrast, bone turnover only slowly reverses upon cessation of the commonly used bisphosphonates or anti-RANK-ligand antibodies.
The Data Monitoring Committee stopped a phase III study in 16,227 postmenopausal osteoporotic women early due to "robust efficacy on all fracture end-points and favourable benefit-risk" and advised to continue the planned, blinded long-term safety, as relatively few patients had been treated for > 3 years. Publication and FDA submission is pending collection of these data. Presuming long-term safety and efficacy are confirmed, it seems that odanacatib will be another powerful tool in the osteoporosis treatment armamentarium with the advantage of proportionally less reduction of bone formation and rapid offset of action, which has not been evident with other effective therapies.

P113

Concern and risk perception for osteoporosis and fracture among post-menopausal Australian women: Results from the Global Longitudinal Osteoporosis Study in Women (GLOW) Cohort.

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Aim: To identify factors associated with concern and perception of risks of osteoporosis and osteoporotic fractures and determine whether bone mineral density (BMD) testing influenced concern and risk perception.
Methods: Study subjects (n=1082, age: 55-94 years) were female Australian participants of the Global Longitudinal study of Osteoporosis in Women (GLOW). Self-administered questionnaires were sent annually from 2007-2010. Study outcomes included 'concern about osteoporosis', 'perception of getting osteoporosis' and 'perception of fracture risk' compared to similar aged women. The closest post-BMD testing or baseline questionnaires were used for women with and without BMD testing respectively. Multinomial logistic regression was used for the analysis.
Results: BMD testing, prior fracture after age 45, younger age and lower self-reported general health were significantly associated with being 'very' or 'somewhat concerned' about osteoporosis and having a 'much higher' or 'little higher' risk perception of osteoporosis and fractures. A poorer BMD result was associated with higher concern and higher risk perceptions. The presence of comorbidities, having ≥2 falls in the preceding year and maternal osteoporosis were associated with higher concern. Maternal osteoporosis, presence of comorbidities, weight loss of ≥5kg in the preceding year and low body mass index were associated with higher perceptions of osteoporosis risk.
Conclusion: Women's concern and risk perception of osteoporosis and osteoporotic fractures were reasonably well founded. However, increasing age, height loss, smoking and drinking were not associated with concern and perception despite being known osteoporosis risk factors. These factors

P114

DEXA screening of patients over the age of 70 followed by videoconferencing substantially increases the recognition and treatment of patients with osteoporosis

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Aim: 1) To investigate the frequency of osteopoenia and osteoporosis in pts over the age of 70 who took part in GP surgery based DEXA screening programs. 2) To investigate the number of patients who took up treatment for osteoporosis following videoconferencing (VC) these pts.
Methods: 1) Lunar DEXA units were sited in GP surgeries in Perth, South Australia (SA) and NSW and available in a mobile DEXA unit outside Perth in SA and NSW or at fixed sites throughout WA. 2) GP surgery staff recruited pts by a letter drop, phone call or direct contact to participate in a DEXA screening program 3) VC was then conducted with eligible pts, a specialist with expertise in osteoporosis and the GP to determine if investigation and treatment of osteoporosis was needed.
Results: Between Sep 2011 - Feb 2013, 2520 VC's have been conducted on eligible pts and the results of the first 884 pts were analysed. At the spine, 37% of pts had a normal T score, 47% were osteopoenic and 16% were osteoporotic. The respective numbers at the total hip were 35%, 44% and 21%. At the total forearm the numbers were respectively 20%, 41% and 39%. Only 17% of pts had vitamin D levels < 50 nmole/l. 30% of pts were advised to commence a specific anti-osteoporotic treatment, 22.5% continued their current treatment, 10% were advised to cease current treatment.25% continued calcium and vitamin D, and 10% treatment recommendations depended on further investigations
Conclusions: 1) More than 50% of patients were recognized to be osteoporotic at least 1 site after a screening GP based DEXA program. 2) A subsequent videoconference of high risk pts identified more than 50% where significant changes of treatment occurred as a result of the VC.

P115

Assessing cortical porosity requires adjustment for the age, sex, and racial differences in the region of interest

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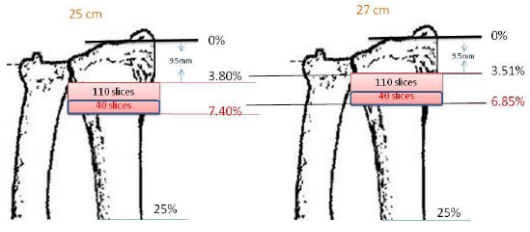
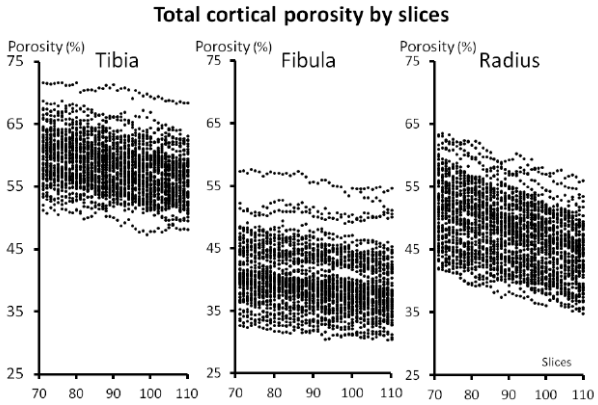
High-resolution peripheral quantitative computed tomography (HR-pQCT) measures micro-architecture in a region of interest (ROI) at the distal radius and tibia. Bone width and micro-architecture vary slice by slice along the length of a bone so differences in micro-architecture by age, pubertal stage, sex and racial group may be the result of differences in the placement of the ROI rather than the characteristics of the subjects. To assess the slice-by-slice variation in cortical porosity we used images of the distal tibia, fibula and radius acquired by HR-pQCT and assessed cortical porosity using Strax 1.0 software in 69 women aged 40 to 61 years.
The mean (SD) cortical porosity at the distal tibia, fibula and radius were 57.6% (5.4), 38.1% (7.3) and 46.9% (6.2). Each 1 mm (12 slices) more distal ROI increased porosity as shown:

	Tibia Mean ±SE	Fibula Mean ±SE	Radius Mean ±SE
Total	**1.22%±0.06**	**0.89% ±0.06**	**2.43%±0.09**
Compact cortex	**1.27%±0.06**	**0.68%±0.09**	**2.01%±0.08**
Outer transitional zone	**0.67%±0.11**	**0.51%±0.12**	**1.25%±0.08**
Inner transitional zone	**0.16%±0.05**	**0.58% ±0.10**	**0.34%±0.05**

We infer that a more distal ROI has a significant effect on cortical porosity which may result in erroneous age, sex and racial differences being reported. This variation needs to be considered when interpreting data in persons who differ in bone length.



Scanning ROI placement difference between short and long forearm



P116

Hip, spine and radial bone mineral density (BMD) in men: inter-machine relationship

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Aim: To validate published inter-machine BMD conversion equations in men.
Methods: Fifty men [mean age (SD) 48.1 (16.9)] underwent femoral neck (FN), total hip (TH), lumbar spine (L2-L4) and radial DXA using Hologic and Lunar. Hologic BMD (H) was converted to Lunar using published equations derived from women (Genant 1994, 2002). Actual (A-Lunar) was compared to Genant calculated (G-Lunar) BMD using Altman analysis and paired t-test. Conversion equations in men for Hologic to Lunar BMD were derived using regression analysis. A range of Hologic BMD values was generated and converted to Lunar values using both published Genant equations (G-Lunar BMD), and conversion equations derived in men (M-Lunar BMD).
Results: Mean FN, TH, L2-L4 and radial BMD for A-Lunar and G-Lunar are shown. G-Lunar BMD was significantly lower ($p<0.01$) than A-Lunar BMD at L2-L4 and the radius. There was no significant difference at FN and TH. Using the two conversion equations, generated M-Lunar BMD was up to 6 and 75 % higher than G-Lunar in the spine and radius respectively.
Conclusion: Published conversion equations for FN and TH from Hologic to Lunar BMD are applicable to men. Calculated Lunar L2-L4 and radial BMD are under-estimated. The impact on predicted young normal mean, and T-scores, has implications for inter-manufacturer conversion of male reference ranges obtained using one manufacturer (e.g. NHANES-III).

	FN	TH	L2-L4	Radius
A-Lunar BMD mean (SD) g/cm^2	0.993(0.154)	1.048(0.145)	1.228(0.185)	0.951(0.092)
G-Lunar BMD mean (SD) g/cm^2	0.984(0.140)	1.051(0.138)	1.168(0.169)	0.624(0.062)
Regression equation for M-Lunar BMD	0.068+1.112*H	-0.016+1.082*H	0.011+1.173*H	-0.061+1.302*H

P117

Rapid response to denosumab in fibrous dysplasia of bone: Report of two cases

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Background: Fibrous dysplasia (FD) of bone is a non-heritable genetic condition characterized by bone pain, skeletal deformities and pathological fractures. The only treatment shown to significantly alleviate pain, suppress bone turnover and improve radiological features in FD has been bisphosphonate therapy. We report on clinical and biochemical outcomes in two patients with active polyostotic FD treated with the RANKL inhibitor, denosumab, following unsatisfactory responses to prior long-term bisphosphonate therapy.
Patient and Methods: A 44-year-old female who had received a cumulative dose of 20mg zoledronic acid over 2.5 years, and a 48-year-old male who had received a cumulative dose of 45mg zoledronic acid over 8 years both experienced minimal reductions in pain scores and markers of bone turnover. Following initiation of denosumab 60mg s.c, changes in bone pain, bone turnover (assessed by serum amino-terminal propeptide of type I collagen (PINP) and urinary deoxypyridinoline (DPD)) and whole body radioisotope bone scans were monitored over a period of 14 and 6 months in the female and male patient, respectively.
Results: Following denosumab administration, both patients demonstrated a rapid and pronounced biochemical response. Within 4-7 weeks, bone turnover markers fell to within the respective reference range. The female patient experienced a reduction in pain whilst the male demonstrated a reduction in tracer uptake on bone scan. Dosing intervals varied significantly between the two patients, depending on disease activity at baseline.
Conclusions: Our observations suggest that denosumab is a potential therapeutic option for fibrous dysplasia, particularly in patients who respond insufficiently to treatment with bisphosphonates.

P118

Deterioration of cortical and trabecular bone quality and microarchitecture during and after lactation

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Introduction: Postmenopausal estrogen deficiency increases the volume of bone resorbed and decreases the volume of bone deposited by each remodeling unit, and increases remodeling rate. We examined whether lactation, an estrogen deficient state, produces irreversible bone loss and microstructural deterioration.
Methods: Distal tibial microarchitecture was measured using high-resolution peripheral quantitative computed tomography in 46 women prior, during and after lactation, and 26 controls.
Results: Five months exclusive lactation reduced cortical bone by 29.1 mg HA (95% CI 18.6-39.5), increased cortical porosity by 2.6% (95% CI, 1.7-3.5), decreased cortical mineralization by 0.25% (95% CI, 0.15-0.35), trabecular bone by 22.1 mg HA (95% CI, 12.0-32.2), and trabecular number by 5.5% (95% CI, 2.6-8.5), (all $P < 0.001$). Despite 24 months of total follow-up, 17 months of restored monthly menses and 11 months after cessation of lactation, there was further 14.2 mg HA (95% CI, 2.3-26.0) cortical and 10.2 mg HA (95% CI, 2.9-17.5) trabecular bone loss (all $p < 0.05$). Total bone loss of 75.7 mg HA (95% CI, 55.4-96.0) was ~50% of bone lost across menopause. Cortical bone loss was three times that in controls (43.2 vs. 12.8 mg HA, $P < 0.01$) and twice that in women breastfeeding when they were over 33 years of age compared with women breasting when they were under 33 years of age; 56.9 mg HA (95% CI, 38.5-75.3) vs. 26.9 mg HA (95% CI, 10.1-43.8; $P = 0.005$).
Conclusion: Lactation produces cortical porosity and loss of trabecular connectivity that may be irreversible.

P119

Bone marrow adiposity is associated with non-vertebral fractures in postmenopausal women

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The relationship between fat and bone is complex, but an increasing body of evidence suggests that increased marrow adiposity may signal a switch in stem cell differentiation towards adipogenesis and may be linked to bone loss. We therefore wanted to study whether marrow adiposity was associated with fracture risk.
In a nested case-control study in Tromsø, Norway, 162 postmenopausal women aged 54-94 years with fractures (hip, humerus and forearm) and 221 controls, had bone marrow fat proportion quantified as the proportion within the total bone with attenuation below that of water in QCT images of the subtrochanteric femur using StrAx 1.0 software. Femoral neck (FN) BMD was assessed using DXA. For the analysis we used linear and logistic regression analyses adjusted for age, height and weight.
Marrow adiposity increased by 0.20 and 0.29 SD for each SD increase in age and height, and by 0.14 and 0.48 SD for each SD decrease in weight and FN aBMD, respectively (all $p<0.01$). Women with fractures, exhibited a higher proportion of marrow fat than controls; 31.2% (SEM 0.5) vs. 27.5% (SEM 0.4), $p<0.001$. Marrow adiposity was associated with fractures independently of FN aBMD, with OR of 1.55 (95% CI 1.17-2.04) for each SD increase in marrow adiposity, while each SD decrease in FN aBMD had OR of 1.72 (95% CI 1.26-2.33). Marrow adiposity significantly contributed to the variance in fracture risk (increment in R^2 was 0.02, $p=0.001$).
Our study suggests that marrow adiposity is an independent risk factor for bone loss and fracture.

P120

Analysis and visualization of bone mineral metabolism in chronic kidney disease by vibrational spectroscopy

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Chitose Institute of Science and Technology

Chronic kidney disease (CKD) causes bone and mineral disorder. In previous works, we characterized the bone quality of femurs in the end stage kidney disease (ESKD) and adynamic bone disease, and then found the bone quality was different between them. In this study, we analyzed and visualized the bone quality of femur in 4/5-nephrectomy CKD model rat by FTIR imaging and Raman spectroscopy due to explore the bone metabolism in CKD. Thirteen-week male rats induced 4/5-nephrectomy and sham-operated were bread 15 weeks, and the kidney and the femur were removed from them. The transverse section of the kidney in the CKD rat was enlarged to approximately 1.5 times, and accumulation of phosphate was observed in the whole kidney tissue, and small calcium deposits was observed by FTIR imaging. The FTIR images and Raman spectra show trabecular bone loss and epiphyseal tissue destruction of femur in the CKD rat. Mineral to matrix ratio, carbonate to phosphate ratio, crystallinity, secondary structure of collagen in the femur were assessed by FTIR images, we found the decrease of mineral to matrix ratio, the decrease of carbonate to phosphate ratio and the change of secondary structure of collagen in trabecular bone and cortical bone in the CKD rat. Whereas, the changes in bone quality of femur in the CKD rat were small as compared with that of femur in the serious ESKD rat. We conclude the bone quality is gradually changed with decreasing of the kidney function.

P121

Comparison of gene expression between osteoblasts from patients of Polynesian and Caucasian ethnicities

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Polynesians have higher BMD and lower rate of hip fracture compared to age-matched Caucasian in New Zealand, and anecdotal evidence suggests that bones of Polynesian patients heal much faster than those of Caucasians. We compared gene expression in osteoblasts cultured from patients of Polynesian and Caucasian origin, in order to identify genes and pathways that contribute to the greater density and accelerated healing of Polynesian bones. RNA was extracted from primary osteoblasts cultured from bone samples obtained during orthopaedic surgery from 30 Polynesian and 30 Caucasian patients (age, sex and BMI matched). Global gene expression was determined in 10 samples from each group using PrimeView GeneChip microarrays (Affymetrix). A total of 171 genes showed two-fold or greater difference between the groups. A number of the genes were further investigated by real-time PCR in the larger group of samples. The levels of *NOV* (nephroblastoma overexpressed), *EFNB2* (ephrin B2), *BGLAP* (osteocalcin) and *EFHD1* (EF-hand domain family, member D1) were significantly lower in the Polynesian group, with approximately two-fold difference between the groups, whereas *KRT34* (keratin 34) was over three-fold higher in the Polynesian group. Significant differences have been identified between osteoblasts of the two ethnic groups and hypotheses about the contribution of the candidate genes to the higher BMD and accelerated healing of Polynesian bone can be formulated and tested.

P122

"Sarco-osteoporosis": The prevalence and functional outcomes of co-morbid sarcopenia and osteoporosis in community-dwelling older adults

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Introduction: Co-morbid sarcopenia and osteoporosis ("sarco-osteoporosis"; SOP) may result in substantially increased risk of functional decline, falls and subsequent fractures. We aimed to compare lower-limb strength and falls risk in community-dwelling older adults with SOP, osteoporosis, sarcopenia, or with neither condition (controls).
Methods: A cross-sectional study of 582 community-dwelling volunteers aged 60-80 years (47% female). Participants completed whole-body, hip and lumbar spine scans by Dual-Energy X-ray Absorptiometry (Hologic, USA), lower-limb strength tests by dynamometer, and the Physiological Profile Assessment for falls risk. Sarcopenia was defined using previously reported sex-specific cut-points for appendicular lean mass normalised to height. Osteoporosis was defined as total hip and/or lumbar spine bone mineral density T-score ≤ -2.5. Generalised linear models calculated differences in lower-limb strength and falls risk scores for SOP, sarcopenia and osteoporosis alone, compared with controls.
Results: The majority (70%) had neither condition; 8% had osteoporosis alone; 19% had sarcopenia alone, and 3% had SOP. After adjustment for potential confounders, estimated lower limb strength was 7 and 19kg lower in participants with sarcopenia alone and SOP, respectively, compared with controls (both $P < 0.05$). Only participants with SOP had significantly increased falls risk scores compared with controls (0.65; 95% CI 0.23 – 1.03).
Conclusions: Older adults with SOP may experience greater decreases in muscle strength and increases in falls risk than those with sarcopenia or osteoporosis alone. Further research is required to determine if this increased falls risk confers an increased fracture risk in this population.

P123

Community dwelling men with dementia are at high risk of hip fracture: Findings from the CHAMP study

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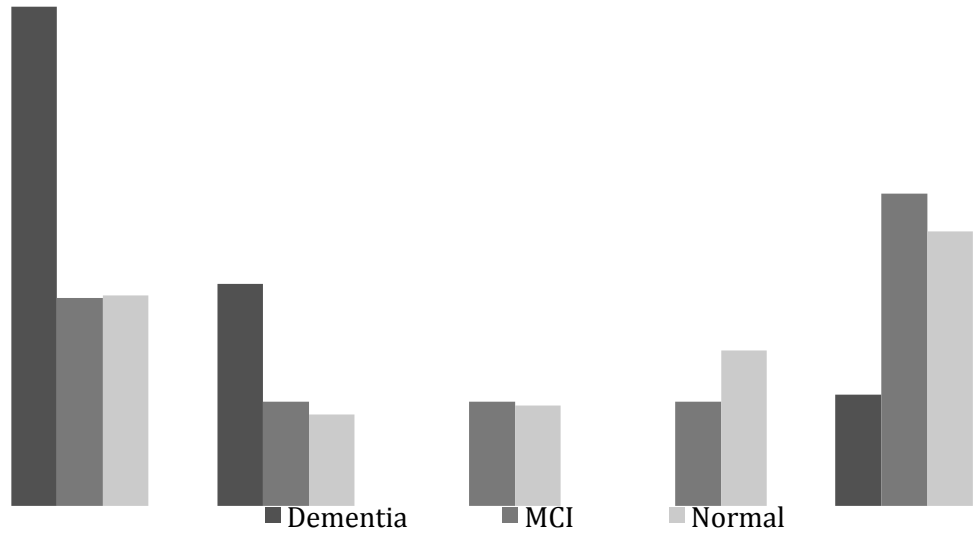
Aim: The aim of this study was to examine the association between cognitive status and fractures in older community dwelling men after accounting for other risk factors for fracture.

Method: In the CHAMP study, 1541 community dwelling men aged 70 to 97 were screened for dementia using the Mini-Mental State Examination (MMSE) and the Informant Questionnaire on Cognitive Decline. Screen positives were then assessed for dementia, mild cognitive impairment (MCI) or no cognitive impairment by a panel of geriatricians and a neuropsychologist. During a mean follow-up of 4.4 years, data were collected on radiologically verified fractures. Data collected at baseline included potential predictors of fractures such as bone mineral density (BMD), falls' history, lifestyle, medical, functional, anthropometric, visual, and serum 25(OH)D measures. The relationship between fractures and cognitive status was analyzed using Cox's Proportional Hazard regression.

Results: 93 (6%) men had dementia and 120 (7.0%) mild cognitive impairment (MCI). There were 114 (7.4%) first incident fractures, including 34 hip fractures. Dementia, but not MCI, was a very strong predictor of fracture, especially hip fracture. Almost 10% of men with dementia suffered a hip fracture compared to 3 % without dementia. Men with dementia had no upper limb fractures but had 5.8(2.5 to 13.7) fold greater risk of hip fracture than men with no cognitive impairment adjusting for age, BMD, falls history, medications, balance and strength.

Conclusion: Dementia was a high risk for hip fracture. This was not related to BMD and only partially explained by poorer balance and gait.

Figure 1: Proportion of fractures by cognitive status according to site of fracture



P124

The role of fat and lean mass in bone loss in older men:Findings from the CHAMP study

Kerrin Bleicher[1], Robert Cumming[2], Vasikaran Naganathan[3], Markus Seibel[4], Fiona Blyth[5], David Le Couteur[3], David Handelsman[4], Louise Waite[5], Thomas Travison[6]

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Aim: The aim of this study was to examine the association between bone mineral density (BMD), fat mass and lean tissue mass in older men.

Methods: BMD of the total hip, whole body lean mass and fat mass were measured with Dual X-Ray Absorptiometry in 1,114 men aged 70-97 in the CHAMP study. Multivariate linear regression models were used to assess relationships.

Results: Over 2.2 years, a third of men lost more than 2% of their body weight (0.8 kg/year of lean body mass, 0.9 kg/year of fat mass). Weight losers lost 1.0%/year of hip BMD while men who gained weight, lost only 0.2% BMD/year. After accounting for age, weight, physical activity, medication and lifestyle factors, lean mass consistently showed a stronger relationship than fat mass with BMD in cross-sectional regression models. In contrast, change in fat mass had the stronger relationship with change in hip BMD. The relationship was stronger in older men (Interaction effect between age and fat mass: p =0.0008). Each standard deviation (1.2 kg) loss of fat mass was associated with 0.2%/year BMD loss in men aged<80 and 0.5%/year BMD loss in men aged ≥80. Each standard deviation(0.8 kg) loss of lean mass was associated with 0.1%/year loss in hip BMD in younger men and 0.2%/year in older men (p<0.001). (Table 1)

Conclusion: Maintaining body weight is important for bone health in elderly men. Body fat plays an important role in this relationship, which may reflect the additional metabolic function of adipose tissue.

Table 1: Longitudinal age stratified [a] models: Standardised associations between annualized change in fat mass (Δ FM) and change in lean mass Δ LM with annualized percentage change in hip BMD (%/yr).

		Age adjusted models		**Multivariate models**	
	SD	Annualised % change of hip BMD (95% CI)	p	Annualised % change of hip BMD (95% CI)	p
Younger					
Δ FM	1.2	0.27 (0.2,0.4)	<.0001	0.20 (0.1,0.3)	<.0001
Δ LM	0.8	0.11(0.02,0.2)	0.02	0.12(0.03,0.2)	0.008
Older					
Δ FM	1.2	0.48 (0.3,0.6)	<.0001	0.48(0.3,0.7)	<.0001
Δ LM	0.8	0.19(0.02,0.4)	0.03	0.23(0.05,0.4)	0.01

[a] Age Stratification: Younger <80 years (n=844); Older 80+ years (n=270). Interaction effect between age and ΔFM p=0.0008.

P125

Benefits of dairy intake on bone structure and muscle mass in elderly women

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Aim: Some studies suggest that dairy intake may play a role in preventing bone and muscle loss with ageing, but there are limited data in the old old (usually after the age of 75). This study aimed to evaluate the association between dairy intake and bone structure and lean body mass in elderly women.
Methods: The study participants were 564 women aged 84.7±2.5 years from the CAIFOS/CARES cohort. Assessments included dairy consumption (milk, yoghurt and cheese) by a validated food frequency questionnaire, 15% tibia BMC, area, and volumetric BMD (vBMD) by pQCT, and body composition and whole body, total hip and femoral neck areal BMD (aBMD) by DXA. Women were categorized according to tertiles of dairy intake: tertile 1 (≤1.5 servings/day), tertile 2 (1.5-2.2 servings/day) and tertile 3 (≥2.2 servings/day).
Results: Compared to the tertile 1, women in the third tertile of dairy intake had 5.4% greater total BMC (190.8±34.4 vs. 181.1±32.9 mg/mm; P=0.024), 5.8% higher total vBMD (410.1±81.6 vs. 387.7±81.3 mg/cm^3, P=0.021) and 7.7% higher trabecular vBMD (223.8±59.1 vs. 207.8±64.0 mg/cm^3; P=0.048) at the 15% tibia. When confounding factors were adjusted in the analyses, these associations remained and tertile 3 had 4.0% higher femoral neck aBMD compared to tertile 1 (P=0.022). Women in the third tertile of dairy intake had 3.4% higher lean body mass (P=0.036) and 4.0% higher appendicular muscle mass (P=0.044) compared to tertile 1.
Conclusion: Our results support previous studies identifying beneficial associations of dairy intake on site specific bone structure and muscle mass in elderly women.

P126

Fracture epidemiology from cradle to grave

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Introduction: To reduce the burden of fracture, bone fragility and injury prevention need to be considered. Examining patterns of fracture in the population irrespective of trauma is therefore informative. The aim of this study was to determine age- and sex-specific fracture incidence rates for males and females residing in the Barwon Statistical Division during 2006-2007.
Methods: Incident fractures (all cause) were identified using keyword searches of radiology reports.
Results: Traditional osteoporotic fractures, vertebral, hip and distal forearm in women, demonstrate stable fracture incidence through early adult life, with an exponential increase beginning in postmenopausal years for forearm followed by vertebral and hip fractures. A similar pattern is observed for the pelvis, humerus, femur and patella. Fractures of the distal forearm, humerus, other forearm and ankle show peaks in incidence during childhood and adolescence. Among men, age-related changes mimic the female pattern, albeit at a lower incidence, for vertebra, hip, rib, pelvis and humerus. A similar childhood-adolescent peak is seen for the distal forearm and humerus. For ankle fractures, there is also an increase in incidence during childhood and adolescence but this extends into early adult life; in contrast to women, there are no further age-related increases. An adolescent-young adult peak incidence is observed for fractures of the face, clavicle, carpal bones, hand, fingers, foot and toe, without further age-related increases.
Conclusion: Examining patterns of fracture in the population provides an evidence base for projecting fracture burden, and for targeting strategies that address risks related to bone fragility and injury.

P127

Comparison of self-reported diagnosis and bone mineral density results in the identification of osteoporosis

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Aim: Osteoporosis can be defined based on areal bone mineral density (BMD) and World Health Organisation criteria (WHO). BMD assessment is not practical for many large-scale epidemiological studies resulting in the reliance on self-report methods to ascertain diagnostic information. The aim of the study was to assess the validity of self-report of osteoporosis against the BMD criteria in a population-based study.
Methods: This study examined data collected from 906 men and 843 women, aged 24 to 98 years, participating in the Geelong Osteoporosis Study (GOS). Women were attending the 10-year GOS follow up and men the 5-year GOS follow up. Self-reported osteoporosis was obtained via a medical conditions questionnaire. BMD results were categorised using WHO criteria and cut points from the Australian reference ranges. Validity was examined by calculating sensitivity, specificity, positive predictive value, negative predictive value and overall level of agreement.
Results: Osteoporosis was self-reported by 118 (6.7%) participants and identified using BMD results for 64 (3.7%) participants. Specificity and negative predictive value were good (95.1% and 96.0%, respectively), whereas sensitivity and positive predictive value were poor (35.9% and 31.4%, respectively). The overall level of agreement (kappa) was 0.92.
Among those who did not fulfil BMD criteria for osteoporosis but reported this diagnosis, 69 of the 81 (85.2%) met WHO criteria for osteopenia.
Conclusion: Due to the poor sensitivity of self-reported osteoporosis against BMD results reported in the current study, relying on self-report methods for identification of osteoporosis is not recommended. These results also support the notion that BMD results may be misunderstood.

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Sex-differences in bone mineral density (BMD) t-scores in older adults referred for DXA: Data from the Barwon region

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Background: Osteoporosis in men is gaining greater recognition as a clinical and public health concern. Although more prevalent in women, men also face high rates of osteoporosis-related morbidity and mortality but frequently remain untreated for the disease. Little is known regarding whether a sex-difference exists in BMD among older men and women referred for dual energy X-ray absorptiometry (DXA) scans.
Methods: BMD T-scores were ascertained for patients aged ≥70 years attending the Geelong Bone Densitometry Service, south-eastern Victoria between 2001-10 (n=5442, 78.5% female). BMD T-scores ≥2.5 standard deviations below the young adult mean were defined as osteoporotic. Age was grouped into five year age strata.
Results: In men and women referred for DXA, mean BMD t-scores were -1.60 ± 1.41 and -2.29 ± 1.10, respectively. The proportion of men and women classified as normal, *osteopenic* and osteoporotic was 30.2%, 44.3%, 25.5% and 10.9%, 47.6%, 41.4%, respectively. After age adjustment, men were ~half as likely to have osteoporosis compared to women (OR 0.45, 95% CI 0.39-0.52). Holding the 70-75 years age group as referent, a dose-response was observed across age strata; OR 1.34 (95%CI 1.17-1.54) for the 75-79 year age group, to OR 5.42 (95%CI 1.09-26.94) for those aged ≥95 years. Results were sustained when excluding those patients referred for fragility fracture or the monitoring of low BMD.
Conclusion: Further work is required to examine whether the observed gender differences in osteoporotic BMD T-scores suggests a sex-bias in DXA referrals, or is explained by sex specific proportions of osteopenia and osteoporosis.

P129

Subgroup analysis for the risk of cardiovascular disease with calcium supplements

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Background: Calcium supplements have been reported to increase the risk of myocardial infarction. We wished to determine whether the effects of calcium supplements on cardiovascular risk vary across different population groups.
Methods: We modelled the effect of calcium (with or without vitamin D) on the time to incident cardiovascular events in pre-specified subgroups based on age, dietary calcium intake, body mass index (BMI), smoking history, history of hypertension, diabetes, and prevalent cardiovascular disease, using interaction terms in Cox proportional hazards models in two randomized controlled trial datasets- our re-analysis of the Women's Health Initiative Calcium and Vitamin D study (WHI CaD), and our pooled patient-level meta-analysis of trials of calcium supplements with or without vitamin D.
Results: For women in WHI CaD not taking calcium supplements at randomization (n=16718), we found no significant interactions between treatment allocation, the risk of myocardial infarction, stroke, or coronary revascularization, and any of the baseline variables. In the pooled patient level dataset of six trials of calcium with or without vitamin D (n=24869), there were also no significant interactions between treatment allocation, risk of myocardial infarction or stroke, and any of the baseline variables.
Conclusion: We found no evidence that the increased cardiovascular risk from calcium supplements differs across varying patient sub-populations.

P130

The association between hip bone marrow lesions and bone mineral density: A cross-sectional and longitudinal population-based study

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Objective: To describe the cross-sectional and longitudinal association between hip BMLs and bone mineral density.
Design: 198 subjects with a right hip MRI and dual-energy x-ray absorptiometry (DXA) scans conducted at two time points, approximately 2.6 years apart were included. MR images were used to assess hip BML presence and size (cm^2) while DXA scans were used to determine bone mineral density (BMD of the total hip, spine and femoral neck.
Results: Fifty-five subjects (28%) had either a femoral and/or acetabular BML. Cross-sectionally, acetabular BMLs were associated with 5-6% lower total hip [p=0.01] and femoral neck BMD [p<0.001]. Resolving acetabular BMLs were associated with a 1-2% increase in BMD at hip [p=0.05] and femoral neck [p=0.01]. In contrast, resolving femoral BMLs were associated with a 4% lower and incident femoral BMLs with 3% higher femoral neck BMD [p=0.04, p<0.001 resp.]. Finally, each 1 cm^2 change femoral BMLs was associated with increase in femoral neck BMD: +0.03 g/cm^2, 95% CI: +0.00, +0.05, and enlarging acetabular BMLs was associated with decrease in hip: - –0.01 g/cm^2, 95% CI: –0.03, –0.00 and femoral neck BMD:- –0.01 g/cm^2, 95% CI: –0.03, –0.001.
Conclusion: Hip BMLs were associated with local BMD (hip and femoral neck) but not with spine BMD and these associations vary according to site. BML prevalence and change was low in this study, hence these findings need confirmation. However, we hypothesize that these associations represent a combination of changes related directly to the BML pathology or changes adjacent to the disease process.

P131

Associations between serum 25-hydroxy vitamin D concentrations and multiple health conditions, physical performance measures, disability and all-cause mortality: The Concord Health and Ageing in Men Project.

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Background: The role of serum 25 hydroxyvitamin D (25(OH)D) for bone and mineral homeostasis is established and there is emerging evidence that poor vitamin D status is associated with a wide range of non-communicable diseases. However, there is disagreement about the optimum 25(OH)D level.
Objectives: To explore associations between serum 25(OH)D levels with a wide range of health conditions, physical performance measures, disability and mortality within one large epidemiological study to identify an optimum range for 25(OH)D.
Design: Cross-sectional study, with additional prospective data on falls and mortality.
Setting: Men aged ≥70 years, living in the community in Sydney, Australia.
Participants: 1659 community dwelling men aged ≥70 years, taking part in the Concord Health and Ageing in Men Project (CHAMP).
Measurements: Serum 25(OH)D levels, general health status, self-reported diseases, physical performance measures, disability (ADLs and IADLs) and falls.
Results: Fair, poor and very poor health, self-reported diabetes, hyperglycaemia, depression, muscle weakness, poor balance and all-cause mortality were all associated with serum 25(OH)D levels<50 nmol/L, even after adjustment for confounding. Our findings also suggest that among older men, for a wide range of health conditions, physical performance measures, disability, falls and mortality, the optimum range for 25(OH)D is between 50 and 75 nmol/L, with no additional benefit and potentially harmful effects for 25(OH)D levels >75nmol/L.
Conclusion: Programs aimed at achieving an optimum range for serum 25(OH)D at levels between 50-74.9 nmol/L may have overall health benefits and appears adequate for older men. Levels above 75nmol/L have no additional benefits and may be harmful.

P132

Effects of habitual physical activity on tibial cortical bone mass, structure and its distribution in pre-pubertal boys and girls

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Cortical bone is a non-uniform tissue, with its apparent mass and density varying around the bone cross-section. Targeted weight-bearing activities during the pre-pubertal years can improve bone mass and structure, but less is known about the influence of habitual physical activity (PA). This study examined the effects of gender and habitual PA on cortical bone density, geometry and its mass distribution in pre-pubertal children. 245 girls and 241 boys (7-9 years) had a pQCT scan to measure tibial mid-shaft total and cortical area, density, SSI_p and the mass/density distribution through the bone cortex (radial distribution) and around the centre of mass (polar distribution). PA quartiles were generated based on daily step counts (pedometer, 7-days). There were no gender differences in total and cortical bone area or density, but cortical mass in the posterior-lateral and posterior-medial region were 3-9% ($p<0.001$) greater in boys compared to girls. In contrast, peri-cortical density (outer third of cortical bone) was greater (0.6%, $P<0.05$) in girls. There were no differences in any of the bone parameters across the PA quartiles for either gender, with the exception that boys in the highest versus lower quartile had 7% ($p<0.001$) greater cortical mass at the posterior-lateral region. In conclusion, this study indicates that: 1) total cortical bone structure and density is no different between pre-pubertal boys and girls, but there are small region-specific differences in the mass/density distribution, and 2) habitual PA results in small regional specific gains in cortical bone in pre-pubertal boys, but not girls.

P133

A case of skeletal fragility determined by microstructural investigation

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Cortical porosity increases with advancing age. It is believed to be a factor in skeletal fragility in the elderly, but is not known to contribute to skeletal fragility in children. We studied a child, aged 10½ years, who sustained four fractures in the previous 8 years. She was phenotypically normal, with no signs of osteogenesis imperfecta. Her bone density and biochemical markers were normal. She was diagnosed with epilepsy at the age of 3 and treated with valproate until age 7 without recurrence of seizures. To try and clarify the cause of her skeletal fragility we assessed a tetracycline labeled transiliac bone biopsy. Bone histomorphometric measurements were performed using Osteomeasure. Micro-CT analysis of the biopsy was undertaken and 3-dimensional bone parameters and a 3-D model generated.
Histomorphometric and micro-CT analyses showed marked cortical porosity and decreased trabecular bone volume. There was no mineralization defect and tetracycline-based dynamic measurements were normal. The cortical osteons appeared active.
After sustaining a further fracture at age 11 the patient was treated with intravenous pamidronate infusions for two years. She had no further fractures over this period, but histomorphometric and micro-CT analysis of a repeat bone biopsy, age 13, showed persistence of marked cortical porosity with some increase in trabecular bone volume.
This case suggests a novel mechanism for skeletal fragility in children. It also reminds us that quantitative bone histomorphometry can identify tissue level abnormalities that cannot be detected by standard imaging and biochemical tests.

P134

Sarcopenic obesity and dynapenic obesity: Five-year associations with falls risk in community-dwelling older adults

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Background: High fat mass may exacerbate functional deficits associated with age-related declines in muscle mass and strength. We aimed to determine whether sarcopenic (low muscle mass) and dynapenic (low muscle strength) obesity result in increased falls risk over five years in older adults.
Methods: 674 community-dwelling volunteers (mean ± SD age 61.4 ± 7.0 years; 48% female) completed assessments at baseline and 5.1 ± 0.5 years later. Sarcopenia and dynapenia were defined as the lowest sex-specific tertiles for dual-energy X-ray (DXA)-assessed appendicular lean mass/height squared and dynamometer-assessed lower-limb strength, respectively. Obesity was defined as the highest tertiles of DXA-assessed trunk fat mass and waist-hip ratio (WHR). A validated Physiological Profile Assessment assessed falls risk.
Results: 141 (21%) participants had 243 diagnoses of sarcopenic obesity (86) and/or dynapenic obesity (157). Falls risk scores significantly increased over five years for men with dynapenic obesity (z-score: 0.32, 95% CI 0.07 - 0.56), and for women with dynapenic obesity (0.38, 95% CI 0.14 - 0.62) and dynapenia alone (0.22, 95% CI 0.01 - 0.43), compared to non-dynapenic, non-obese participants. Similar results were observed regardless of whether trunk fat or WHR was used to define obesity. Sarcopenic obesity was not associated with an increase in falls risk ($P > 0.05$).
Conclusions: Dynapenic obesity, but not sarcopenic obesity, is predictive of increased risk of falling community-dwelling older adults in community-dwelling older adults five years later. Dynapenic obesity can be easily assessed and may be a useful component of falls risk assessments in the clinical setting.

P135

Osteocalcin, muscle strength and indices of bone health in older women

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Exercise increases total and undercarboxylated osteocalcin (TOC and ucOC) and improves glucose control. We investigated associations between TOC, ucOC and ucOC/TOC ratio (ucOC%) and indices of both muscle and bone health. We hypothesized that in 90 women aged 70+ years (1) ucOC and ucOC% is associated with increased muscle strength and (2) TOC, but not ucOC, is associated with other bone turnover markers and bone quality.

A chemiluminescence immunoassay was used to quantify TOC, with hydroxyapatite pre-treatment for ucOC. Lower-limb muscle strength was measured at three sites using the Manual Muscle Testing System. Heel ultrasound assessed speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI). Regression statistics were used for analysis.

ucOC% was associated with muscle strength (age-adjusted; $p<0.05$) and remained significant at two of the three sites after adjustment for 25(OH)D levels and BMI. Neither TOC, nor P1NP or CTx were associated with muscle strength ($p=0.15$ to 0.72). Alpha-1-antichymotrypsin (α-ACT), was inversely associated with ucOC% ($p=0.03$) only. TOC was positively associated with both P1NP and CTx ($p<0.001$), while ucOC% was weakly negatively associated with both P1NP and CTx (both $p = 0.06$). With higher TOC levels there was a corresponding lower BUA, SOS and SI; all $p<0.04$).

TOC is associated with bone health. An association between ucOC% (but not TOC) and muscle strength is novel and supports a distinct metabolic musculoskeletal role for ucOC in humans. This and the negative correlation between ucOC% and α-ACT are consistent with improved glucose control mediated through a musculoskeletal interaction.

P136

High-impact physical activity participation estimated by the BPAQ is associated with bone mass and cardiovascular risk factors

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Physical activity participation that engenders high magnitude or rapid loads is associated with benefits to bone mass and strength. The association between such participation and risk factors for cardiovascular disease, however, has not been established. **Methods:** We recruited 94 men and women (mean age 34.0 ±13.3 years) to undergo measures of cardiovascular disease risk (body mass index, total cholesterol, fasting blood glucose, waist-to-hip ratio, and mean arterial pressure) and DXA measures of bone mass (femoral neck, lumbar spine, and whole body BMD) and body composition (whole body lean mass and fat mass). Physical activity participation was estimated using the bone-specific physical activity questionnaire (BPAQ). **Results:** Current BPAQ score was positively associated with lumbar spine BMD and predicted 6% of the variance in the measures ($P = 0.02$). When participants were ranked in tertiles by current BPAQ score, those in the highest tertile had greater lumbar spine BMD than those in the bottom tertile (1.230 ±0.154 vs. 1.069 ±0.277 g/cm^2, $P = 0.008$). Current BPAQ score was inversely related to total cholesterol ($r = -0.49$, $P = 0.001$), waist-to-hip ratio ($r = -0.29$, $P = 0.003$), and mean arterial pressure ($r = -0.30$, $P = 0.002$). Those in the highest tertile for current BPAQ score also exhibited lower total cholesterol, waist-to-hip ratio, and mean arterial pressure than those in the middle and bottom tertiles ($P < 0.05$). **Conclusion:** Physical activity that applies high magnitude or rapid loads may be additionally beneficial for attenuating measures of cardiovascular disease risk.

P137

PTH IN THE ELDERLY: ARE THE NORMAL RANGES METHOD DEPENDENT? PRESERVED RENAL FUNCTION ESTIMATION BY USING DIFFERENT INDEXES

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The aim of the present collaborative work was to re-evaluate normal ranges of parathyroid hormone in the elderly in order to include subjects over 80 years old, as life expectation has risen in the last years. We investigated retrospectively data from patients attending to the various hospitals integrating the Biochemistry Department. The patients were selected with the following inclusion criteria: preserved renal function, non-diabetic, normal levels of calcium, both in serum and urine, Vitamin D ≥ 30 ng/ml, euthyroid, no treatment with corticoids, anti resorptives or estrogens, and normal arterial tension. They were divided into 3 groups according to age: A (50-60 years), B (61-70 years) and C (over 71 years). Methods for PTH included in the comparison were ICMA Immulite™ Siemens, ECLIA Cobas™ Roche and ICMA Architect™ Abbott. The number of subjects included in each age group per method was ICMA Immulite n: 145 (Group A: 54, Group B: 39, Group C: 52); ECLIA Cobas n: 93 (Group A: 40, Group B: 30 and Group C: 23) and ICMA Architect n: 168 (Group A: 54, Group B: 52 and Group C: 62). Two different formulas were used to estimate renal function, MDRD and CDK-EPI. Statistical analysis included Anova, Kruskall-Wallis, Bland-Altman for methods´ differences, and Spearman Ranks correlation for evaluation of relationship between PTH and age. The results showed no significant differences between age groups assayed by the different methods: Group A (50-60 years), median PTH ICMA Immulite 48.5 pg/ml vs. median PTH ECLIA Cobas 43.8 pg/ml vs. median PTH ICMA Architect 46.6 pg/ml; Group B (61-70 years), median PTH ICMA Immulite 57.4 pg/ml vs. median PTH ECLIA Cobas 50.5 pg/m vs. median PTH ICMA Architect 53.1 pg/ml; Group C (71-89 years), median PTH ICMA Immulite 56.6 pg/ml vs. median PTH ECLIA Cobas 50.4 pg/ml vs. median PTH ICMA Architect 50.8 pg/ml . Also no significant differences were found between age groups assayed by the same method, and no association was found between age and PTH level. We conclude that PTH levels are similar between age groups evaluated, making it possible to use the adult reference range for the elderly. The condition for use is that renal function is preserved (evaluated with creatinine clearance and formulae for glomerular filtration rate) and that adequate Vitamin D levels are present.

P138

Does wearing "Barefoot" footwear improve musculoskeletal adaptations to high impact exercise?

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Aim: To compare lower extremity musculoskeletal responses to an osteogenic training program between limbs wearing a 'barefoot' or cushioned sports shoe.
Methods: A within-subject pilot exercise intervention was conducted. Previously sedentary young adults were randomly assigned one 'barefoot' and one regular athletic shoe in which they performed 45-60 min high impact exercise training 3-4 times per week for 6 months. Anthropometry and parameters of bone and muscle strength, including femoral neck BMD, content, area and cross sectional moment of inertia, and lower extremity lean and fat mass (XR-800, Norland), trabecular density at the distal tibia, cortical thickness at the 38% tibial site and calf muscle area (XCT3000, Stratec) were measured. The effects of training and shoe type were examined by 2-way ANOVA, controlling for age, weight, calcium and compliance.
Results: Six young adults (4F, 2M, 23±2.4 years) were recruited. All bone parameters improved over the training period, except tibial trabecular density, with greater improvements observed in the barefoot limb; however, no significant differences in effect were detected between limbs. A 4.5% increase in lower extremity lean mass (p=0.01) and 10.1% decrease in fat mass (p=0.002) occurred in the barefoot limb only.
Conclusion: Power was low in this pilot trial, thus trends for positive skeletal responses to high impact training did not reach statistical significance in either limb. By contrast, greater increases in lean mass and decreases in fat occurred in the 'barefoot' than regularly shod limb. The novel within-subject design provided effective control of individual variation with no adverse effects.

P139

Immobilisation Hypercalcaemia

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Aims: To highlight the importance of hypercalcaemia screening and management in patients requiring prolonged hospital admissions.
Methods: Case reports
Results:
Case 1: 36yo morbidly obese female with multiple comorbidities presented with intra-abdominal bleeding and collections. Required multiple laparotomies throughout 7 month ICU stay. Day 196: new onset of nausea, polydipsia and abdominal pain with corrected calcium 3.10mmol/L. Intravenous fluids failed to normalise calcium, given 30mg IV pamidronate. No recurrence of hypercalcaemia with increasing mobility.
Case 2: 36yo female with cardiogenic shock and acute renal failure in setting of intravenous drug use. Developed tetraplegia and bulbar weakness secondary to ICU myopathy/neuropathy. No improvement during prolonged admission; discharged to nursing home day 200 with continuous PEG feeding. Hypercalcaemia noted by day 67, managed with increased fluid through PEG. Day 200: corrected calcium 3.24mmol/L, given IV 30mg pamidronate. Has remained normocalcaemic despite ongoing immobility.
Case 3: 67yo man admitted with sepsis and back pain found to have multiple epidural collections. Urgent decompression performed with only partial improvement of lower limb paraplegia. Asymptomatic hypercalcaemia from day 42, peak 3.22mmol/L day 89. 30mg IV pamidronate given. Remains normocalcaemic with improvement in mobility.

Other causes of non-PTH mediated hypercalcaemia excluded in all patients (malignancy, myeloma, granulomatous disease).

Table 1

	Patient 1	Patient 2	Patient 3
Admission calcium corr (2.15-2.65mmol/L)	2.64	2.47	2.68
Peak calcium corr	3.10	3.24	3.22
Day of peak calcium	196	200	89
Phosphate (0.8-1.4mmol/L)	1.8	1.16	1.24
Vitamin D nmol/L	40	73	72
1,25(OH)2 vitamin D (78-190pmol/L)	26	-	28
PTH (1.5-7.6pmol/L)	<0.3	0.7	1.3
Creatinine (40-80umol/L)	100	68	93
24hr urinary calcium excretion (2.0-7.5mmol/24hs)	9.7	1.93	-
Calcium post pamidronate	2.42	2.50	2.59
C-telopeptide pre pamidronate (100-600 ng/L)	2311	-	
C-telopeptide post pamidronate	1568	-	826
P1NP pre pamidronate(>80ug/L indicates high bone turnover)	488	-	
P1NP post pamidronate	218	-	303

Conclusion: Immobilisation should be considered in a rehabilitation setting as a differential for hypercalcaemia. Intravenous bisphosphonates are highly effective in this condition. Bone turnover markers can be markedly elevated, demonstrating bone resorption as the pathogenesis. Classically, hypercalciuria develops with suppressed PTH and low 1,25 $(OH)_2$ vitamin D.

P140

Effects of Circulating Osteocalcin on Bone Remodelling

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Aim: Osteocalcin (OCN) is a protein synthesized solely by osteoblasts. While circulating OCN may play a role in regulating glucose metabolism, its functional role in bone is unclear. As osteocalcin knock-out mice display increased bone formation[1], we hypothesized that an increase in circulating OCN would lead to decreased bone formation.
Methods: Using in-vivo gene therapy [2], we transfected a wild-type osteocalcin (wtOCN) or a mutant osteocalcin construct (mOCN) which cannot be carboxylated into hepatocytes of 7-week-old mice. Empty vector (EV) was used as control. Tibiae were harvested at d21 post-transfection for mCT, histomorphometry and gene expression analysis.
Results: Compared to EV, total serum OCN levels were increased by 30% (wtOCN) and 40% (mOCN) on d21. BV/TV decreased from 17.9±1.8% in EV to 11.5±1.2% and 9.4±1.1% in wtOCN and mOCN receiving mice, respectively ($p<0.01$). While trabecular spacing was increased, trabecular number and cortical thickness were significantly decreased by both OCN vectors. Trabecular thickness was unchanged. Histomorphometry revealed that compared to EV controls, both osteoblast and osteoclast surfaces were significantly increased in wtOCN and mOCN vector receiving mice. Furthermore, hepatic expression of the mOCN vector was associated with significant increases in Runx2 (1.5-fold), osterix (2-fold), BMP-4 (1.5-fold increase), BMP-7 (1.7-fold increase) and osteocalcin (1.5-fold) mRNA expression. . RANKL mRNA expression was significantly increased in both OCN vector-receiving mice.
Conclusion: Increasing circulating osteocalcin via gene therapy is associated with bone loss and accelerated bone turnover in mice.

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